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RESEARCH ARTICLE

The Nexus of Economic Growth and Environment: A Challenge for SDG-13 with Reference to India

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ABSTRACT

The current study uses Co-Integration Analysis to estimate the Environmental Kuznets Curve for GHG Emission in India for the years 1985 to 2018. This analysis relies on secondary data from the World Bank's World Data Indicator for Gross Domestic Product per capita, trade intensity, and greenhouse gas emissions. The evidence suggests that there is a 'N' shaped link between environmental deterioration and GDP growth. Due to the inclusion of trade intensity, trade liberalization appears to increase Greenhouse gas emissions in India. Acceptance of EKC implies that there is environmental degradation at an early stage of development, but that at a broad level of development, per capita GHG emissions begin to decline, implying that a nation can choose the present value of higher future growth and a clean environment at the expense of the current rate of environmental damage.

Keywords: Trade intensity, Environmental quality, Environmental Kuznets Curve, Greenhouse gas emission

INTRODUCTION

EKC is a well-known hypothesis in the area of Environmental Economics. The study "Environmental Impact of North American Free Trade Agreement" by Gene Grossman and Alan Krueger indicates that urban air concentrations of SO2 and two kinds of Suspended Particulate Matter (SPM) have an inverted U-shaped connection with national income level. Environmental quality deteriorates with economic growth at low income levels, but over a specific threshold level of about $4000 to $5000 per capita per year, air quality improves with economic growth. After 1995 work by Simon Kuznets hypothesizing a link between economic growth and income distribution, this inverted U relationship was coined the EKC (Kuznets, 1995).

Following that, other studies were released that took into account various variables such as nations, time, economic indicators, and pollutants, resulting in varied forms of EKC. These diverse shapes of EKC, whether growing, U-shaped, Inverted-U-shaped, N-shaped, inverted N-shaped, etc., are sensitive to the context, model specification, explanatory variables, turnaround points, time-period, location, and so on, either supporting or rejecting this theory. Understanding the income-environment link is the first step toward analysing the impact of economic expansion on the environment. In this study, an attempt is made to integrate explicit trade considerations for a better understanding of the income-environment connection. The impact of commerce on the environment can occur through both income and non-income channels (Frankel & Rose, 2002). The EKC concept is based on the interplay between economic growth and environmental deterioration. According to Grossman (1995), the amount of environmental damage in a country at any moment in time is endogenous and is determined by the country's wealth level. (Grossman and Kruger, 1995; Selden and Song, 1994)This impression may be achieved through three channels: scale, composition, and technical effect.

The scale effect quantifies the increase in pollution that would result if economies were simply scaled up while keeping the mix of commodities produced and manufacturing technology constant. To fuel expansion, the need for natural resources grows, and as a result, direct and indirect use of natural resources is turned into a manufacturing process that creates industrial waste. This by-product of economic expansion is a major hazard to the environment. With a rise in income, the industrial structure of an economy undergoes a shift, as does the composition of an economy. The composition impact is reflected by changes in the percentage of filthy items in national GDP while maintaining the economy's scale and emission intensities constant. During this period, the secondary sector begins to mature, and the economy begins to transition toward greener technology. In this sense, economic expansion has a positive effect on the environment, causing the economy to become more knowledge intensive rather than capital intensive.

Current status of world trade

Trade has grown tremendously, over the last two centuries which have completely altered the world economy. The export amounts to 1/4th of sum of world production. This alteration procedure has played a significant role in generating gains by trade which also possessed important distributional consequences. The graphs-1& 2 show the value of world exports and value of export with share of GDP (%). These values are based on constant price level, which means they are adjusted to remove the effect of inflation level. Here, 1913 has been taken as a base year.

Current status of Indian trade

The graphs 3, 4 & 5 show the value of Indian merchandise trade as percentage of GDP, Import volume index (2000=100) & Export volume index (2000=100) over the period 1960-2016, 1980-2016 and 1980-2016 respectively. India liberalized its trade policy in 1991 motivated by its role in economic growth. The growth rate of export was -1.1% in 1991 which was increased to 20.2% due to various reforms. However, since July 1996 it starts to decline. Effects of liberalization are visible as post liberalization growth is higher, then the pre liberalization. Foreign trade now accounted for 48.8 % of India's Gross Domestic Product (2015), remarkably exceeding than 13 per cent in 1990-91.

The repercussions of trade on growth have 2 sides of coin; First, explained earlier i.e., international trade increases the opulence of a country by permitting the buyers to buy more and better-quality goods at lowest price level, enhanced efficiency, drive competitiveness, increase innovation and economic growth. Second side of coin, shown the picture of global competitiveness in international trade which leads to more industrialization, resulted into the emission of greenhouse gases, contaminated water resources, over exploitation of natural resources, generating pollutant which leads to affect the environmental standards. These burning issues also raised in on Global environmental forum and took the main agenda place in Kyoto protocol 2020 targets.

MATERIAL AND METHODS

At the outset, on the basis of aforementioned explanation, we can show the relationship among international trade, economic growth and environmental quality by below mentioned functional equations as;

Economic Growth = f (International Trade)..... (1.1)

+verelation

Means if rate of international trade increases the rate of economic growth also increases.

Also, Economic Growth = f (population need) .....(1.2)

Domestic demand for goods and service = f (population needs) .....(1.3)

So, from equation 1.5.2 & 1.5.3

Economic Growth = f (Domestic demands for good and service) .....(1.4)

Therefore, from equation 1.5.1 & 1.5.4

International trade = f (Domestic demands for good and service) .....(1.5)

And vice versa

Demand for goods and service = f (International Trade).....(1.6)

Therefore, International trade, economic growth has negative relation with Environmental Quality therefore, for better quality of environment, we have to control international trade and economic growth. But as mentioned above in equation 1.2, therefore, as population, taste and preference of the consumers increase economic growth will also be increase. Many studies focused on the second side of the relation among the economic growth, international trade and quality of environment by investigating existence of EKC Hypothesis. To identify the relationship between international trade & environmental quality. This research paper examines three effects, which are as follows-

Scale Effect- It represents the situation when the scale of economy increases or decreases over the years. It is represented by RGDP/ Area.

Composition Effect- It explains the use of gross fixed capital formation and labour force to produce different goods and hence the composition of product. It is represented by GFCF/L.

Technique Effect- It explains the change in total product due to technological advancement and hence the effect of change in technology. It is represented by RGNP.We have calculated these three-effects using the data from 1985-2018. Fig. 5 describe this clearly.

METHODOLOGY

To check the shape of EKC for greenhouse gas emission this study uses cubic specification of EKC model. As per the specification model to derive the shape of EKC, model must have followed the first & second order validation conditions. The model is as follow:

$$\ln GHG_t = c_0 + c_1 \ln GDP_t + c_2 \ln GDP_t^2 + c_3 \ln GDP_t^3 + c_4 \ln T_t + \varepsilon_t \quad \text{[Model]}$$

Where GHG is Greenhouse gas emission and GDP denotes gross domestic product.

Analysis of Unit Root Test After standardization of selected variable time series data, to check The stationarity we applied ADF/KPSS test. When we applied unit root test its very important to design the hypothesis and for this, we designed two hypotheses which are as follows-

H_0: series is stationary or trend stationary.

H_0: I (0)

H_1: series is non- stationary.
H_1: I (1)
For ADF-
H_0: $|\phi| < 1; \delta < 1 \Rightarrow Y_t \sim$ I (0), series is stationary.
H_1: $|\phi| = 1; \delta = 0 \Rightarrow Y_t \sim$ I (1), series is non-stationary.
For KPSS
H_0: $\sigma_\varepsilon^2 = 0$; I (0), series is stationary.
H_1: $\sigma_\varepsilon^2 > 0$; I (1), series is non-stationary.

When we applied these hypotheses, condition for the acceptance and rejection of null hypothesis is mentioned in table number 4 After applying test on our selected time series variables, we get these results which are present in table number 2.

RESULTS AND DISCUSSION

Interpretation

In the output table of ADF and KPSS test, we have examined the stationarity assumption under different scenarios that is no constant, constant only, constant+trend and constant+trend+trend^2. As per the result mentioned in table 2, calculated value for GHG emission is 0.4 which is more than critical value (negative 2) therefore, null hypothesis rejected, and alternate hypothesis accepted which means series is non-stationary and having unit root for no constant and constant only and constant plus trend. While as per the result of KPSS test P-values for GHG emission are 79.8% for no constant which is greater than 5% therefore we rejected the null hypothesis of stationarity for no constant. Again, under KPSS test P-values for GDP emission is71.1% under the assumption of no constant which are greater than 5% so we rejected the null hypothesis of stationarity so time series is not stationary. Results showed that series is non-stationary which confirmed the existence of unit root, then it is necessary to check the order of stationarity. So, in the fourth step, to check the order of stationarity subsequent difference equation method was applied on the selected variables. After the first difference we again run the ADF & KPSS test & the results are mentioned in table 3. The output table of ADF & KPSS for different variables showed that all the variables are stationary in nature after first differencing. So, from above result we concluded that all-time series data are not stationary in nature having unit root problem.

Analysis of Johansen's Cointegration Test

As there are two components of Johansen test, "trace test" and "maximum eigen test". After checking stationarity results, we run the Johansen cointegrating test. The hypotheses of these two tests are mentioned below.
For Trace test-
H_0: $K = K_0$; $K_0 = 0$, There is no cointegration between the series. (r=0)
H_1: $K > K_0$; $K_0 \neq 0$, There is cointegration between the series. (r>0)
For Maximum Eigen Test-
H_0: $K = k_0$; $K_0 = 0$, there is only one possible combination of non-stationary variables to yield stationary series.
H_1: $K = k_0+1$; $K_0 \neq 0$, there is more than one possible combination of non-stationary variables to yield a stationary process.
When we applied these hypotheses, condition for the acceptance and rejection of null hypothesis is mentioned in table number 4. We have tested this model through Johansen cointegration test. The result is listed below in table 5. Table 5 shows that the calculated values for trace test under different condition no constant, constant-only and constant+trend are 65.2, 93.2, 107.7 which are greater than critical values 40.2, 47.9, 55.2, respectively. Hence, we rejected the null hypothesis of no cointegration which means gross domestic product and GHG are cointegrated in long run for model. Now under maximum eigen value test calculated value1.4, 1.1, 1.1 are less than the critical values 4.1, 3.8, 3.8. they all failed. Therefore, we rejected the null hypothesis which means there are more than one

combination (number of combinations are three) to generate stationarity in the process and hence the conclusion is, variables are cointegrated for model. The value of r (co-integrating vector) is 3 for model which is less than the no. of variables used in model (4).It satisfied the condition of eigen value test hence we concluded that four variables are connected to each other in long run for model 3.1 in the form of maximum three linear combination which will give stationary process. Based on the above results we concludes that there is significant relationship between economic growth and environmental quality.

Analysis of EKC for GHG Emission in India

To check the shape of EKC for greenhouse gas emission this study uses cubic specification of EKC model. As per the specification model to derive the shape of EKC, model must have followed the first & second order validation conditions. The model and validation conditions are as follow:

$\ln GHG_t = c_0 + c_1(\ln GDP_t) + c_2(\ln GDP_t^2) + c_3(\ln GDP_t^3) + c_4 \ln T_t + \varepsilon_t$ ..... (1.1)

First order conditions

1. If $c_1 = c_2 = c_3 = 0$ indicates no growth-pollution association.
2. If $c_1 > 0$, $c_2 = c_3 = 0$, it indicates linearly increasing growth- pollution association between the variables.
3. If $c_1 < 0$, $c_2 = c_3 = 0$, it indicates linearly decreasing growth- pollution association between the variables.
4. If $c_1 > 0$, $c_2 < 0$, $c_3 = 0$, it indicates inverse U shape growth- pollution association between the variables.
5. If $c_1 < 0$, $c_2 > 0$, $c_3 = 0$, it indicates U-shape growth- pollution association between the variables.
6. If $c_1 > 0$, $c_2 < 0$, $c_3 > 0$, it indicates N shape growth-pollution association between the variables.
7. If $c_1 < 0$, $c_2 > 0$, $c_3 < 0$, it indicates inverse N shape growth-pollution association between the variables.

First order differentiation is given by-

$\partial(GHG_t)/\partial(GDP_t) = \alpha_1 + 2\alpha_2 GDP_t + 3\alpha_3 GDP_t^2$ ..... (1.2)

For the "Environmental Kuznets Curve" to be N-shape or inverse N shape, equation 1.1 must have local maxima & minima at two distinct values of Y. Which can be found at

$$Y = \frac{-2\alpha_2 \pm \sqrt{4\alpha_2{}^2 - 12\alpha_1\alpha_3}}{6a_3} \quad \text{or } Y = \frac{-\alpha_2 \pm \sqrt{\alpha_2{}^2 - 3\alpha_1\alpha_3}}{3a_3}$$

As mentioned above the necessary condition for the EKC to be N-shape or inverse N shape is that $c_1c_3 > 0$, $c_2 < 0$ and $c_1c_3 < 0$, $c_2 > 0$, respectively. However, this is not the sufficient condition as we cannot explain anything about the validity of model from this. For validity we have to do the second order differentiation of equation 1.1. which is given below:

$\partial^2(GHG_t)/\partial(GDP_t)^2 = 2\alpha_2 + 6\alpha_3 GDP_t =$ ..... (1.3)

The validity of second order condition is also provided by Eq 1.3

$\partial(GHG_t/\partial GDP_t) = 0$; $\partial^2(GHG_t)/\partial(GDP_t)^2 >$ or < 0, For concavity and convexity we must have to check the max. and min. which exist in equation 1.2, to find out the two conditions, which are for maxima GDP should be less than zero and for minima value of GDP should be gather than zero.

If first order condition is being fulfilled but 2nd order condition does not satisfy then model cannot be estimated. Now equating Eq. 1.3 equal to zero. It will give point of inflection and validity of model is provided by $4\alpha_2^2 - 12\alpha_1\alpha_3 > 0$ or $\alpha_2^2 - 3\alpha_1\alpha_3 > 0$. The parameters of difference equation for GHG emission in India is mentioned below in table number 6.After applying cubic specification model and fulfilment of first and second order conditions we calculated result which is mentioned in table-5 & shape of EKC shown in figure 4 which is N- shaped.With increase in the level of income of Indian household, demands for cleaner goods increases as a result EKC curve turns out to be N- shape curve where technical and composition effect out weighted the scale effect at the beginning for GHG.

CONCLUSION

The relationship between economic growth & drivers of economy is very complex as they contribute a great deal in the development of an economy but at the same time, they adversely affect the environmental quality. International trade is one among the other major drivers of economic growth. As the world economy started growing, a significant part of this growth was driven by international trade. It is observed that countries which are open to international trade tend to grow faster. They tend to improve their productivity & provide higher levels of income and opportunities to their citizens. Thus, international trade played an important role in shaping the global economy. But at the same time with the increase in production process the environmental quality started deteriorating. The shape of EKC for GHG emission were turned out N-shaped which represents that GHG emission increases in India after 1991 and after reaching at the threshold limit it started decreasing. As the GDP level start increasing the quality of environment start improving in long run.N-shaped represents that among all the three technical effects emerged as a stronger effect and it became a reason of the turning point of EKC.

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Table 1.1: Condition for the acceptance and rejection of null hypothesis under ADF & KPSS Test

Test	Condition	Result
ADF	If calculated value > Critical value	Rejection of H_0
KPSS	If P- value < 5%	Acceptance the H_0

Table-1.2: Output table of ADF & KPSS for different variables

Variables	Test	Calculated Value	P-Value	Critical Value	Stationary?
GDP	No Constant	0.1	71.1%	-2.0	F
	Constant-Only	-4.8	0.3%	-3.2	T
	Constant +Trend	-6.0	0.0%	-1.6	T
	Constant+Trend+Trend^2	-5.9	0.0%	-1.6	T
GHG Emission	No Constant	0.4	79.8%	-2.0	F
	Constant -Only	-2.6	13.5%	-3.2	F
	Constant +Trend	-0.8	19.9%	-1.6	F
	Constant+Trend+Trend^2	-3.9	0.0%	-1.6	T

*all values are at 5% level of significance. **(**Source – Author's calculation)

Table 3: Output table of ADF & KPSS for different variables after first differencing

Variables	Test	Calculated Value	P-Value	Critical Value	Stationary?
GDP	No Constant	-3.8	0.1%	-2.0	T
	Constant-Only	-4.8	0.3%	-3.2	T
	Constant +Trend	-6.0	0.0%	-1.6	T
	Constant+Trend+Trend^2	-5.9	0.0%	-1.6	T
GHG Emission	No Constant	-4.3	2.8%	-2.0	T
	Constant-Only	-3.4	3.5%	-3.2	T
	Constant +Trend	-2.1	1.9%	-1.6	T
	Constant+Trend+Trend^2	-3.9	0.0%	-1.6	T

*all values are at 5% level of significance.

(Source – Author's calculation)

Table 4: Condition for the acceptance and rejection of null hypothesis under Trace test and Maximum Eigen Test

Test	Condition	Result
Trace test	If calculated value > Critical value	Rejection of H_0
Maximum Eigen Test	If calculated value < Critical value	Rejection of H_0

Table 5: Cointegration for Model 1

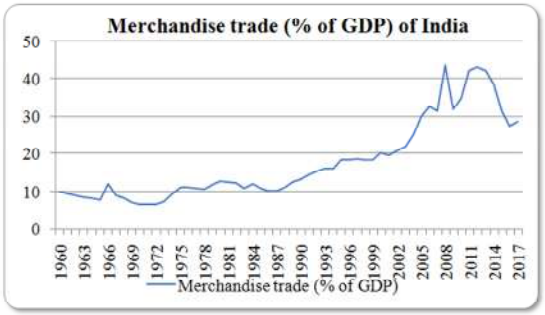
Test	Calculated Value	Critical Value	Passed?
Trace test (r = 0)	**0**		**r >0**
No Constant	65.2	40.2	T
Constant-Only	93.2	47.9	T
Constant +Trend	107.7	55.2	T
Maximum Eigenvalue test (r=3)	**3**		**r = 3**
No Constant	1.4	4.1	F
Constant -Only	1.1	3.8	F
Constant +Trend	1.1	3.8	F

(Source – Author's calculation)

Table 6: Parameters estimates of difference equation for GHGs emission in India

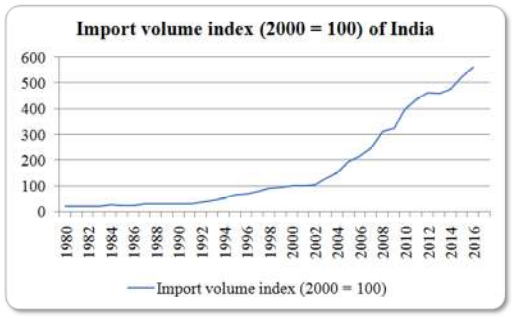
Estimators	Coefficient
c_0	1.36
c_1	1.21
c_2	-.50
c_3	.01
c_4	3.29
$c_2^2 - 3c_1c_3$	.22>0

(Source – Author's calculation)



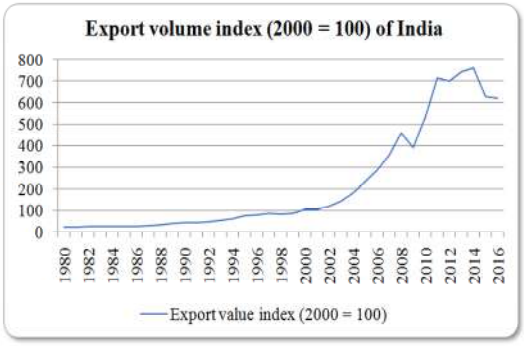
(Source – Author's calculation)

Fig. 1. Growth of merchandise trade (% of GDP) of India from 1960-2016



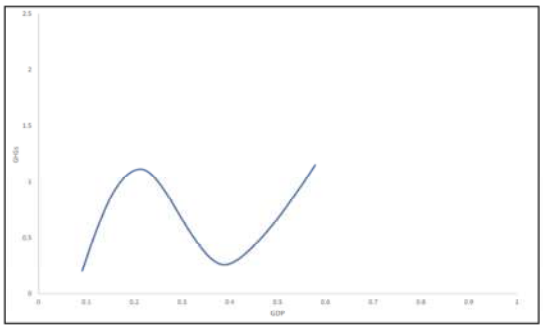
(Source – Author's calculation)

Fig. 2. Import volume index (2000 = 100) of India from 1980-2016



(Source – Author's calculation)

Fig. 3. Export volume index (2000 = 100) of India from 1980-2016



(Source – Author's calculation)

Fig. 4. Cubic specification of environmental Kuznets curve for GHGs

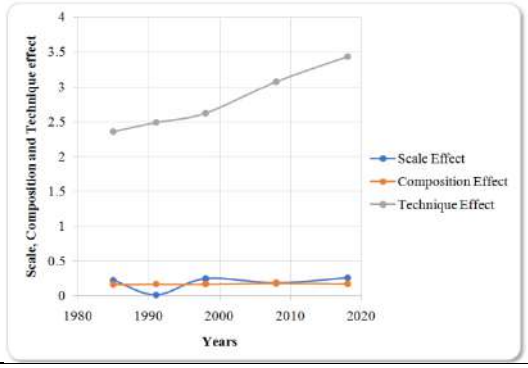


Fig. 5. Scale, Composition and Technique effect of trade on the shape of EKC

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RESEARCH ARTICLE

Mathematical Modeling of Humoral Responses

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ABSTRACT

There are several mathematical models that were developed to study the biological aspects using specific mathematical principles. In this paper, I had developed a mathematical model to study the humoral response. I will consider multiple steady states in developing this model. I have used discrete transition principle to effectively model humoral response activities.

Keywords: T – Cells, Boolean Variables, Immune Reaction Sequence, Discrete Transition, Differential Equations.

INTRODUCTION

Several mathematical models were proposed in recent times to study and understand the behavior of biological aspects of humans thanks to the development of study of genetics and technology. Ever since Charles Darwin proposed his theory of species, there was huge research carried out globally to understand the biological process. In this paper, I had made presented a mathematical model to study the humoral responses by considering T – cells. The behavior of humoral responses were determined through six differential equations obtained in this paper.

Description of the Model

In this paper, I consider the model for humoral response, in which the influence, and mutual influence, of T_H, helper, and T_S, suppressor, T-cells is taken into account. Note that the lymphokines, or molecular messengers, not indicated explicitly. The purported observations that we need to consider in this model are the following:

(*a*) There is a negative feedback loop between T_H and T_S.
(*b*) T_H and T_S have autocatalytic feedback loops.
(*c*) Virgin (unactivated) B-cells are sensitive to negative signaling.

We also wish to predict multiple steady states: virgin, memory, and nonresponsive, as well as the kinetics of primary and secondary response, and the reaction phenomena of high Ag dose and low Ag dose paralysis. For the latter, one needs a primitive graded structure for the Ag, which we impose by attaching two Boolean variables to the Ag population: $e_1 = 0$ for no dose, $e_1 = 1$, $e_2 = 0$ for low dose and $e_2 = 1$ for high dose.

The species in this model as can be seen from Figure 1 are given by E: antigen (e_1, e_2); B_1: virgin B-cells (b_1); B_2: mature activated B-cells (b_2); T_H: helper T-cells (h); T_S: suppressor T-cells (s); A: antibody (a). The suggested form is shown schematically in Figure 1, with $\Rightarrow$ indicating the necessity of e_2 for stimulation, $-\rightarrow$ for the mopping up of Ag by Ab, which we will not now include, preferring to control the Ag population by hand, $\textcircled{B_1}$ to indicate slow B_1 disappearance, and $\boxed{T_S}$ to indicate slow T_S growth.

Mathematical Representation of the Model

If a, b are Boolean variables then we know that $ab = 1$ only if $a = b = 1$ (3.1) and
$a + b = 1$ if $a = 1$ and/or $b = 1$ (3.2)

In view of (3.1) and (3.2), we can write the following equations

$$\left.\begin{aligned} b_1' &= \bar{e}_1,\ b_2' = b_1 e_2 h \\ h' &= e_1 \bar{s} + h,\ s' = h + s,\ a' = b_2 e_1 h \end{aligned}\right\} \quad (3.3)$$

With five control variables (at specified e_1, e_2), there are $2^5 = 32$ possible states, making our situation with more situations. In this model, I just consider sample transitions for the three antigen levels $\bar{e}_1, e_1\bar{e}_2, e_2$.

A typical immune reaction sequence for slow b_1 disappearance and s growth would be

$$10000 \xrightarrow{e_2} \bar{1}0\bar{0}00 \rightarrow \bar{1}0\bar{1}0\bar{0} \rightarrow \bar{1}111\bar{0} \rightarrow \bar{1}1111 \quad (3.4)$$

In fact, this model, with timed reaction rates, satisfies the initial requirements quite well. Completing the discrete-continuum transcription, now including e removal by a, we get

$$\left.\begin{aligned} &\dot{b}_1 = k_1 F_1^-(e) - d_1 b_1,\ \dot{b}_2 = k_2 b_1 F_2^+(e) h - d_2 b_2, \\ &\dot{h} = k_3 F_3^+(e) F_3 F_3^-(s) + m_3 F_3^+(h) - d_3 h,\ \dot{s} = k_4 h + m_4 F_4^+(s) - d_4 s, \\ &\dot{a} = k_5 b_2 e h - k_6 q a^q e^b - d_5 a,\ \dot{e} = -k_5 p a^q e^b - d_6 e \end{aligned}\right\} \quad (3.5)$$

Note that several replacements corresponding to the transitions of the form $F^+(x) \rightarrow x^n$ were incorporated in forming equations (3.5). Solving the equations in (3.5), we would determine the required factors corresponding to knowing the humoral responses.

CONCLUSION

In this paper, using the basic idea of discrete transition of Boolean variables, I had presented a basic mathematical model through which we can determine the parameters corresponding to determining the humoral responses.

In particular, considering the two Boolean variables e_1, e_2 and three specified antigen levels $\overline{e}_1, e_1\overline{e}_2, e_2$, I had created six differential equations corresponding to the parameters b_1, b_2, h, s, a, e .

Being simultaneous differential equations, we can use numerical methods techniques to solve these six equations thereby, determining the required six values. These six values help us to know the behavior of humoral responses explicitly. We can improve this model by considering more antigen levels apart from the three discussed in this paper.

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REVIEW ARTICLE

An Overview on Ethnobotanical Studies

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ABSTRACT

This study represents a systematic attempt to explore the knowledge of the native people about plants, which they use to cure diseases. Several systematic field visits and questionnaire surveys were carried out in selected sites of the study area to gather relevant information from the local community. Ethnobotany is a distinct branch of natural science dealing with various aspects such as anthropology, archaeology, botany, ecology, economics, medicine, religious, cultural and several other disciplines. Recently ethno-botanical studies have gained importance during recent years.

Keywords: Ethnobotany, traditional and complementary medicine, medicinal plants.

INTRODUCTION

Harshberger in 1895 coined the term ethnobotany to indicate plants used by the aboriginals. It included the study and evaluation of plant-human relations in all phases and the effect of plant environment on human society. Ethnobotany defined as "the study of the relationship which exists between people of primitive societies and their plantenvironment". Globally, about 60–80% of the people rely on herbal medicine as for primary healthcare needs. Subsequently, the number of plants being recommended for use as herbal medicines has increased.In areas where there is perceived high cost of medical care, especially in Asia and Africa, medicinal plants have gained more recognition. Traditional medicine is defined as indigenous medicine that is used to maintain health and to prevent, diagnose, and treat physical and mental illnesses differently from allopathic medicine based on theories, beliefs, and experiences. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka, Thailand, and Korea. In China, traditional medicine accounts for around 40% of all health care delivered and is used to treat roughly 200 million patients annually.

MATERIALS AND METHODS [8-12]

STUDY AREA

Berbere district is situated 526 km southeast of Addis Ababa, in Bale Zone of Oromia Regional State. This district has 17 kebeles which are characterized by undulating highlands in the north and lowlands in the south. Madhupur Forest is situated at the central part of Bangladesh and offers dense vegetation due to its tropical moist climate.Maximum and minimum annual temperature is 33.8 and 11.6 1C, respectively. Annual relative humidity ranges between 22.8% (average minimum) and 97.4% (average maximum). Chail valley, located in district Swat of Khyber Pakhtunkhwa province, Pakistan, near the border of Afghanistan. Winteris extremely severe with coldest months of Decemberto February and mean minimum recorded temperatureis −2.4°C. Comparatively, summer, is fairly moderate withmean maximum recorded temperature is 36.32°C.

POPULATION, SAMPLE SIZE, AND SAMPLING

In purposive sampling, the participants are selected on the basis of some specific criteria that are judged to be essential. The researcher deliberately selected community members with a long period of resident in the community, which signify knowledge of the natural environment and the use of natural resources to fulfill basic needs.

RESEARCH INSTRUMENTS

Information on identification and use of local plant species was conducted through a census with seven traditional healers who were present during the time of study., equestionnaires were designed to answer the following research questions: (1) which medicinal plant species do you know in the wild? (2) what are the plants used for? and (3) which plant parts are used?. Local medicinal knowledge of plants use was obtained from questionnaires administered to the local community members.

ETHNOBOTANICAL DATA COLLECTION

The techniques employed for data collection were semistructured interviews, group discussion, guided field walks, and observations with participants. Semistructured interviews were undertaken based on checklist of questions prepared in English and translated to 'Afaan Oromo', the language of local people. Information was carefully recorded during an interview with a participant. The discussions were conducted on threats to medicinal plants, conservation of the medicinal plants, and transferability of knowledge in the community. Brief group discussions were made with participants regarding the medicinal plants in the study area.

DATA ANALYSIS

In paired comparison, ten participants were selected and asked to choose the best item from every pair according to personal perception in treating wound. Te total number of possible pairs [15] was obtained by applying the formula n (n-1)/2, where n is the number of medicinal plants being compared. Informants consensus factor (ICF) was calculated for each category to identify the agreements of the participants on reported cures for the group of ailments.

VOUCHER SPECIMEN COLLECTION

The voucher specimens were collected onsite during guided field walk, numbered, pressed, dried, and deep frozen for identification. Identification of specimens was carried out both in the field and in the herbarium. Finally, the identified specimens were stored at the National Herbarium of the Addis Ababa University, Ethiopia.

VALIDITY AND RELIABILITY OF RESEARCH INSTRUMENTS

Reliability of the research instruments was performed during pilot through the split half technique and Cronbach alpha coefficient computed. Here, the instruments were provided to a total of 24 household heads divided into 2

groups. ,e reliability of the items was based on the estimates of the variability of responses between the two groups. In this study, the reliability coefficient was found to be 0.85, which was very good for the analysis.

DATA ANALYSIS

Quantitative data were cleaned, coded, and entered into Statistical Package for Social Science (SPSS) version 23 for analysis. Descriptive and cross tabulations were carried out. On the other hand, qualitative data were analyzed through synthesized text summaries and frequency distributions.

PILOTING

A reconnaissance visit was conducted for four days to gain basic understanding of the potential respondents for the study. After the initial visit, a week was spent preparing interviews and questionnaires for the survey and another week for training of research assistants on how to effectively administer the instruments. The services of a translator were employed where necessary. A total of 24 questionnaires were piloted.

ETHICAL CONSIDERATIONS

This study adhered to the Ethical Standards of the University of Eldoret. Informed consent was sought and obtained before the study. Anonymity was ensured by not collecting identifying information of individual subjects. Confidentiality was ensured by not divulging the identity of the respondents or their organizations.

FIDELITY LEVEL (FL)

Fidelity level (FL) expresses the preference a species is givenover others in the management of a particular ailment. It was calculated according to the formula:

FL=Ip/Iu*100

where Ip is the number informants stating the use of a species for a
particular ailment category while Iu is the number of informants.

SYSTEMATIC DESCRIPTIONS OF SOME HERBS[13]

Family: Araceae

Acorus calamus L.

Local name: Nag Russ

Ethnomedicinal uses:useful in bronchitis and remittent fever. Fresh rhizome is inhaled in common cold as anti-allergic.

Family: Asteraceae

Artemisia maritima L.

Local name: Mooin

Ethnomedicinal uses: The leaves are used in stomach problems. The leaves are also useful in expelling worms from the intestine

Family: Balsaminaceae

Impatiens glandulifera Royle

Local name: Hillu Ethnomedicinal

uses: The roots and leaves are crushed and applied on forehead, hands and foot to provide cooling effect. Leaves decoction is used in stress and mental tension. Flowers used against snake bite.

Family: Berberidaceae

Berberis aristata D.C.

Local name: Kareelkaimbal

Ethnomedicinal uses: Ripe fruits are edible and given as a mild laxative to children.

Family: Cannabinaceae
Cannabis sativa L.
Local name: Bhang
Ethnomedicinal uses: This plant yield 'charas' and 'ganza' which in action are sedative, appetizer, narcotic and toxic. Leaf juice removes lice and dandruff.

Family: Dioscoreaceae
Disocoreadeltoidea Wall. Ex. Kunth.
Local name: Kinns
Ethnomedicinal uses: The rhizomes yield a steroid, 'cortisone', which has proved of great value in the treatment of a large variety of diseases, particularly in rheumatic diseases; certain ophthalmic disorders and allergic states.

HERBAL DRUG PREPARATION AND UTILIZATION [14-20]
In the present survey, we found that almost all parts of the different species were used against common diseases. The most commonly used plant parts in herbal preparations were leaves (33%), followed by roots (17%), fruits (14%), whole plants (12%), rhizomes (9%), stems (6%), barks (5%), and seeds (4%) (Figure 4).In many cases, more than one part of the same species, generally leaves and aerial parts (comprising stems, branches and flowers) are used in different herbal preparation and remedies. Leaves as frequently used organ in traditional herbal drugs is also reported in previous ethnobotanical studies. In addition to this, leaves are the main photosynthetic organs in plants and are considered to be the natural pharmacy for synthesis of many active constituents those are pharmacologically more active against certain diseases. With similar to previous reports, it is also noted in this work that roots are frequently used part, second to leaves. The utilization of fruit by locals in Chail valley after leaves and roots is found to be widespread as compare to the whole plant use. This agrees with Rashid et al.who also reported the rich diversity of edible wild fruits with medicinal uses in Swat region of northern Pakistan.

THE TRIBALS [21-29]
Tribals are the oldest ethnological groups which live far away from the civilized world. They prefer to live in forested areas, follow primitive customs and occupations, profess primitive religions, have common language and social culture, are economically dependent on each other. About 500 tribal communities are representing 7.76 per cent of the total population of the country. It is spread over 19 per cent of the total area of the nation. The total tribal population of Rajasthan state is 5,474,881 which is 12.44 per cent of the total population of this state. The tribals of Rajasthan constitute 8.07% of the total population of tribals in India. Several tribes inhabited in the state of Rajasthan, namely – 'Bhil', 'Bhil-Meena', 'Garasia' 'Damor', 'Dhanaka',, 'Kathodia', 'Meena', 'Patelia' and 'Saharia'. Besides these, there are some nomadic, semi-nomadic tribes and denotified communities also. Nomadic tribes are 'Banjara', 'Gadia–Lohar' and 'Kalbelia', whereas semi-nomadic tribes are 'Rebari', 'Jogi' and 'Masani'. 'Bori', 'Kanjer', 'Sansi', 'Bhat' are included in denotified communities. On the basis of distribution of various tribes the state can be divided into four different zone. Different zone/districts of Rajasthan.

I. First Zone: In this zone the districts of southern areas are included. These districts are Banswara, Dungarpur, Udaipur and Chittorgarh where 'Bhils' and 'Damors' are residing.
II. Second Zone: It includes Sirohi and Pali districts where 'Garasia' is the dominating tribe.
III. Third Zone: It has Jaipur, Sikar and Alwar districts where 'Meena' tribes reside dominantly.
IV. Fourth Zone: In this zone Tonk, Bundi, Jhalawar and Kota districts are included where 'Bhil' and 'Meena' form the dominating tribal population. The Meena population (3,68025) found majority in Jaipur district and also in other tribal population e.g. 'Bhil', 'Kalbelia', 'GadiaLohar', 'Banjara', 'Kanjar', 'Sansi' and 'Bauria' found in minority. Several wild plant species are used by tribals as fodder.

VEGETATION [30,31]

The vegetation of the area has been classified as "scrub jungle". Plants which can either adapt themselves to high temperatures or to low temperatures and discouraging conditions of soil and rainfall can be found. The trees are commonly lacking, shrubs are the dominant perennials, most of which form thickets e.g. *Crotalaria burhia,* Leptadeniapyrotechnica, Saricostomapauciflorum and Zizyphusnummularia. This perhaps is the reason for a very low percentage of tree species.

PREPARATION, ROUTES OF ADMINISTRATION AND APPLICATION OF MEDICINAL PLANTS [32,33]

Most remedies were processed by crushing (34%), chewing (12%), or boiling (8%) or used in unprocessed form (8%). The majority (59%) of remedies were prepared from fresh materials (Abraha et al., 2013). According to Giday and Ameni (2003), 79% of the remedies were prepared without the use of solvents or diluents. When solvents are needed, however, it was water that was frequently used. Human saliva and urine as well as milk and butter are also used as solvents or additives, to some degree, in the preparation of remedies. The local people employ several methods of preparation of traditional medicines.

FACTORS THREATENING MEDICINAL PLANTS[34]

Fire wood was ranked first by selected key informants followed by agricultural expansion and drought respectively; the least one is fodder (Abdurhman, 2010). However, of five (agricultural expansion, drought incidence, fire wood extraction, overgrazing, and construction) provided threats to medicinal plants, agricultural expansion (26.7%) is the main threat to wild plant species. Overgrazing was relatively perceived to be least destructive factor (11.1%).

THREATENED MEDICINAL PLANTS[35]

Olea europaea supsp. cuspidata is the most threatened followed by *Clerodendrum myricoides* and *Myrica salicifolia* and the least threatened one is *Acokanthera schimperi* (Abdurhman, 2010). This disagrees with other studies elsewhere in the region (Ref????), the wild plants *Grewia villosa, Berberis holistii, Pittosporum viridiflorum*, and *Maerua angolensis* are the most scarce medicinal plants in the area.

MARKETED MEDICINAL PLANTS[36,37,38]

The medicinal plant material found being marketed in the open markets for medicinal purpose was *Hagenia abyssinica*. In addition to this, some medicinal plants are sold in the market for other purposes and most of them are sold as food. Medicinal plants in the market are not a common cultural activity in local markets of the study area. However, medicinal plant like *Hagenia abyssinica* (dry flower) is sold in the market for its medicinal purpose. Some fresh collection of Artemisia absinthim and Rutachalepensis are also marketed in a local community for their aromatic and spice value respectively (Abdurhman, 2010). However, elsewhere in the region fruits Opuntia *Ficus indica* and *Ziziphus spina-christi* were marketed. They were sold primarily for other purposes (Giday and Ameni, 2003). This is in line with Teklay et al. (2013), there were no reports of medicinal plants being sold in open markets solely for their medicinal use. However, some medicinal plants were indicated to be sold in local market but for their uses as food, spices, and beverages. These include *Allium sativum* (spice), Carica papaya (food), Citrus aurantifolius (food), *Lepidium sativum* (spice), *Lycopersicum esculantum* (food), *Opuntia ficusindica* (food), *Rhamnus prinoides* (additive for fermented beverages), *Ruta chalepensis* (spice), *Sorghum bicolor* (food), *Trigonella foenum-graecum* (spice), *Vicia faba* (food), *Zingiber officinale* (spice) and *Ziziphus spinachristi* (food).

TRANSFERRING OF INDIGENOUS KNOWLEDGE [39,40]

The commonest way of transferring knowledge of traditional medicinal plants orally is to the eldest son who received 25 (29.6%) votes followed by the all children of the family 20 (23.80%) and 15 (17.86%) for eldest daughter. This agrees with most local knowledge systems, information on ethnoveterinary medicinal plants in the study area is rarely codified in written form; they are accepted orally from their forefathers and transmitted similarly to their children, most often to the first-born from generation to generation and Majority (69%) traditional healers transfer their indigenous knowledge to their selected family verbally, some (23%) through showing the medicinal plant in the

field and the remaining (8%) through demonstration including remedy preparation methods. Nevertheless, study elsewhere in the region showed that they obtained their knowledge from the books or any other accessible literature, while illiterate farmers had to rely on knowledge of their fathers and their own experimental treatment methods.

FUTURE IMPACT OF THE STUDY[41,42]

The study will provide a sense of social and economic responsibility among the common people, conserving the local flora. This valued information will also motivate the local community to attract tourism in the valley by preserving its natural beauty, which will enhance the socioeconomic prosperity and wellbeing of the rural community. The participation of the local community will help in conserving the floral diversity, promote trade and tourism. But meanwhile experts engaged in the policy making could possibly address the issues relate to the floristic composition and conservation. Besides the pharmaceutical and food industry could invariably exploit the local medicinal flora, which could be used for the public health and socioeconomic uplift of the regi

ETHNOBOTANICAL USES [43-45]

Soil conservation

Traditionally some plants are kept on the fields by farmers as they know their potential benefits through generations. Khejiri(*Prosopis cineraria* Linn.) is most common tree in the Thar desert of Rajasthan. They are grown all over the crop fields. Crops like millets, moth (*Phaseolus aconitifolius* Jacq.),Currybeans (*Phaseolus lunatus* Linn.), moong *(Phaseolus mungo* Linn.) leaves and oil seeds grown well in combination with it. Recent researches indicate that it brings up moisture and nutrients from underground soil for crop grown above. The leguminous plants or trees in field also fix nitrogen by nitrogen fixing bacteria in the root nodules and green manure to the soil by their leaf fall.

Plant Dyes

Dyes are also obtained from flowers of *Butea monosperma* O. Kuntze. (Palas), *Caesalpinia sappana* Linn.(Bakam) and leaves of Tectona grandes. Red dyes from *Caesalpinia coriaria* Willd. (Divi-divi.). Indian ink is prepared from the bark and leaves of *Terminalia catappa* Linn. Blue dyes from leaves of *Indigofera tinctoria* Linn. (Neel) and the root of *Petrocarpus santalinus* Linn. (RaktaChandan).

Cosmetic uses

Since early age plants have served for human adornment for the millenia and people have been using various kinds of herbs to maintain their beauty. The study revealed that the use of plants as herbal cosmetics is prevalent among the tribal communities and represent not only a part of their ethnic culture but also witness the use of plants in their regular health care practices since ancient times. All kinds of skin and hair problems are frequently treated through external application of the herbal preparations in the form of paste, powder, lotion, body massage oil and hair oil. Every individual of tribal community is able to provide some sort of information about the herbs used as cosmetics.

Plants in Fabric Printing

The pink city Jaipur, is also known for its excellent block printing, throughout the world. Sub-towns, like 'Sanganer' and 'Bagru', of the district developed into a printing centre in last centuries. Their subtle colourways and stylized floral and other motifs were developed to meet the needs of a selected and elite clientele. The "Chhipa" community of the district carried out this block printing work from ancient times. The colours are prepared from plant materials and prepared blocks of local flora and fauna as designs from locally available specific woods.

Folk medicine

Descriptions of trees and flowers are found profusely in folk songs and there are songs of worship of plants. Religious songs have references to offering of flowers and fruits. Folk songs in praise of Bamboo (*Bambusa vulgaris* Schard. ex. J.C.Wendl), Basil (*Ocimum sanctum* Linn.), and Amaltas (*Cassia fistula* Linn.) are sung, believing these plants are the abode of several Gods and Goddess. On auspicious occasions, such as birth of babies, thread

ceremonies, marriages and other religious functions, all have associations with mandaps made from bambooand the decoration of doors with mango leaves.

CONCLUSION

Most of the communities in the region live in remote areas away from towns and have only limited access to the modern healthcare facilities. For the daily need of medical treatments they heavily depend on ethnobotanical practice.ack of attention towards medicinal plants and their traditional uses were found amongst young generation because of numerous reasons. This study established that the traditional medicinal knowledge of medicinal plant use. It is important to collect this information and develop a database of medicinal plants for future research and potential development of new drugs. These medicines have less side effects as compared to the allopathic medicines and are also eco-friendly. So there would be a need to conserve this knowledge and distribute it. this study clearly indicated that these areas need much attention for medicinal plants documentation, conservation and protection by the government, non-government organizations.

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RESEARCH ARTICLE

Survey of Invasive Alien Flora in Kattathurai Panchayat, Kanyakumari District, Tamil Nadu, India

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ABSTRACT

Invasive alien species are exotic organisms which can alter the biodiversity of ecosystems. A floristic survey was conducted in Kattathurai Panchayat, Kanyakumari district, Tamil Nadu, India to record alien invasive flora. A total of 121 angiosperm species representing 98 genera and 47 families were recorded through floristic survey. The family Asteraceae dominated the list of invasive plants with 13 species followed Caesalpiniaceae (12); Convolvulaceae and Euphorbiaceae (9); Amaranthaceae (6); Solanaceae (5); Malvaceae, Mimosaceae, Poaceae, Cleomaceae and Verbenaceae (4); Apocynaceae, Asclepiadaceae, Lamiaceae, Acanthaceae, Papilionaceae (3); Nyctaginaceae, Onagraceae, Araceae, Oxalidaceae and Portulacaceae (2). Other families like, Liliaceae, Papaveraceae, Passifloraceae, Polygonaceae, Rubiaceae Typhaceae, Tiliaceae, Lythraceae, Sterculiaceae, Balsaminaceae, Boraginaceae, Cactaceae, Bignoniaceae, Utricaceae, Ponteridiceae, Combretaceae, Salviniaceae, Utriculaceae, Schorphulariaceae, Zygophyllaceae, Turneraceae, Commelinaceae, Salviniaceae, Caricaceae, Rhamnaceae, Crassulaceae were represented by just single species. Among 98 genera, *Cassia* with 8 species dominated the study area followed by *Ipomoea (*6) and *Euphorbia* (3); *Cleome* (4); *Physalis* (2) and *Catharanthus, Ludwigia, Portulaca, Solanum and Tagetus and Alternanthera* (2 species each). Among the six life forms, herbs dominated the flora with 88 species followed by shrubs (16 species each); aquatic herb (03); wet land herbs (02), climbers (04) and trees (08 species). Sixty three species from tropical America, nine from tropical Africa, three from Central America, two each from Europe, West Indies, three each from Brazil, Asia, America, 5 from China, one each from Africa, Malaya, Mediterranean, Australia, Peru, Central America have invaded into Kattathurai Panchayat, Tamil Nadu, India.

Keywords: Invasive alien species, exotics, biodiversity, Kattathurai Panchayat.

INTRODUCTION

Plants are introduced by humans intentionally or accidentally from one region to another. The plants which are introduced into a new habitat are known as alien, introduced or exotic taxa (1). If the introduced plants have the potential to grow well in the introduced environment, they are invasive. Invasive alien species are non-native exotic organisms which can reduce the biodiversity and cause changes to the ecosystem. The establishment of alien flora may lead to the endangerment of the local flora.

Invasion by alien plants (i.e. plant species introduced by humans to regions outside their native distribution) is an important aspect of the Anthropocene (2). More than 13,000 plant species (4% of the extant global vascular flora) have become naturalized somewhere on the globe as a result of human activity and many of them represent a threat to biodiversity (3). Biological invasions by alien species are a significant component of anthropogenic environmental change, often resulting in a significant loss in the economic value, biological diversity and change in aspects of functioning of invaded ecosystems (4). Globalization and rapid modification of natural habitats have particularly accelerated the pace of invasion during the past century. At the continental and global scale, species invasions have diminished the regional distinctiveness of flora and fauna (5). In many continental areas, 20% or more of the plant species are now non-indigenous. At least 10% of the world's vascular plants (300,000) have the potential to invade other ecosystems and affect native biota in direct or indirect ways (6). About 18% of the Indian flora constitutes aliens, of which 55% are American, 30% Asian and Malaysian and 15% European and Central Asian species (7). Inderjit *et al*. (8) have reported 471 naturalized species in India, which represents 2.6% of the total flora of this country and Tamil Nadu has the highest number of naturalized species (332) in India among Indian states.

A perusal of literature showed that a proper survey pertaining to the presence of invasive plant species has not been reported from the study area. Therefore, an attempt has been made in the present study to provide the baseline information on the invasive plant species in and around Kattathurai Panchayat, Kalkulam Taluk, Kanyakumari District.

MATERIALS AND METHODS

Study Area

The study area, Kattathurai Panchayat is situated in Kalkulam taluk, Kanyakumari district Tamil Nadu, India. The average temperature does not exceed 30^{0}C. The days are hot in summer in May, but the nights are much cooler. In late May and in June there are thunder storms. The rainfall is due to South West monsoon and also due to North East monsoon from the Bay of Bengal. The flora here is very rich containing dry deciduous, semi-evergreen and some moist evergreen species.

An extensive field survey was conducted from June 2019 to May 2020 to record the alien flora growing in the different parts of Kattathurai Panchayat. One visit was made after every two months. Thus a total of six visits were made for the field observations in a year. Visits were made to all the practically possible places in the study area in search of alien members. The plant specimens collected were identified and their identity further confirmed by using standard taxonomic floras published by Gamble (9) and Mathew (10).

RESULTS

A total of 121 angiosperm species in 98 genera and 47 families were recorded through qualitative floristic survey in the selected study area between June 2019 to May 2020 as alien flora. The members of the family Asteraceae dominated the alien vegetation with 13 species followed Caesalpiniaceae (12); Convolvulaceae and Euphorbiaceae (9); Amaranthaceae (6); Solanaceae (5); Malvaceae, Mimosaceae, Poaceae, Cleomaceae and Verbenaceae (4);

Apocynaceae, Asclepiadaceae, Lamiaceae, Acanthaceae, Papilionaceae (3); Nyctaginaceae, Onagraceae, Araceae, Oxalidaceae and Portulacaceae (2). Families such as Liliaceae, Papaveraceae, Passifloraceae, Polygonaceae, Rubiaceae Typhaceae, Tiliaceae, Lythraceae, Sterculiaceae, Balsaminaceae, Boraginaceae, Cactaceae, Bignoniaceae, Utricaceae, Ponteridiceae, Combretaceae, Salviniaceae, Utriculaceae, Schorphulariaceae, Zygophyllaceae, Turneraceae, Commelinaceae, Salviniaceae, Caricaceae, Rhamnaceae, Crassulaceae were represented by a single species in study area.

Among 98 genera *Cassia* with 8 species dominated the study area followed by *Ipomoea (*6) and *Euphorbia* (3); *Cleome* (4); *Physalis* (2) and *Catharanthus, Ludwigia, Portulaca, Solanum and Tagetus and Alternanthera* (2 species each). Among six life forms, herbs dominated the flora with 88 species followed by shrubs (16 species each); aquatic herbs (03); wet land herbs (02), climbers (04) and trees (08 species). Sixty three species from tropical America, nine from tropical Africa, three from Central America, two each from Europe, West Indies, three each from Brazil, Asia, America, five from China, one each from Africa, Malaya, Mediterranean, Australia, Peru, Central America have invaded into study area (Tables 1 to 4; Fig. 1).

The family Asteraceae was represented by a large number of species in the study area. With acapacity to quickly produce large number of seeds and efficient seed dispersal mechanisms, the members of Asteraceae have established themselves in a wide range of climatic conditions (11).*Ageratum conyzoides* is found to grow extensively as a weed in the study area as also *Parthenium hysterophorus* which was introduced to India through import of wheat during 1950s'(12).

During the field visits, some of the most noxious invasive plants studied were *Lantana camara*, *Eichhornia crassipes*, *Parthenium hysterophorus*, *Prosopis juliflora*, *Xanthium indicum*, *Argemone mexicana*, *Ageratum conyzoides, Hyptis suaveolens*, *Pedalium murex* etc. *Lantana camara*, a shrub species introduced as an ornamental in India during 1809–1810 is also a common weed in the study area now. *Eichhornia crassipes* (introduced from Brazil during 1914-1916) is now found extensively from the northern Himalayas to Cape Comorin (the southern tip of India) as an invasive weed which clogs the waterways across India. *Ipomoea carnea* was also introduced as an ornamental from tropical America. Now it grows aggressively in aquatic ecosystems all over the Indian subcontinent. However, the quantitative impact of these species on the indigenous flora and invaded ecosystems is yet to be studied. The study revealed that some of the invasive species like *Ocimum sanctum,Catharanthus roseus, Cardiospermum halicacabum* are used as medicinal purposes. Some are used as ornamentals *Bougainevillea spectabilis)*, avenues trees (*Delonix regia*) and for fuel wood (*Prosopis juliflora*).

Ageratum conyzoides, *Cassia alata*, *Lantana camara*, *Cassia tora*, *Parthenium hysterophorus*, *Xanthium* sp., *Datura metel*, *Hyptis suaveolens* were some noxious species found during the study. *Parthenium hysterophorus* was one of the highly noxious and abundantly growing plant species followed by *Ageratum conyzoides* and *Lantana camara*. The study revealed that Asteraceae was the most dominant invasive family which dominated all other species due to the adaptive nature of its seeds in different areas. The Asteraceae species have high reproductive potential to produce minute seeds quickly which disperse in new areas through wind, air, and water. Asteraceae was more invasive in other areas of India too (13) and also all over the world. *Parthenium hysterophorus* was another noxious plant of this family which could cause disease. It grows very rapidly as its seeds disperse and grow fast in new areas, qualities which help it to become an efficient invasive plant. Kumar *et al*. (14) reported herbs to be the more dominant plant group found in Rourkela flora. Herbs have more tolerance to harsh conditions which helps them to become more invasive than others. Annuals showed dominance over perennials among the invasive species as annuals complete their life cycle in a short period within a year. The habit-wise distribution showed that herbaceous plants are more invasive than shrubs, climbers, and trees.

CONCLUSION

Invasive species are non-native and exotic which are introduced intentionally or accidentally to a new area. The invasive species have a good adaptive response due to which they are able to reproduce and disseminate quickly in an alien environment. Due to their rapid spread, they can change the composition of the flora in the native ecosystem and become a threat to the native ecosystem. Due to the rapid population growth of the invasive plants, they encroach the crop fields, waterways and water bodies, wastelands, and barren lands. The invasion by alien species directly affects the agricultural economy and the biodiversity. However, the utilization of hidden medicinal potential can make invasive alien species beneficial to the people of the region. Moreover, the effect of invasive alien species in the economy, biodiversity, and human health is yet to be assessed. This study is based on diversity of invasive plant species found in different areas of Kattathurai panchayat, Kalkulam Taluk, Kanyakumari District. This study will help in the compilation of invasive flora of Kattathurai Panchayat.

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Table 1. List of alien species in the study area.

Sl. No	Species	Family	Habit	Nativity
1	*Acacia auriculiformis* L.	Mimosaceae	Tree	Australia
2	*Aerva lanata* (L.) Juss. ex. Schult.	Amaranthaceae	Herb	Madagascar
3	*Ageratum conyzoides* L.	Asteraceae	Herb	Tropical America
4	*Aloe barbadensis* Mill.	Liliaceae	Herb- succulent	Mediterranean
5	*Alternanthera pungens* Kunth	Amaranthaceae	Herb	Tropical America
6	*Alternanthera sessilis* (Linn) DC.	Amaranthaceae	Herb	Tropical America
7	*Amaranthus spinosus* L.	Amaranthaceae	Herb	Tropical America
8	*Antigonon leptopus* Hook. & Arn.	Polygonaceae	Climber	Tropical America
9	*Argemone mexicana* L.	Papaveraceae	Herb	Tropical Central & South America
10	*Urena lobata* L.	Malvaceae	Herb	Tropical Africa
11	*Asclepias curassavica* L.	Asclepiadaceae	Herb	Tropical America
12	*Bidens pilosa* L.	Asteraceae	Herb	Tropical America
13	*Borassus flabellifer L.*	Asteraceae	Tree	Tropical Africa
14	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	Shrub-climbing	Brazil
15	*Caesalpinia pulcherrima* (L.) Sw.	Caesalpiniaceae	Shrub	Tropical America
16	*Calotropis gigantea* (L.) R.Br.	Asclepiadaceae	Shrub	Tropical Africa
17	*Calotropis procera* (Ait.) R. Br.	Asclepiadaceae	Shrub	Tropical Africa
18	*Capsicum annum* L.	Solanaceae	Shrub	Tropical America
19	*Cardiospermum halicacabum* L.	Sapindaceae	climber	Tropical America
20	*Carica papaya* L.	Caricaceae	Tree	Mexico
21	*Cassia alata* L.	Caesalpiniaceae	Shrub	West Indies
22	*Cassia hirsuta* L.	Caesalpiniaceae	Herb	Tropical America
23	*Cassia obtusifolia* L.	Caesalpiniaceae	Herb	Tropical America
24	*Cassia occidentalis* L.	Caesalpiniaceae	Herb	Tropical South America
25	*Cassia pumila* Lam.	Caesalpiniaceae	Herb	Tropical America
26	*Cassia rotundifolia* Pers.	Caesalpiniaceae	Herb	Tropical South America
27	*Cassia tora* L.	Caesalpiniaceae	Herb	Tropical South America
28	*Cassia uniflora* Mill.	Caesalpiniaceae	Herb	Tropical South America
29	*Catharanthus pusillus* (Murray) Don.	Apocynaceae	Herb	Tropical America
30	*Catharanthus roseus* (Linn) G. Don	Apocynaceae	Shrub	West Indies
31	*Celosia argentea* L.	Amaranthaceae	Herb	Tropical Africa
32	*Chloris barbata* Sw.	Poaceae		Tropical America
33	*Chromolaena odorata* (L.) King & Robinson	Asteraceae	Herb	Tropical America
34	*Cleome gynandra* L.	Cleomaceae	Herb	Tropical America
35	*Cleome monophylla* L.	Cleomaceae	Herb	Tropical Africa
36	*Cleome rutidosperma* DC.	Cleomaceae	Herb	Tropical America
37	*Cleome viscosa* L.	Cleomaceae	Herb	Tropical America
38	*Crotalaria retusa* L.	Papilionaceae	Herb	Tropical America
39	*Cynodon dactylon* (L.) Pers.	Poaceae	Herb	Tropical America

40	*Datura metal* L.	Solanaceae	Shrub	Tropical America
41	*Delonix regia* (Boj.) Raf.	Caesalpiniaceae	Tree	Madagascar
42	*Digeria muricata* (L.) Mart.	Amaranthaceae	Herb	Asia
43	*Duranta repens* L.	Verbenaceae	Shrub	America
44	*Echinochloa colona* (L.) Link	Poaceae	Herb	Tropical America
45	*Eclipta prostrata* (L.) Mant.	Asteraceae	Herb	Tropical America
46	*Eichhornia crassipes* (C. Martius)	Pontederiaceae	Herb -aquatic	Tropical America
47	*Emilia sonchifolia* (L.) DC.	Asteraceae	Herb	Tropical America
48	*Euphorbia heterophylla* L.	Euphorbiaceae	Herb	Tropical America
49	*Euphorbia hirta* L.	Euphorbiaceae	Herb	Tropical America
50	*Euphorbia thymifolia* L.	Euphorbiaceae	Herb	Tropical America
51	*Evolvulus nummularius* (L.)L.	Convolvulaceae	herb	Tropical America
52	*Heliotropium indicum* L.	Boraginaceae	Herb	America
53	*Hibiscus rosa-sinensis* L.	Malvaceae	Shrub	China
54	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	Herb	Tropical America
55	*Impatiens balsamina* L.	Balsaminaceae	Herb	Tropical America
56	*Ipomoea carnea* Jacq.	Convolvulaceae	Shrub	Tropical America
57	*Ipomoea eriocarpa* R.Br.	Convolvulaceae	Herb	Tropical Africa
58	*Ipomoea hederifolia* L.	Convolvulaceae	Herb	Tropical America
59	*Ipomoea obscura* (L.) Ker.-Gawl.	Convolvulaceae	Herb	Tropical Africa
60	*Ipomoea pes-tigridis* L.	Convolvulaceae	Herb	Tropical East Africa
61	*Ipomoea quamoclit* L.	Convolvulaceae	Herb	Tropical America
62	*Jatropha gossypifolia* L.	Euphorbiaceae	Shrub	Brazil
63	*Justicia gendarussa* Burm.f.	Acanthaceae	Herb	China
64	*Kalanchoe pinnata (*Lam.) Pers.	Crassulaceae	Herb	Tropical Africa
65	*Lagerstroemia indica* L.	Lytharaceae	Shrub	China
66	*Lantana camara* L.	Verbenaceae	Herb	Tropical America
67	*Leonotis nepetifolia* (L.)R.Br.	Lamiaceae	Herb	Tropical Africa
68	*Leucaena leucocephala (*Lam.) de Wit	Mimosacceae	Herb	Tropical America
69	*Ludwigia adscendens* (L.) Hara	Onagraceae	Wetland Herb	Tropical America
70	*Ludwigia octovalvis* (Jacq.) Raven	Onagraceae	Wetland Herb	Tropical Africa
71	*Mimosa pudica* L.	Mimosacceae	Herb	Brazil
72	*Mirabilis jalapa* L.	Nyctaginaceae	Herb	Peru
73	*Ocimum americanum* L.	Lamiaceae	Herb	Tropical America
74	*Opuntia stricta* (Haw.) Haw.	Cactaceae	Herb succulent	Tropical America
75	*Oxalis corniculata* L.	Oxalidaceae	Herb	Europe
76	*Parthenium hysterophorus* L.	Asteraceae	Herb Tropical	Tropical North America
77	*Passiflora foetida* L.	Passifloraceae	climber	Tropical South America
78	*Pedalium murex* L.	Pedaliaceae	Herb	Tropical America
79	*Triumfetta rhomboidea Jacq.*	Tiliaceae	*Herb*	Tropical America
80	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne	Caesalpiniaceae	Tree	Malaya
81	*Peperomia pellucida* (L.) Kunth	Oxalidaceae	Herb	Europe

82	*Peristrophe paniculata* (Forssk.) Brummitt	Acanthaceae	Herb	Tropical America
83	*Phyla nodiflora* (L.) Greene	Verbenaceae	Herb	Tropical America
84	*Phyllanthus tenellus* Roxb.	Euphorbiaceae	Herb	Mascarene Islands
85	*Physalis angulata* L.	Solanaceae	Herb	Tropical America
86	*Physalis minima* L.	Solanaceae	Herb	Tropical America
87	*Pilea microphylla* (L.) Liebm	Urticaceae	Herb	Tropical South America
88	*Pistia stratiotes* L.	Araceae	Aquatic Herb	Tropical America
89	*Sida acuta Burm. f.*	Malvaceae	Herb	Tropical America
90	*Pithecellobium dulce* (Roxb.) Benth.	Mimosaceae	Tree	Mexico
91	*Portulaca oleracea* L.	Portulacaceae	Herb	Tropical South America
92	*Portulaca quadrifida* L.	Portulacaceae	Herb	Tropical America
93	*Quisqualis indica* L.	Combretaceae	Climber	Malaya
94	*Rhoeo discolor* Hance.	Commelinaceae	Herb	Central America
95	*Ricinus communis* L.	Euphorbiaceae	Shrub	Africa
96	*Ruellia tuberosa* L.	Acanthaceae	Herb	Tropical America
97	*Saccharum spontaneum* L.	Poaceae	Herb	Asia
98	*Salvinia molesta* D. S. Mitch.	Salviniaceae	Aquatic Herb	Tropical America
99	*Scoparia dulcis* L.	Scrophulariaceae	Herb	Tropical America
100	*Sesbania bispinosa* (Jacq.) Wight	Papilionaceae	Shrub	Tropical America
101	*Sida acuta* Burm.f.	Malvaceae	Herb	Tropical America
102	*Solanum torvum* Sw.	Solanaceae	Shrub	West Indies
103	*Solanum torvum* Sw.	Solanaceae	Shrub	West Indies
104	*Spermacoce hispida* L.	Rubiaceae	Herb	Tropical America
105	*Stachytarpheta jamaicensis* (L.) Vahl.	Verbenaceae	Herb	Tropical America
106	*Stylosanthes hamata* (L.) Taub.	Papilionaceae	Herb	Tropical America
107	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	Herb	West Indies
108	*Tagetes erecta* L.	Asteraceae	Herb	Mexico
109	*Tagetes patula* L.	Asteraceae	Herb	Mexico
110	*Tamarindus indica* L.	Caesalpiniaceae	Tree	Tropical America
111	*Tecoma stans* (L.) Juss. ex Kunth	Bignoniaceae	Tree	America
112	*Thevetia peruviana* (Pers.) Merrill	Apocynaceae	Tree	Tropical America
113	*Thuja occidentalis* L.	Cupressaceae	Tree	China
114	*Tribulus terrestris* L.	Zygophyllaceae	Herb	Tropical America
115	*Tridax procumbens* L.	Asteraceae	Herb	Tropical Central America
116	*Typha angustata Bory. & Choub.*	Typhaceae	Aquatic Herb	Tropical America
117	*Turnera subulata* J.E. Smith.	Turneraceae	Herb	Tropical America
118	*Waltheria indica* L.	Sterculiaceae	Herb	Tropical America
119	*Xanthium strumarium* L.	Asteraceae	Herb	Tropical America
120	*Zinnia elegans* Jacq.	Asteraceae	Herb	Mexico
121	*Ziziphus mauritiana* Lam.	Rhamnaceae	Tree	China

Table: 2 Habit-wise distributions of invasive alien plant species

S. No.	Habit	No. of sps.
1	Herb	88
2	Shrub	16
3	Tree	08
4	Climber	04
5	Aquatic herb	03
6	Wetland herb	02

Table: 3 Different geographic nativities of the invasive alien plants.

S. no.	Nativity	No. of species
1	Tropical North America	04
2	Tropical America	63
3	Tropical south America	05
4	China	05
5	Europe	02
6	West Indies	02
7	Australia	01
8	Brazil	03
9	Tropical Africa	09
10	Africa	02
11	America	03
12	Mexico	06
13	Malaya	01
14	Madagascar	02
15	South America	04
16	Peru	01
17	Asia	03
18	Central America	01
19	Mediterranian	02
20	Mascarane island	02

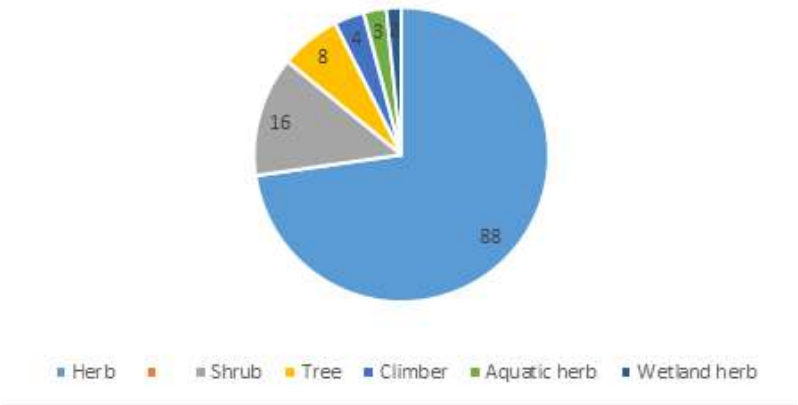

Fig 1.Habit wise distribution of invasive plant species.

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REVIEW ARTICLE

A Review on Natural's Boon: Mushrooms

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ABSTRACT

The present study was to explain the medicinal importance of mushroom at the same time describe the mushroom poisoning. It is the fungus originated species but considered as plant like form. Mushroom plays the important role in the term of nutraceuticals which means nature substance consume as a food and also for the medicinal importance. Mushrooms can edible but not all, some of them poisonous that can produce severe effects like GIT disorders, nerve disorders, muscular disorders and sometimes fatal. Edible mushrooms consumed for their nutritional value as well as health benefits. A melon grower was discovered mushrooms growing on his growth fertilizer in Paris at 1650. Mushrooms were introduced into Netherlands for the primary time at the start of the 19th century. The button mushroom, milky mushroom and oyster mushrooms are commercially cultivated in an India.

Keywords: Mushrooms, Edible, Noxious, Cultivation.

INTRODUCTION

A mushroom is that the plant organ produced by some fungi. It somewhat just like the fruit of a plant, except that the "seeds" it produces are actually many microscopic spores that form within the gills or pores underneath the mushroom's cap. The spores blow away into the wind, or are spread by other means, like animal feeding. If they land on an appropriate substrate (such as wood or soil) spores will germinate to make a network of microscopic rooting threads (mycelium) which penetrate into their new food source. Unlike the mushroom, which pops up then passes away quickly, the mycelium persists, often for many years, extracting nutrients and sending up its annual crop of mushrooms [1]. Mushrooms are fungi. They belong in a kingdom of their own, separate from plants and animals. Fungi differ from plants and animals in the way they obtain their nutrients. Generally, plants make their

food using the sun's energy (photosynthesis), while animals eat, then internally digest, their food. Fungi do neither: their mycelium grows into or round the food source, secretes enzymes that digest the food externally, and therefore the mycelium then absorbs the digested nutrients. There are exceptions to those generalizations; some organisms are placed into their respective kingdoms supported characteristics aside from their feeding habits. Mushrooms aren't really plants; they're sorts of fungi that have a "plantlike" form - with a stem and cap (they have cell walls as well). This is often really just the "flower or fruit" of the mushroom - the reproductive part which disperses the spores. The larger portion of the many fungi is underground, and may be acres in size! Mushrooms aren't plants because they do not make their own food [2].

The nutritional and medicinal values of mushrooms have long been recognized. In recent times, however, mushrooms have assumed greater importance in the diets of both rural urban dwellers. It is conceivable that the increased demand for mushrooms is contingent upon the phenomenal rise within the unit costs of the conventional sources of meat. Edible mushrooms are placed into five categories consistent with the derivation of their names, viz., those named according to the taste, morphology, growth habit, texture, and habitat [3]. Examples in each category are: taste (*Volvariella volvacea, Volvariella esculenta* and *Termitomyces clypeatus*: Morphology (*Termitomyces manniformis, Termitomyces robustus, Schizophyllum commue* and *Agrocyber broadway*; growth habit (*Termitomyces globulus* and *Pleurotus tuber-reguim*); texture (*Pleurotus squarrouslus* and *Psathyrella atroumbonata)*.

In addition to the above, the natives have observed the expansion of the various fungi on differing types of dead wood and have named each fungus after the wood on which it grows. Besides the edible mushrooms, the natives also recognize some poisonous or none edible fungi a couple of which are listed as *Coprinus africans*, *Phallus aurantiacus, Phallus industiatus, Phallus rubicundus*, *Mutinus bambustnus* and *Coprinus enphemerus* [4]**.**

HISTORY OF THE MUSHROOM

Already as early because the Roman times, fungi weren't only popular in Europe, but they were also consumed centuries ago in Middle and South America. They were considered special and mysterious and were often utilized in age-old rituals [5]. The word mushroom springs from the French word for fungi and molds. One day, around 1650, a melon grower near Paris discovered mushrooms growing on his growth fertilizer. He decided to cultivate this new exotic delicacy commercially and to introduce it in exclusive Parisian restaurants. It was at that point that the mushroom was given the nickname 'Parisian mushroom'. Later on, the French gardener, Chambry, discovered that the caves had just the proper cool and moist environment for cultivating mushrooms, after which a large-scale mushroom cultivation developed within the caves around Paris [3]. In Europe, the primary cultivated fungi, the mushroom, was introduced within the 17th century [5]. Mushrooms were introduced into Netherlands for the primary time at the start of the 19th century, but it had been not be until after the 1900s that they were cultivated on a large-scale within the marl mines in Limburg. Within the early years, the mushroom was still very exclusive and only available to the elite. However, since then, better and simpler methods are developed and there has been an enormous increase in mushroom cultivation [6].

GROWING SKILLS

The most important thing to know about mushrooms is that they're simply the above-ground fruiting bodies of fungi that sleep in the soil. The overwhelming majority of fungal mass is below ground where it goes unseen and unnoticed until mushrooms emerge. Most mushrooms don't damage lawns or gardens; they're simply an unsightly nuisance. Mushrooms only grow when environmental conditions are good. Prolonged periods of wet, humid weather, like we've had over the past few weeks, cause fungi to send up fruiting structures. Fungi disperse to new areas via windblown spores. When the spores land during a suitable location they develop in to new fungi which can grow mushrooms given enough time. Mushrooms will get away on their own once the weather dries out. The fungus will still grow and persist as long as there's many organic interest feed on. Mushrooms will emerge again as soon because the growing conditions are right, which cannot be for an additional year.

If you're unwilling to attend for mushrooms to travel away on their own, you'll remove them by hand or with the mower. Although removing the mushrooms themselves does nothing to affect the fungi within the soil, it'll reduce the amount of spores released into the environment and therefore the number of latest mushrooms in several areas of the lawn and garden. Never eat an unknown mushroom unless you're absolutely confident of your identification skills. If you are doing plan to try eating wild mushrooms, be very cautious and only eat a little amount initially. Even mushrooms that are purportedly edible can make some people very ill [7].

CULTIVATION HISTORY

Unlike plants, mushrooms couldn't be cultivated initially and were collected for an extended period of your time. Even today, relatively few species of mushrooms are often cultivated compared to the amount of edible species. Mushrooms were thought to be special and supernatural in origin – 4600 years ago, the Egyptians believed mushrooms to be plants of immortality. The Romans thought mushrooms were the food of the gods. Many of us collect mushrooms for the aim of consumption, but many myths and false concepts still survive today. The Chinese and Japanese have utilized mushrooms for medicinal purposes for thousands of years. Research in Japan claims that this mushroom has medicinal use – Shiitake was combined with AIDs drugs to spice up immune reaction, combat chronic fatigue and induce antibody formation to Hepatitis B; it also stimulated antitumor activity. *Auricularia polytricha*, "ear fungus", was first cultivated in ancient China around 300 to 200 B.C. This mushroom is now cultivated in many South Pacific countries [8].

Different cultures cultivated different species – cultivation of mushrooms in Western cultures was first recorded in Paris, France, around 1650. *Agaricus bisporus*, the quintessential "shop mushroom", was first observed growing in melon crop compost. This mushroom was cultivated in open fields for 160 years then moved underground into caves, excavated tunnels or quarries – this type of cultivation remains utilized in France today. From France, the gardeners of England found *A.bisporus* a really easy crop to grow which required little labor, investment and space. By 1865, the US began mushroom cultivation. There are two widely known genetic variants of *A.bisporus* – these are Portobello and Crimini. These subterranean mushrooms can't be "cultivated" within the usual sense because they form a mycorrhizal (symbiotic) relationship with the roots of trees [9].

MUSHROOM FEATURES GLOSSARY

- **Cap (Pileus):** the expanded, upper part of the mushroom; whose surface is the pileus
- **Cup (Volva):** a cup-shaped structure at the base of the mushroom. The basal cup is the remnant of the button. Not all mushrooms have a cup.
- **Gills (Lamellae):** a series of radially arranged (from the center) flat surfaces located on the underside of the cap. Spores are made in the gills.
- **Mycelia threads:** root-like filaments that anchor the mushroom in the soil.
- **Ring (Annulus):** a skirt-like ring of tissue circling the stem of mature mushrooms. The ring is the remnant of the veil. Not all mushrooms have a ring.
- **Scale:** rough patches of tissue on the surface of the cap.
- **Stalk:** the main support of the mushroom; it is topped by the cap. Not all mushrooms have a stalk [10]**.**

CLASSIFICATION OF MUSHROOMS

Mushroom may be a fleshy plant organ of some fungi arising from a gaggle of mycelium buried in substratum. Most of the mushrooms belong to the Sub- Division: Basidiomycotina and a couple of belong to Ascomycota of Kingdom-Fungi. it's reported that there are about 50,000 known species of fungi and about 10,000 are considered as edible ones. Of which, about 100 and eighty mushrooms are often tried for artificial cultivation and seventy are widely accepted as food. The cultivation techniques were perfected for about twenty mushrooms and about dozen of them are recommended for commercial cultivation. However, only six mushrooms are widely preferred for large-scale cultivation. They are [11]

- Paddy straw mushroom - *Volvariella* spp.
- Oyster mushroom - *Pleurotus* spp.
- Button mushroom - *Agaricus* spp.
- Milky mushroom - *Calocybe* spp.
- Shiitake mushroom _ *Lentinulla* spp.
- Jews ear mushroom –*Auricularia* spp

An atypical mushroom is that the lobster mushroom, which may be a deformed, cooked-lobster-colored parasitized fruit body of a Russula or Lactarius, colored and deformed by the myco parasitic Ascomycete, Hypomyces, lactifluorum Other mushrooms aren't gilled, therefore the term "mushroom" is loosely used, and giving a full account of their classifications is difficult. Some have pores underneath (and are usually called boletes), others have spines, like the hedgehog mushroom and other tooth fungi, and so on. "Mushroom" has been used for polypores, puffballs, jelly fungi, coral fungi, bracket fungi, stinkhorns, and cup fungi. Thus, the term is more one among common application to macroscopic fungal fruiting bodies than one having precise taxonomic meaning. Approximately 14,000 species of mushrooms are described [12].

NOXIOUS MUSHROOMS

Only about 3% of known mushroom varieties are poisonous, and therefore the symptoms of poisoning can vary from gastrointestinal discomfort to liver failure and death, counting on the sort of toxin ingested. Acute liver failure from food poisoning is comparatively less common, but it does happen. And within the majority of cases, it's because an amateur mushroom hunter or backyard forager misidentified a mushroom. The most common dangerous mushrooms are those belonging to the Amanita genus, especially death cap, aptly called "death cap" mushrooms. They contain toxic compounds called amatoxins that damage liver cells. Every mushroom expert repeats an equivalent mantra: "Never eat a mushroom unless you'll positively identify it". Identification isn't easy, though. Mycologists have catalogued approximately 14,000 different mushroom species worldwide and classified them into variety of distinct genera.

Each genus typically includes edible and inedible species, and lots of of those look similar. The Amanita may be a case in point. It includes the poisonous Destroying Angel (*Amanita virosa*), the delicious Caesar's mushroom (*Amanita caesera*) and therefore the hallucinogenic Fly Amanita or toadstool (*Amanita muscaria*). Mushrooms with white gills are often poisonous. So are those with a hoop round the stem and people with a volva. Because the volva is usually underground, it is vital to dig round the base of a mushroom to seem for it. Mushrooms with a red color on the cap or stem also are either poisonous or strongly hallucinogenic. The foremost notorious red-colored mushroom is fly agaric, which has been consumed for thousands of years to supply visions. In large doses, even this "magic mushroom" are often lethal. Other Amanita species even have this coloration, and that they are far less benign [13].

World deadliest mushrooms [14]

1. Death Cap (*Amanita phalloides*)
2. The Destroying Angels (*Amanita virosa*)
3. False Morel (*Gyromitra esculenta*)
4. Autumn Skullcap (*Galerina marginata*)
5. Deadly Webcap (*Cortinarius rubellus*)
6. Conocybe Filaris (*Pholiotin arugosa)*
7. Deadly Dapperling (*Lepiota*sp.)
8. Angel Wing (*Pleurocybell aporrigens*)

MUSHROOM POISONING AND TREATMENTS [15]

Signs of mushroom poisoning:

Nausea, vomiting, abdominal pain and diarrhea are the foremost common initial food poisoning symptoms. Within the case of *Amanita* food poisoning, symptoms are usually delayed for six to 24 hours, by which era the toxins are completely absorbed. After an initial phase of digestive symptoms, the patient may appear to recover for 2 to 3 days, followed by relapse with liver and renal failure, which could lead on to massive bleeding and death. Eating poisonous mushrooms may cause differing types of reactions which may broadly be classified as follows:

1. **Gastric disorder:** The poison causes serious gastric disturbance, it chiefly acts by exciting and then paralyzing the central nervous system as by *Amanita muscaria*.
2. **Nervous disorder:** It causes degeneration of cells, especially of the nervous system and glandular parenchymatous tissues like liver as in case of *Amanita phalloides*.
3. **Muscular disorder:** There may be exciting of the muscular system, especially the smooth muscular fiber as it is there in the uterus, vessels etc.
4. **Hemolytic disorder:** There can be destruction of blood or hemolysis as in case of *Amanita rubescens***.**

Treatments

A regional poison center should be contacted to debate likely mushroom species ingested based upon clinical findings, identification of any mushrooms available for analysis, and treatment of specific toxic effects. Most poison control centers maintain active call lists of mycologists who are knowledgeable concerning local prevalence of mushroom genera and species and may assist in mushroom identification. Supportive care and gastrointestinal decontamination with activated carbon suffice for correct management of most patients with food poisoning [16].

The patient should be made to hide his body with a blanket, lie calmly and given the first-aid treatment till the arrival of the doctor. Removal of poison from the stomach: The stomach should be completely washed by means of a stomach tube. One also can give some sedatives like warm water, 4-5 tablespoonful of warm milk, and two tablespoonful of vegetable oil beaten with the yolk of an egg etc. elimination of the toxin.

EDIBLE MUSHROOMS

Edible mushrooms are the fleshy and produce bodies of several species of macro fungi. They will appear either below ground or above ground where they'll be picked by hand. Edible mushrooms are consumed for his or her nutritional and culinary value. Mushrooms, especially dried shiitake, are sources of umami flavor from granulate. Mushrooms consumed by those practicing folk medicine are referred to as medicinal mushrooms. While psychedelic mushrooms are occasionally consumed for recreational or entheogenic purposes, they will produce psychological effects, and are therefore not commonly used as food. There's no evidence from high-quality clinical research that "medicinal" mushrooms have any effect on human diseases [17].

Pleurotus species are commonly grown at industrial scale

- *Lentinula edodes*, the Shiitake mushroom
- *Auricularia auricula-judae*, the Jew's ear, wood ear or jelly ear mushroom
- *Volvariella volvacea*, the paddy straw mushroom or straw mushroom
- *Flammulina velutipes*, golden needle mushroom, seafood mushroom, lily mushroom, winter mushroom, velvet foot, velvet shank or velvet stem
- *Tremella fuciformis*, the snow fungus, snow ear, silver ear fungus and white jelly mushroom.
- *Hypsizygus tessellatus, Hypsizygus marmoreus*, the beech mushroom, also known in its white and brown varieties.
- *Cyclo cybeaegerita* or black poplar mushroom

PRODUCTION OF MUSHROOMS

Fungi culture is the cultivation of mushrooms and other fungi. A mushroom farm is in the business of growing fungi. Mushroom cultivation can help reduce vulnerability to poverty and strengthens livelihoods through the generation of a fast yielding and nutritious source of food and a reliable source of income.

- 1651 Discovery of the mushroom in the vicinity of Paris by sprinkling the waste from melon crops with leachate from ripe mushrooms.
- 1707 First controlled cultivation of "edible fungi" in the vegetable garden.
- 1825 Mushrooms are cultivated on a country estate near Haarlem.
- 1934 First scientific study of mushroom culture in the Research Station, The Netherlands.
- 1950 Construction of the first modern aboveground mushroom nurseries with several cultivation areas. The trays were made of concrete.
- 1955 Cultivation in wooden boxes in a tray.
- 1960 Cultivation in wooden beds on metal trays.
- 1975 Cultivation in fully metal trays, with mechanization of inserting and removing the mushroom compost [18].

BUTTON MUSHROOM CULTIVATION

Making Compost

The first step for growing mushrooms is composting which is completed within the open. Compost yard for button mushroom cultivation is ready on clean, raised platforms made from concrete. They need to be raised in order that the run-off water doesn't get accumulated at the heap. Although the composting is completed within the open, they need to be covered to guard from rain. Compost prepared is of two types, viz. natural and artificial compost. The compost is ready in trays of dimensions 100 X 50 X 15 cm.

Synthetic Compost for Mushroom Farming

The ingredients for synthetic compost are wheat straw, bran (rice or wheat), urea, gypsum, calcium nitrate or ammonium sulphate. The straw must be chopped to 8-20 cm. in length. it's spread uniformly to make a skinny layer on the composting yard. The straw is then drenched thoroughly by sprinkling water. Subsequent step is to combine all other ingredients like gypsum, urea, bran, and nitrate with the wet straw and heap them into a pile. The piling is often done by hand or with a stick. Lookout to not compactly compress the straw although it should be compressed firmly. The pile must be turned regularly as per the subsequent schedule During every turn, ensure to drench the heap by sprinkling water so on structure for the water lost thanks to evaporation.

Natural Compost for Mushroom Farming

Horse dung, wheat straw, poultry manure and gypsum are the ingredients. Wheat straw must be chopped finely. Horse dung must not be mixed thereupon of other animals. Additionally, it must be preferably freshly collected and not exposed to rain. Once the ingredients are mixed, they're uniformly spread on the composting yard. Water is sprinkled on the spread surface to wet the straws sufficiently. It's heaped and turned like that for synthetic manure. This is often a sign that the compost has opened. The heap is turned every 3 days and sprinkled with water. During the 3rd and 4th turning, 25 Kg gypsum to per ton of compost is added. 10mL Malathion to 5L water is sprayed in to the heap during the ultimate turn king.

Filling the Compost into Trays

The ready compost is dark brown in color and has no odor. It smells like fresh hay with an almost neutral or neutral ph. While filling the compost into trays, it must be neither too wet nor too dry. Just in case the compost is dry then sprinkle a couple of drops of water. If too wet, then allow some water to evaporate. When the compost has the proper amount of water, a couple of drops of water would exude when a little amount of the compost is pressed between the palms.

The size of the trays for spreading the compost is often as per convenience. However, it must be 15-18 cm deep. The trays must be made from soft wood. They're given pegs in order that when the trays are stacked one above the opposite, there's sufficient air gap. The trays must be crammed with compost to the brim and leveled on the surface [19].

Day 0: during this stage, the above Ingredient except Gypsum is mixed well and make a 5-feet-wide, 5-foot-high stack. With wooden box help or the other equipment in grow room. The stacks length depends on the quantity of fabric, but the peak and width shouldn't be more or but the measurements are written above, and It kept because it is for five days. Water is spray as per the need of lower moisture within the outer layers. This stacks temperature in about two to 3 days gets around 65-70 ° C, which may be a good sign.

First turnaround (6th day): On the sixth day, start the primary rotate. confine mind that every a part of the stack should be thoroughly mixed during the turning point, and enough air circulates in order that humidity to urge obviate each bit of compost. If the compost's moisture content is reduced, then the water is sprayed as per the need. The dimensions and size of the new pile are almost like the primary one.

Second turnaround (10th day): The second turnaround is as almost like the primary turnaround
Third turnaround (13th day): within the third turnaround, add Gypsum follow an equivalent procedure because the first turnaround me & mix completely

Fourth turnaround (16th day): Same process because the first turnaround
Fifth turnaround (19th day): Same procedure because the first turnaround
Seventh turnaround (25th day): Sprinkle Nuwan or Malathion (0.1%).

Eighth turnaround (28th day): Check Ammonia and moisture in compost on the twenty-eighth day. If the fingers become wet on the press, but the water with the compost does not squeeze, in this condition, the humidity level is appropriate in the compost in this situation, in the compost, 68-70% moisture is present, suitable for seed production. When the smell of ammonia is finally finished, and the sweet aroma comes from the compost, then compost is spread on the floor and cools down to 25°C.

Spawning: The method of sowing the mushroom mycelium into the beds is named spawning. After spawning the trays are covered with old newspaper sheets. The sheet surface is then sprinkled with water to take care of moisture and humidity. The trays maybe stacked one above the opposite with spacing of 15-20 cm between 2 trays [20].

Temperature and Other Conditions: The space temperature must be maintained at 25^0C. Humidity and moisture level must be maintained by sprinkling water on the walls and therefore the floor of the space. During the amount of spawn run, there should be no fresh air entering the space. Hence, it must be kept closed. On a mean the spawn run lasts for 12-15 days although it's going to take longer if the temperature is lower.

Sterilizing Casing Soil Using Formalin: For sterilization of 1 KL casing soil using formalin, half a liter of formalin in 10L water is sufficient. The soil is cover with plastic sheet and sprinkled with formalin. After 2 days, it's turned frequently for every week. Once the casing soil is freed from all traces, there would be no smell of formalin left behind.

Cropping: 15 to twenty days after casing, pinheads start becoming visible. White colored, small-sized buttons develop within 5-6 days of this stage. Mushrooms are ready for harvest when the caps are sitting tight on the short stem. Opened button mushrooms are considered to be inferior in quality.

Average Yield: The typical production of button mushroom is 3-4 Kg per tray. In favorable conditions the yield can go up to 6 Kg also.

Harvesting: While harvesting, the cap must be twisted off gently. For this, it's held gently with the forefingers, pressed against the soil then twisted off. The bottom of the stalk wherein mycelia threads and soil particles cling must be chopped off.

Storage: Once harvested, the mushrooms must be consumed fresh. While storing in fridge it's advisable to stay them wrapped in moist towel [21].

OYSTER MUSHROOM CULTIVATION:

This type of mushroom is grown where the climatic conditions are not favorable for button mushrooms. Being very low in fat content it is recommended for controlling obesity and patients suffering from diabetes, blood pressure, etc.

Growing material: Unlike button and paddy mushrooms, this type of mushroom can be grown on farm wastes high in cellulosic content like cotton waste, banana pseudo stems, cereal straws, etc. However, the most commonly used substrate is paddy straw.

Step 1: Ensure that the sawdust or straw is free from micro-organisms or anything that may disturb the growth of the mushrooms.
Step 2: Spread your medium into a container that can provide enough room for proper growth of the mushrooms.
Step 3: Mix the mushroom spawn with the medium inside the container.
Step 4: The mushroom spawn needs a certain amount of heat to enable its roots settle in the medium. Therefore, you must heat the container with the mushroom spawn and the medium or place it in direct sunlight for a while.
Step 5: After heating the medium and the spawn, the next thing to do is to put the mixture in a dark room. It could be a cabinet or drawer, but it must be a darkened environment where the temperature is not too high.
Step 6: When you have done all that you need to do, you must leave the mixture in the drawer or cabinet for about three to four weeks for it to grow. Meanwhile, cover the mixture with soil and continue to spray with water to keep it damp even as the mushrooms grow [22].

GROWTH TECHNIQUES
There are two types of growth techniques followed in oyster mushroom cultivation.

Polythene bags: The paddy straw is chopped into small pieces of 5 cm length, soaked in water for eight hours and the water is squeezed out. The paddy is placed in polythene bags that are 45 cm in length and 30 cm in diameter perforated with holes. About 200 grams of grain spawn is mixed 5-6 Kg of straw in these polythene bags. The spawning is done up to 2/3rd of the bags and the mouth is tied. The bags are then placed in shelves in the growing room having a temperature of 24-26°C.

Rectangular blocks: Bottomless wooden trays of 50 X 33 X 15 cm in dimensions are needed for this purpose. A transparent polythene sheet is spread on the bottom of the tray such that it becomes the bottom of the tray as well as covers it from the sides on the inner side. The wet, chopped paddy straw is filled in the tray to form a thick layer of 5 cm and the spawn is scattered uniformly. Lay another 2 layers of straw and repeat spawning after every layer. A last layer of straw is added and compressed firmly. About 200 grams of spawn is sufficient for 2 blocks.

Spawn run: The spawn run under ideal conditions get completed in 10-12 days. Once complete, the cottony white mycelia growth permeates through the straw. This makes the straw compact and it does not split while being handled. The polythene cover is cut and the sheets are untied at this stage. The bags are removed and the straw

bundles look like a neat cylinder. The cylinders are then arranged on the shelves and watered twice a day to maintain moisture.

Mushrooming: The first set of mushroom appears after 20 days of spawning. Further 2-3 flushes appear within a time interval of one week. They are harvested once the cap folds.

Harvesting and storage: Harvesting of mushroom is done by cutting it with a sharp knife or twisting it off with the fingers. They are best consumed fresh. However they can also be dried under a mechanical drier or under the sun and packed in polythene bags.

Yield: 1 Kg of oyster mushrooms can be obtained from 5-6 Kg of wet straw [24].

TISSUE CULTURE TECHNIQUE

Base spawn / nucleus culture: Tissue culture technique is used to bring the edible mushroom to pure culture so that the mushroom fungus can further be used to prepare spawn, which is an essential material for mushroom cultivation. This nucleus culture is grown on Potato Dextrose Agar medium in test tubes.

Procedure

- Select well grown, disease free button mushroom early in the morning and keep it on a clean paper for 2-3 hrs.
- Keep the sterilized PDA slants, razor blades, forceps etc. inside the chamber and put on the UV light.
- After 20 minutes put off the UV light and start working after 5 minutes.
- Take in the mushroom and split open the mushroom longitudinally into two halves.
- Using a blade cut a small piece of tissue from the center of the spilt mushroom at the junction of pileus and stipe.
- Remove the cotton plug of the agar slant and the tissue is aseptically placed inside the slant by using a sterilized forceps and closes it immediately.
- After transferring tissues from the mushroom , the tube are arranged in a wire basket and kept in a clean room at room temperature for the growth of the fungus
- Observe the tube at periodical intervals and remove the contaminated ones. The tubes will be ready for further use within another ten days. The base spawn is used for preparation of mother spawns.

Mother spawns: Mother spawn is nothing but the mushroom fungus grown on a grain based medium. Among the several substrate materials tested by TNAU, Coimbatore, sorghum grains are the best substrate for excellent growth of the fungus. Well-filled, disease- free sorghum grains are used as substrate for growing the spawn materials. Take out the bags after cooling and keep them inside the culture room and put on the UV light. After 20 minutes put off the UV light and start working in the culture room. Cut the fungal culture into two equal halves using an inoculation needle and transfer one half portions to a bag.

Bed spawns: The method of preparation of bed spawn was same as that of mother spawn. The cooking, filling and sterilization were similar to that of mother spawn. After sterilization, the bags are taken for inoculation**.**

NUTRITION OF MUSHROOMS

Raw brown mushrooms contain 92% water, 4% carbohydrates, 2% protein and less than 1% fat. In a 100 gram raw mushrooms provide 22 calories and are a rich source of B complex vitamins, such as riboflavin, niacin and pantothenic acid, selenium and copper, and a moderate source of phosphorus, zinc and potassium. They have minimal or no vitamin C and sodium content.

HEALTH BENEFITS [25]

- They also contain B vitamins as well as a powerful antioxidant called selenium, which helps to support the immune system and prevent damage to cells.
- Mushrooms can help to lower cholesterol, particularly in overweight adults, as well as phytonutrients that can help prevent cells from sticking to blood vessel walls and also reducing plaque build-up.
- White button mushrooms are one sources of vitamin D.
- Certain varieties of mushrooms to have potential in protecting against cancer and management of neurodegenerative diseases**.**

CONCLUSION

Mushroom cultivation is the most important microbial technology, after the yeast fermentation. It promises to supply food with good quality protein produced from cheapest lingo cellulosic wastes. Cultivation of edible mushrooms is the current stage that results the production of protein rich food with very minimal environmental pollution. Nutrition value knowledge are increased nowdays in this food we can get more nutrients as well as good immunization.

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Fig: 1 – Mushrooms

Fig: 2 - Autumn Skullcap (*Galerina marginata*)

Fig: 3- Conocybe Filaris

Fig: 4 -Angel Wing

Fig: 5 - Milky mushroom

Fig: 6 - Button Mushroom

Fig: 7- Oyster Mushroom

Fig: 8 - Mushroom compost

Fig: 9 - Mushroom Spawns

Fig: 10 – Button mushrooms

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RESEARCH ARTICLE

Formulation and Evaluation of Combination of Clindamycin and Tretinoin Topical Gel

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ABSTRACT

Topical drug delivery system is defined as drug delivery system which delivers the drug dermally on the local affected regions. Affordability and localized effect are some merits of this system. Topical gel with better efficacy was attempted by changing the polymers involved in formulation.15 formulations were prepared by qualitatively & quantitatively varying polymers. Quality of drug was confirmed using FT-IR spectroscopy, solubility studies, etc.Evaluation studies of finished product batches was donefollowed by short term stability studies. Microbial limit tests were performed to understand efficacy of formulation batches.5 batches were selected for In-vitro drug release. F-2 was the best formulation, whichwas subjected to stability studies. Gel formulation containing Carbopol 0.8% can be taken as ideal formulation.

Keywords: Topical, Carbopol, polymers, anti-microbial, topical gel, formulation

INTRODUCTION

Topical dermal delivery is used for application to skin directly at the affected area to deliver the drug in dermal and the underlying tissue at the site of application [1].Gel is defined as semi-solid system, which consists of either of inorganic or large organic particles[2]. It is classified based upon: [3-6]

i. Colloidal phases
ii. Solvent nature
iii. Physical properties

Economic cost and localized effect are merits.Poor permeability and chances of enzymatic degradation in skin are few disadvantages [7-10].

GelPreparation is performed as follows:

i. Thermal change
ii. Flocculation with neutralizers
iii. Chemical reaction

However, bio-pharmaceutical parameters must be considered during the formulation of topical delivery systems. Drug permeation of the drug happens through sorption by stratum corneum. This is followed by penetration via viable epidermis followed by drug uptake by in dermal papillary layer[11-14].

To achieve an ideal topical drug, Diffusion cells are used for in-vitro studies of semisolid dosage forms [14,15].

Acne can be defined as adolescent dermatological condition. Clindamycin and Tretinoin can be used as topical anti-acneicgels [16-18].

MATERIALS AND METHODS

Melting Point Determination

Melting point is the temperature at which the pure liquid and solid exist in equilibrium. In practice, it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometimes known as normal melting points.

Melting point was found to be for clindamycin at 163°C and for Tretinoin at 180°C which was in compliance with the official value.

FT-Irspectra[19]

IR spectra of drug in KBr pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Perkin Elmer Spectrum one). The comparison of the test spectra with Reference spectra of the drug shows similar peaks.

Organoleptic Characteristics[20]

The colour and odor of the drug were characterized and recorded using descriptive terminology. Tretinoin was found as yellow Crystalline Powder, with odourless and clindamycin was found as white to off white, hygroscopic, crystalline powder with odour less and bitter taste.

Solubility

Clindamycin was freely soluble in water and slightly soluble in dehydrated alcohol. The drug also was very slightly soluble in acetone. But, it was practically insoluble in chloroform; in benzene and in ether and Tretinoinin soluble in water slightly soluble in ethanol (96%) and in chloroform, soluble in ether.

Procedure

Two different procedures based on the type of polymer to be incorporated.

The qualitative and quantitative estimation was performed for the following batches:

1. Firstly 50% of purified water was taken and polymer was added and dissolve with stirring and maintained at the temperature about 60-70ºC.
2. Remaining quantity of purified water was taken in a beaker and Clindamycin phosphate was added and maintained at 40-50ºC and stir well.
3. PEG 400 and Propylene glycol was taken in a beaker and dissolve Tretinoin dissolved on 30-40ºC temperature.
4. The Clindamycin solution was mixed with the polymer solution.
5. Tretinoin-Propylene Glycol mixture is added to the above mixture thoroughly.
6. pH was adjusted to 4.6-6.5 by employing 10% solution of sodium hydroxide.

pH[21,24,25,26,27,28]:

The pH of the prepared gel is determined by using digital pH meter. Proper stabilization was performed by dipping

and stabilizing after dipping into the buffer solutions with pH of 3.99, 7.0 and 9.2. The electrode was dipped into the gel and pH was determined.

Viscosity[21-25]:

Sample was incubated at 25 ºC for at least 16 hour. In a incubator and then run on brook field viscometer using different rpm 0.5, 1,2.5 and 5 rpm. Spindle was chosen to maintain a torque between 10% and 90%. The RV spindle givesviscosity at a single immersion point in the sample. the helipath T bar spindle were rotated down and up in the sample , giving viscosity at a number of point programmed over the run time ,two reaching taken over a period of 60s were average to obtain the viscosity .the viscosity was calibrated using brook field viscosity standard 5000 (100% polydimethylsiloxane).

Extrudability[25]

Gel formulation were filled in standard caped collapsible lamitube and sealed. The tube was weighed recorded. The tube was placed between two glass slides and was clamped. A 500 g weight was placed over the glass slide and then cap was opened. The amount of gel extruded were collected and weighed. The % of gel extruded was calculated; and grades were allotted (+ + + + excellent, + + + Good, + + fair, + Poor).

Assay

Drug assay was conducted using HPLC using acetonitrile and tetrahydrofuran for Clindamycin & Tretinoin respectively, which was diluted with Phosphate buffer pH 2.5 & 3.

Microbial Limit Test

Enrichment was performed using Casein Soya Bean Digest Broth followed by incubation at 35°C to 37°C for 18 to 48 hours. Primary culture was selected based on the type of microbe to be screened. Iodole test was performed as a confirmatory test.

Stability Studies[20]

The stability studies of formulated gels were carried out at 40/75(oC/RH) and at room temperature for one month. The effects of temperature, Humidity and time on the physical characteristics of the gels were evaluated for assessing the stability of the prepared formulations. The stability studies were carried out when the room temperature was 20 to 25oC.

RESULTS AND DISCUSSION

Melting Point Determination

Melting point was found to be for clindamycin at 163 °C and for Tretinoin at 180°C which was in compliance with the official value.

FT-IR Spectroscopy

The FT-IR spectrum of Clindamycin & Tretinoin was taken and is displayed as below:

IR spectra of drug in KBr pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Perkin Elmer Spectrum one). The comparison of the test spectra with Reference spectra of the drug shows similar peaks

Organoleptic Properties

Tretinoin: yellow Crystalline Powder, with characterstic floral odour.

Clindamycin Phosphate: white to off white, hygroscopic, crystalline powder with odourless and bitter taste.

Thus, the drugs comply with the Pharmacopeial limits.

Solubility

Tretinoin: soluble in Dimethylsulfoxide, slightly soluble in propyleneglycol 400, octanol, 100% ethanol. It is practically insoluble in water and mineral oil and it is insoluble in glycerin.

Clindamycin Phosphate: Freely soluble in water, slightly soluble in dehydrated alcohol, very slightly solublein acetone, practically insoluble in chloroform, in benzene and in ether.

pH

The pH of the various formulations was determined and is graphically shown as follows:

Viscosity

The viscosity of the various formulations were determined and is graphically shown as follows:

Extrudability

Extrudability test of 15 formulations were performed and is tabulated as follows:

The 2nd batch has the maximum extrudability while 5 batches (F3, F11, F12, F14 & F15) had the least extrudability.

Assay

5 batches were selected on the basis of physical evalution tests. The *in-vitro* studies of the 5 selected batches of Clindamycin and Tretinoin are as follows:

Microbial Limit Test

Stability Study

Stability study was carried out for the optimized formulation according to ICH guide lines at 40°C/75 %RH for 1 month. The results showed that there was no significant change in physical and chemical parameter of the gel, hence the formulation (F2) was found to be stable.

CONCLUSION

The combination of Clindamycin and Tretinoin gel formulation was optimized on the basis of different physical parameters with comparison of formulations on basis of in-vitro diffusion study and found to be 39.40 % for Clindamycin and 33.08 % for Tretinoin release within 6 hours in dissolution media. Results of all the batches showed that all comply with the pharmacopeial and/or standard references. Results of all the physical and in-vitro dissolution data concluded that formulation F-2 was the most promising formulation. Stability study was conducted of F-2 storing at 40°C/75%RH for one month. Gels were evaluated for appearance, feel on application, pH, viscosity, assay and in-vitro drug release profile after one month. It concluded that Formulation F-2 was stable. From the above results, it concluded that combination of Clindamycin and Tretinoin gel. Gel formulation containing Carbopol 0.8% can be taken as ideal formulation. This gives a ray of hope on development of topical gels of different class of drugs using polymers of various types.0.1 N Hydrochloric acid (for Clindamycin) and Phosphate buffer : methanol in ratio of 65 : 35 (for Tretinoin)

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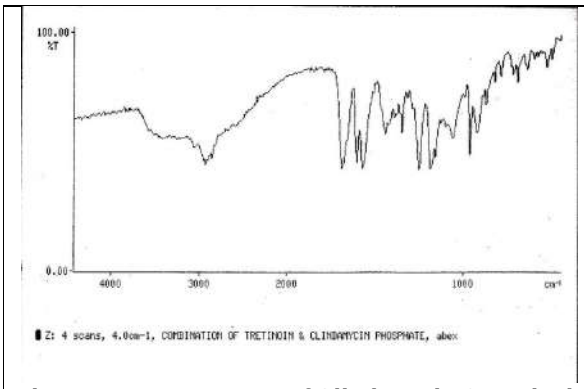

Figure 1. FT-IR spectrum of Clindamycin & Tretinoin

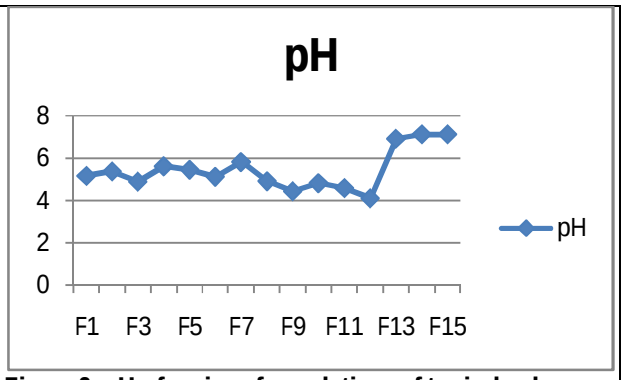

Figure 2. pH of various formulations of topical gel

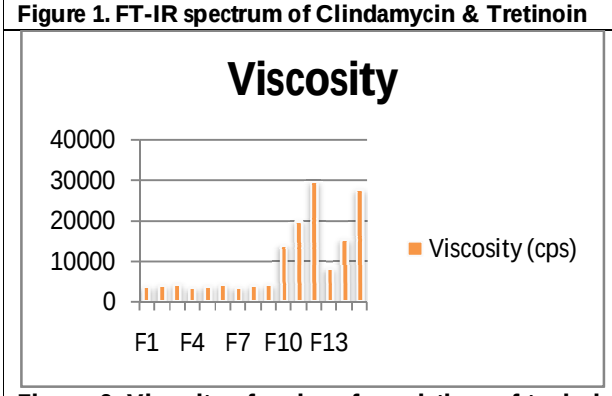

Figure. 3. Viscosity of various formulations of topical gel

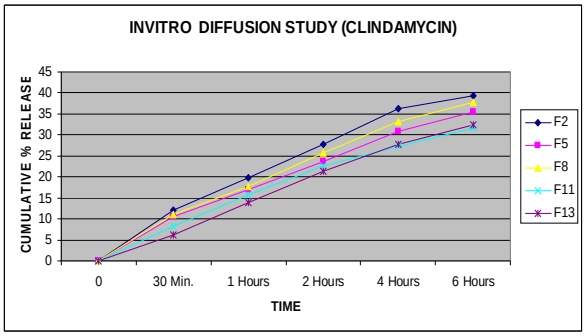

***Figure 4.In-vitro* diffusion study of Clindamycin for the selected formulation batches**

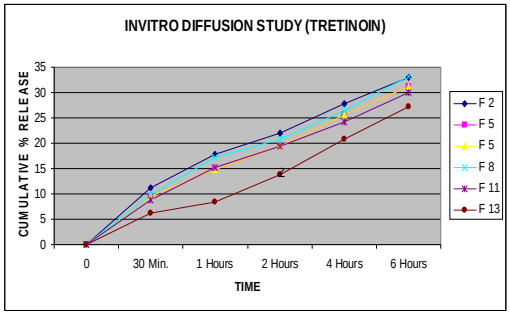

Figure 5.*In-vitro* diffusion study of Tretinoin for the selected formulation batches

Table-1: Formulation of Clindamycin & Tretinoin with varying concentrations of Carbopol polymers

S.No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
		%	%	%	%	%	%	%	%	%
1	Clindamycin	1.234	**1.234**	1.234	1.234	**1.234**	1.234	1.234	**1.234**	1.234
2	Tretinoin	0.025	**0.025**	0.025	0.025	**0.025**	0.025	0.025	**0.025**	0.025
3	disodium edentate	0.02	**0.02**	0.02	0.02	**0.02**	0.02	0.02	**0.02**	0.02
4	Methyl paraben sod.	0.1	**0.1**	0.1	0.1	**0.1**	0.1	0.1	**0.1**	0.1
5	Propyl paraben sod.	0.05	**0.05**	0.05	0.05	**0.05**	0.05	0.05	**0.05**	0.05
6	PEG 400	10	**10**	10	10	**10**	10	10	**10**	10
7	Propylene glycol	2	**2**	2	2	**2**	2	2	**2**	2
8	Carbopol 934	**0.6**	**0.8**	**1**	---	---	---	---	---	---
9	carbopol 940	---	---	---	**0.3**	**0.5**	**0.7**	---	---	---
10	carbopol 980	---	---	---	---	---	---	**0.4**	**0.6**	**0.8**
11	Purified water	85.97	**85.77**	85.57	86.27	**86.07**	85.87	86.17	**85.97**	85.77
12	Sodium hydroxide	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table-2:Formulation of Clindamycin & Tretinoin topical gel with varying concentrations of HPMC K4M & CMC Na

Sr.No.	Ingredients	F10	F11	F12	F13	F14	F15
		%	%	%	%	%	%
1	Clindamycin	1.234	1.234	1.234	1.234	1.234	1.234
2	Tretin	0.025	0.025	0.025	0.025	0.025	0.025
3	disodium edetate	0.02	0.02	0.02	0.02	0.02	0.02
4	Methyl paraben sod.	0.1	0.1	0.1	0.1	0.1	0.1
5	Propyl paraben sod.	0.05	0.05	0.05	0.05	0.05	0.05
6	PEG 400	10	10	10	10	10	10
7	Propylene glycol	2	2	2	2	2	2
8	HPMC K4M	**3**	**3.5**	**4**			
9	CMC Na				**4**	**4.5**	**5**
10	Purified water	83.57	83.07	82.57	82.57	85.07	81.57

Table-3: Extrudability grades of various prepared topical gel formulations

FORMULATION	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
EXTRUDABILITY	++	++++	+	+++	++	+++	+++	+++	++	++	+	+	++	+	+

Table-4: Microbial Limit Test for selected formulations

S.No.	Test	Formulations				
		F2	F5	F8	F11	F13
1	Total microbial count a)Bacterial count b)Fungal count	Nil	Nil	Nil	Nil	Nil
2	Test for *E.coli* *E. coli*	Nil	Nil	Nil	Nil	Nil

3	Test for Salmonella species *Salmonella typhyrium*	Nil	Nil	Nil	Nil	Nil
4	Test for *Staphylococcus aureus* *Staphylococcus aureus*	Nil	Nil	Nil	Nil	Nil
5	Test for *Pseudomonas aeruginosa* *Pseudomonas aeruginosa*	Nil	Nil	Nil	Nil	Nil

Table-5:Stability Parameter of selected formulation

PARAMETERS	**INITIAL**	**AFTER ONE MONTHS 40/75(°C/RH)**
Appearance	Yellowish Translucent	Yellowish Translucent
Feel on Application	Smooth	Smooth
Ph	5.37	5.43
Viscosity	4100	4611
Drug Content (%)	102.542 (Clindamycin)	101.461(Clindamycin)
	106.518(Tretinoin)	104.691(Tretinoin)

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RESEARCH ARTICLE

Ant Diversity in Selected Ecotone Patches Abutting Bannerghatta National Park

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ABSTRACT

Ant is the pioneer species which plays an important role in the basic food chain hence, we tried to study the composition abundance of different species of ants in selected geographical areas of the A Rocha Field study center adjoining the Bannerghatta National Park, Bengaluru. In the three day collection, around 62 individuals were captured belonging from Formicinae, Myrmicinae, Dolichoderinae and Pseudomyrmecinae sub-families.

Keywords: A Rocha, Bannerghatta National Park, Ants, Formicinae, Myrmicinae, Dolichoderinae, Pseudomyrmecinae, Diversity.

INTRODUCTION

Ants impact the biodiversity because of their numerous interactions with different other types of species which further helps in balancing the terrestrial ecosystem. They are also a fantastic biological indicator due to mutualistic behavior with both flora and fauna (Mahalakshmi and Channaveerappa, 2016) and are universally found everywhere except the regions of extreme low temperatures such as Antarctica, Iceland, Greenland, etc. There are about 15000 species found, out of which 152 species are listed in IUCN from India where the Western Ghats harbor a large number of ant species around 455 species from 75 genera (Raj *et al*., 2016). About 226 species of ants of 63 genera and 11 sub-families are reported from the state of Karnataka, India (Varghese, 2009). The main aim of the present survey was to document myrmecofaunal diversity and compare to existing data from A Rocha field study center, adjoining the Bannerghatta National park, Bengaluru, Karnataka.

MATERIALS AND METHODS

The study was undertaken in the campus of A Rocha Field Study Centre (12.8136° N, 77.5673°E) (Map 1),Bannerghatta (Post), Jigani (Hobli), Anekal (Taluk), Bengaluru Urban District, Karnataka, for three consecutive

days from 9th-11th of May, 2019. The collection procedure mainly included pitfall traps with different baits *viz.*, sugar syrup, jam and mango pieces and random handpicking after which specimens were sorted and labeled and finally preserved in 70% ethyl alcohol for identification.

RESULTS AND DISCUSSION

Of the seven traps that were set up, the sugar syrup attracted maximum number of ants which was followed by traps with jam and the mango baits. The collection had a total of around 65 individuals belonging to Formicinae, Myrmicinae, Dolichoderinae and Pseudomyrmecinae sub-families were observed (Table). The Simpson's Index of Diversity (1 – D) was found to be 0.8 (higher diversity) and Shanon-Weiner (H) index was found to be 2.1 (Values range from 0 to 5, usually ranging from 1.5 to 3.5 and rarely reaches 4.5). Subfamily Formicinae was found to have 8 species belonging to 5 different genera viz., *Oecophylla smaragdina*, *Anoplolepis gracilipes*, *Paratrechina longicornis*, *Camponotus compressus*, *C. parius*, *C.angusticollis*, *C.irritans* and *Polyrhachis exercita.*

It was followed by the subfamily Myrmicinae with 3 species belonging to 3 different genera, namely, *Carebara affinis, Solenopsis geminate*, *Pheidole* sp. The members are morphologically distinct which makes identification easier on appropriate magnification. The subfamily Dolichoderinae whose members prefer warm and xeric conditions, have a typical brown red colour body and the size ranges from 1 nm to 7 nm and the subfamily Pseudomyrmecinae, found in warm temperate regions, often nest on the cavities of dead plant tissue were found to have one species each- *Tapinoma melanocephalum* and *Tetrapontera nigra* respectively. The number of individuals of *Tapinoma melanocephalum* species of Dolichoderinae subfamily was the maximum, i.e.15, followed by species *Paratrechinalongicornisn* with around 12 individuals and around 10 individuals of *Anoplolepisgracilipes* belonging to subfamily Formicinae. A similar study was done in the year 2017 in the same locality considering seasonal variations in the occurrence of species and ant diversity. A total of 37 species were found in the span of two seasons (monsoon of 2016 and summer of 2017) and the sub-family Myrmicinae was found to be the dominant one. The abundance of species was found more in the summer season than monsoon (Nikhil *et al.,* 2017). This may be due to the wear and tear of nest sites during rains, leading to water clogged nests (Kolay *et al*., 2015).

The grassy habitat harbors more individuals than dry habitat in both the studies (Nikhil *et al.,* 2017)which was found true in the present study as well. The collection was maximum during the mornings and minimum during afternoons and evenings. This could be due to the fact that, ants are ectothermic and are considered to forage at warm temperatures, but not too warm (Holldobler and Wilson, 1990).The striking difference between the two studies was the number of species collected in the same area during the summer of 2017 was found maximum in the evening whereas in the present study there were almost no ants found in the traps after sunset. This could be due to the various changes in the human induced landscape alterations over a span of two years. The species richness for each week is likely to vary greatly among plots and from week to week because of weather or sampling error (Changlu *et al*.,2000).

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Table 1. List of collected ant species from the study area.

S.No.	Subfamily	Species	Number of individuals
1	Formicinae	*Oecophylla smaragdina*	2
2		*Anoplolepis gracilipes*	10
3		*Paratrechina longicornis*	12
4		*Camponotus compressus*	2
5		*Camponotus parius*	4
6		*Camponotus angusticollis*	1
7		*Camponotus irritans*	3
8		*Polyrhachis exercita*	2
9	Myrmicinae	*Carebara affinis*	3
10		*Pheidole sp.*	5
11		*Solenopsis geminata*	2
12	Dolichoderinae	*Tapinoma melanocephalum*	15
13	Pseudomyrmecinae	*Tetraponera nigra*	1

Map 1: Location of A Rocha field study centre adjoining BNP (Picture Courtesy: Google Maps)

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RESEARCH ARTICLE

Estimating the Land Use Land Cover Matrix and its Impact on Urban Heat Island Effect: A Case Study of Mountainous and Plateau Region

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ABSTRACT

The frenetic growth in the global population has changed its land use land cover pattern which has an unfortunate effect on weather conditions and is resulting in extreme weather events such as Urban Heat Island (UHI). The rapidly spreading Urban Heat Island effect contributes towards extreme heat events causing catastrophic impact on daily weather phenomena such as maximizing the day-time temperature, reduces night-timecooling, escalates air pollution levels and causes heat-related illness. In recent years, with the expansion of settlement in mountains and plateau regions, the disastrous impact of UHI is observed in the cities of elevated topography. Thus, the current study verifies the existence of UHI phenomena in the cities of Bangalore (plateau region) and Dehradun (mountainous region) and their changing Land Use Land Cover pattern for the period 2001-2019. The Land Use Land Cover matrix depicts an increase in heat source landscapes such as built-up area, current fallow land, barren land and sand whereas, a decrease in heat sink landscapes: forest, water and agriculture. As far as Land Surface Temperature (LST) is concerned a drastic increase in surface area under high LST is observed in Dehradun. On the other hand, the increase in surface area under high LST in Bangalore was gradual. Thus, the current study exploring both the LULC matrix and LST can provide unique information for site-specific land planning and policy implementation to mitigate such extreme weather events.

Keywords: Land Surface Temperature, Urban Heat Island, Geospatial Techniques, Remote Sensing, Elevated Topography, Land Use Land Cover Matrix.

INTRODUCTION

The unprecedented growth in urban population has been observed worldwide in recent years. The global share of the population living in urban areas has reached 55.7% in 2019 which is projected to rise to 68% by 2050 (United Nations, Department of Economic and Social Affairs, 2018). Therapid growth in urban population has led to enormous extraction and utilization of natural resources, causing an extreme change in land use patterns in urban areas (Dewan et al.,2009). The rapid process of urbanization has been most pronounced in developing countries of

Asia and Oceania (Helble et al., 2021; United Nation Conference on Trade and Development, 2019). The extraordinary pressure on land created by accelerating population growth has resulted in a marked increase in alteration of natural vegetation and water bodies to build up areas including buildings, roads, pavements and impervious surfaces (Garg, 2017). The expansion of land surface covered by impervious materials characterized by high heat capacity and conductivity contributes towards growing Land Surface Temperature (LST) (Kikon et al., 2016). Land Surface Temperature (LST) also known as radiative skin temperature is referred to the temperature at the top surface in case of bare ground condition and at the surface of the canopy in vegetated areas derived from infrared radiation (ESA, 2020; Hulley et al., 2019). The phenomenon with higher LST in urban areas compared to its surrounding peripheral areas is termed Urban Heat Island (UHI) which is intensely exacerbating over time (Rizwan et al., 2008). The increasing LST in cities has disastrous global consequences such as a rise in global temperature (Alcoforad et al., 2008), triggers storms /precipitation(Bornstein and Lin, 2000; Dixon and Mote,2003), creates an imbalance in the energy demand-supply of cities (Santamouris et al.,2015), contributes towards urbanization induced heat-related mortalities (Hondula et al.,2014) and exacerbates heat waves (Ramamurthy,2017).Thus, proper planning, effective strategies and appropriate policy interventions are required to mitigate these disastrous UHI effects (Chow et al., 2012).

Lately, climatologists and urban scientists have explored and evaluated UHI effects for various cities across the globe. Formerly, to examine UHI effects traditional Canopy Layer Heat Island and Boundary Layer Heat Island methods such as weather stations, fixed-point observation and sport transect methods which required huge human, as well as material resources and substantial financial aid with low accuracy, were applied (Pan, 2015). But,with the emergence of remote sensing, space-borne sensors which provide better spatial coverage with greater resolution and accuracy were used to investigate the UHI effect (Pongracz,2010). The first person to applied satellite-based thermal infrared data to explore UHI was Rao, 1972 and since then many research studies has been conducted using remote sensing data for UHI effect analysis. The high spatial resolutions satellite imageries including Landsat TM (thematic mapper)/ETM (enhanced thematic mapper) and the Terra Aster (advanced space-borne thermal emission and reflection radiometer) data are commonly used to analyze UHI effects and their relation to land use land cover pattern (Ahmed,2017; Weimin Wang,2019; Sekertekin et al.,2020). There are also sensors with higher temporal resolution as MODIS (moderate-resolution imaging spectra radiometer) (Robert et al., 2014) and AVHRR (Sahin et al., 2012) that enables to carry out of studies on the UHI effect at different time scales.

Additionally, LST retrieval algorithms based on temperature, vegetation indices and thermal landscapes (Kumar et al., 2015; Wang et al., 2019) are applied to calculate LST values. The radiative transfer equation is the very first algorithm developed (Berk et al., 1889) and few other methods include the mono window algorithm (MWA) (Qin et al.,), the split-window algorithm (SWA) (Mcmillin et al.,), the single-channel method (SC)(Jimenez-Munoz et al.,) and multi-angle algorithm (Dash et al.,2002). So far, various studies exploring the UHI effect for different landscapes including plain regions (Barat et al.,2018), arid and semi-arid areas (Sofer and Potchter, 2006), coastal cities (Wu et al., 2019), mountainous regions (Li et al.,2018) and polar region(Konstantinov et al.,2014)has been conducted. But studies analyzing the UHI effect in elevated topography: plateau and mountain region has been limited. Currently, over 50 % of the global population are dependent on mountains for water, food and clean energy and around 15% of the human population lives in mountainous areas (United Nations, 2020; Food and Agricultural Organization (UN), 2017). Moreover, cities in plateau regions that are favorable for a living has seen rapid growth in the human settlement in recent decades. Thus, the enormous pressure on mountains as well as plateaus has resulted in climate-induced disaster, climate change, climate variability and extreme weather events as the UHI effect. Thus, the current research study explores the spatio-temporal dimension of the UHI effect in the cities of elevated topography: Bangalore (Plateau Region) and Dehradun (mountainous region). The study exclusively focuses on:

1. To verify the existence of the Urban Heat Island (UHI) phenomenon in Bangalore and Dehradun.
2. To evaluate the spatio-temporal variation of Land Surface Temperature (LST) of Bangalore and Dehradun from 2001 to 2019.
3. To analyze the Land Use and Land Cover matrix of Bangalore and Dehradun for the period 2001-2019.

The research paper begins with an introduction providing a brief on the research problem and signifying the imperative need of analyzing theextreme weather events as UHI effects in elevated topography. The following section provides an account of the study area and the methodology applied. The results are discussed in the next section. Lastly, the conclusion summarizes the main findings and key take away from the research.

Study Area

Bangalore: Bangalore is located at 12°39'32"N to 13°14'13"N latitudes and 77°19'44"E to 77°50'13"E longitudes. It is the principal administrative, cultural, commercial, industrial and knowledge capital of the state of Karnataka with an area of 2208 sq. km. Bangalore is located at an altitude of 920 meters above mean sea level. The mean annual total rainfall is about 880 mm with about 60 rainy days a year over the last ten years. The summer temperature ranges from 18° C – 38° C, while the winter temperature ranges from 12° C – 25° C (Ramachandra, 2010). The fifth-largest metropolis in India is home to a population of 9621551 among which males and females are 5022661and 4598890 respectively. (Census, 2011).

Dehradun: The district Dehradun is one of the thirteen districts of the Uttarakhand state where the capital of the State –Dehradun lies. The district Dehradun encompasses an area of 3088.50 km^2, extends in between 29°57'56.44" N to 31°1'127.13" N latitudes and 77°38'19.57" E to 78°14'24.53" E longitudes. The elevation of the district varies between 288 m to 3096 m from the mean sea level (Rawat et al., 2017).The climate of Dehradun is humid subtropical. The overall population of Dehradun is 1696694 with 892199 male population and 804495 female population having a population density of 550 persons/km2 (Census, 2011).

METHODOLOGY

The methodology adopted to carry out the present research work is briefly outlined in this section. The study utilizes Landsat 5 TM, Landsat 7 ETM+ and Landsat 8 OLI satellite images to calculate Land Surface Temperature and prepare the Land Use Land Cover Map for the years 2001 and 2019 which has been downloaded from the "Earth Explorer" website of the United States Geological Survey (USGS) (Table No.1). Once the preprocessing of the satellite data was over the Erdas Imagine and Arc GIS software has been used for calculating the LST from Landsat 5 TM, Landsat 7 ETM+ and Landsat 8 OLI images and further reclassified in Arc GIS software.

Calculation of LST by Model Maker tool in Erdas Imagine

Step 1- Conversion of the Digital Number (DN) to Spectral Radiance (L)

$L = L_{MIN} + (L_{MAX} - L_{MIN}) * DN / 255$

Where L = Spectral radiance, L_{MIN} = Spectral radiance of DN value 1, L_{MAX} = Spectral radiance of DN value 255 DN = Digital Number

Step 2-Conversion of Spectral Radiance to Temperature in Kelvin

$TB = K_2 / \ln (k_1/L + 1)$

Where K_1= Calibration Constant 1, K_2 = Calibration Constant 2, TB= Surface Brightness Temperature

Step3-Conversion of Kelvin to Celsius

$TB = TB - 273$

Calculation of LST by Raster Calculator in ArcGIS

Step 1-Conversion of the Digital Number (DN) to Top of Atmosphere (TOA) Spectral Radiance

$TOA (L) = M_L * Q_{cal} + A_L$

M_L = Cell value as the radiance of thermal band (band 10), Q_{cal} = Thermal Band (band 10),
A_L = Band specific additive rescaling factor

Step 2- Conversion of the Top of Atmosphere to Brightness Temperature

$BT = (K_2 / (Ln (K_1/L) + 1)) - 273.15$

K_1 = Constant of the thermal band (band 10), K_2 = Constant of the thermal band (band 10),
L =TOA, Ln =Log

Step 3- Calculate NDVI (Normalized Difference Vegetation Index)
NDVI = float (NIR-Red) /float (NIR+ Red)
NIR = Near Infra – Red (band5) Red= Red band (band4)
Step 4- Calculate the Proportion of Vegetation (Pv)
Pv= Square ((NDVI + 1)/ (1=1))
NDVI = Normalized Difference Vegetation Index
Step 5-Calculate Emissivity E = 0.004 * Pv +0.986
Pv = Proportion of Vegetation
Step 6- Calculate Land Surface Temperature (LST)
LST = (BT / (1+ (λ * BT / 14388) * Ln (E)))
BT = Brightness Temperature, λ = Wavelength of emitted radiance,

RESULTS AND DISCUSSION

Land Use Land Cover (2001 and 2019)

Land use land cover (LULC) has been explored in the current study for both the landforms to understand the landscape distribution pattern. The study has been conducted from 2001 to 2019 in both the landforms. The increasing heat source landscapes (built-up area, current fallow, barren land and sand) and decreasing heat sink landscapes (forest, water body and agriculture) from 2001 to 2019 were highlighted in red colour. Likewise, the increasing heat sink landscapes and decreasing heat source landscapes from 2001 to 2019 has been highlighted in green colour in the tables below. Accordingly, in Land use, land cover matrix change from the heat source to heat sink landscapes has been highlighted in green colour and the land use change from the heat sink to heat source landscapes has been highlighted by the red colour in the tables.

Land Use Land Cover of Bangalore (2001 and 2019)

In Bangalore, it has been witnessed that both the heat source landscapes: barren and current fallow have decreased to 4.4 % and 50.1 % respectively throwing a positive impact on controlling the UHI (Table No. 2). But, the increase in built up area to 22% in 2019 and decrease in water bodies and agriculture landscape to 11.1% and 1.1% respectively in 2019 has highly contributed towards intensifying the UHI effects.

Land Use and Land Cover Matrix of Bangalore (2001-2019)

The land transition matrix of Bangalore (2001-2019) depicted in Table No. 3 shows a significant increase in heat source landscapes. It has been observed that 162.72 $km^2$and 44.38 km^2 of agricultural land has been converted to current fallow and built up landscapes. Whereas, considering the heat sink landscapes, there has been a remarkable transformation of barren (21.4 km^2) and current fallow (43.76 km^2.) to forest landscapes. Moreover, there has been a decline in the proportion of water bodies due to its transformation to barren (2.26 km^2) and current fallow (7.56 km^2) landscapes. Thus, the findings above clearly depict that the built up area has increased tremendously from 11.8% in 2001 to 22% in 2019. This increase in built up area has been contributed by the conversion of agricultural land (44.38 km^2) and forest (30.8 km^2) to some extent. Moreover, a decrease in agricultural land is reported from 15.8% to 11% from 2001 to 2019. Therefore, an overall increase in heat source landscape (built up, barren land, current fallow) and a decrease in heat sink sources (forest, agriculture, water body) has been observed (Figure 3).

Land Use Land Cover of Dehradun (2001 and 2019)

Table No. 4, illustrates the distribution of the surface area under each land use class (in %) for the years 2001 and 2019 in Dehradun. It has been observed that there is an increase in heat source landscape: agricultural from 5.7 % to 7.1 % and decrease in heat source landscape: current fallow from 20.2 % to 19.9 % between 2001 and 2019 respectively. But unfortunately, the increasing proportion of sand, built up, barren landscape and decrease in forest landscapes from 001 to 2019 contribute towards the growth of UHI phenomena.

Land Use and Land Cover Matrix of Dehradun (2001-2019)

Table No. 5, basically illustrates the land use land cover transition matrix between two time periods (2001 and 2019) of Dehradun. It has been observed that around 156.42 km^2 and 41.07 $km^2$of forest land cover got converted into barren land and current fallow respectively in 2019 that contributed towards the expansion of heat source landscapes. Moreover, 36.83 $km^2$of the agricultural landscape has been transformed to the current fallow in 2019 which shows a positive growth of heat source landscapes. Comparatively, the transition of heat source landscapes to heat sink landscapes is limited. Around 89 $km^2$of current fallow has been converted to forest landscapes and 11.2 $km^2$of the sand landscape has been converted to forest contributing towards mitigating the severity of the Urban Heat Island effect (Figure 4). Therefore, compare to Bangalore in Dehradun a marginal increase in built up area has been observed from 3.5% to 4.5 %.But, the land use land cover matrix indicates that 19.52$km^2$of agricultural land and 13.78 km sq. of forest land got converted into the built up area. Moreover, a huge amount of forest land was transformed to barren land, current fellow as well as sand landscape. On the other hand, a positive impact can be observed with the conversion of 89.7 km^2 of current fallow land into forest land. Thus, an overall increase in heat source landscape can be seen compared to heat sink landscape from 2001-2019.

Spatio-temporal variation of Land Surface Temperature (LST) (2001-2019)

The current research study evaluated the rising LST for the years 2001 and 2019. The study adopted 10 categories of LST to identify the percentage of earth surface that records a particular range of LST and examine the change in the proportion of land over time (Figure 7).

Spatio-temporal variation of Land Surface Temperature (LST) of Bangalore (2001-2019)

According to the results obtained (Figure 5), in both the years the highest percentage of surface area(40.60%: 2001, 44.60%: 2019) experienced LST ranging between 32^{o}C-36^{o}C. Although the same range of temperature is experienced by the maximum proportion of land in both the years, the percentage of the surface area has significantly increased from 40.60% to 44.60% over the years. Moreover, in 2019 an increase in the surface area towards the high temperature ranges has been observed. For instance, the area under 36^{o}C – 40^{o}C temperature range has increased from 9.61% in 2001 to 11.3%. Additionally, in 2001 not a single proportion of land had experienced temperature ranging between 44^{o}C – 48^{o}C whereas, in 2019, 5.30 % of the land was reported under this category. Furthermore, a decline in the percentage of surface area under the categories of low temperature such as 16^o C- 20^o C, 20^{o}C- 24^{o}C and 24^{o}C- 28^{o}C from 0.86, 8.66 and 16.6 to 0, 3.52 and 0.68 is observed respectively. As far as spatial variation is concerned the disappearance of urban lakes has resulted in the decrease of patches of surface area recording low temperatures such as 20^{o}C- 24^o C and 24^{o}C- 28^{o}C. In the center of the city, a dominance of the temperature ranging from 28^o C- 32^o C is observed which has become more prominent over the years. Whereas, the city peripheral areas experience high temperatures and it is increasing over time showing a reverse phenomenon of the Urban Heat Island effect.

Spatio-temporal variation of Land Surface Temperature (LST) of Dehradun (2001-2019)

Dehradun has shown a drastic change in Land Surface Temperature (LST) from 2001 to 2019 (Figure 6).In 2001, the maximum surface area has recorded a temperature ranging from 16^{o}C-20^{o}C whereas, the surface area went down to 3.18 % in 2019.Moreover, the area under low temperature categories including 8^o C- 12^o C, 12^{o}C- 16^{o}C and 16^o C- 20^o C showed an intense declination from 23.27%, 17.79 % and 36.75% in 2001 to 0% and 3.18 % respectively in 2019. On the other hand, a tremendous increase in surface area under categories of high temperature is reported. In 2001, the surface areas under the categories of 20^{o}C- 24^{o}C, 24^{o}C- 28^{o}C was only 20.43% and 1.66 % which has dramatically increased to 29.33% to 28.5% respectively in 2019.Moreover, in 2001 no surface area was reported under the temperature ranging from 28^o C- 32^o C and 32^o C 36^{o}C. But, in 2019 around 38.99% of surface area has experienced LST ranging from 28^o C-36^{o}C. Spatially, it was observed that the southern region of Dehradun has experienced high LST compare to its northern hilly areas. The increasing conversion of forest area into human settlement and built up area in southern foothills is responsible for rising LST. Moreover, a transformation of a large number of forest areas into barren lands, current fallow and sand is another reason for increasing LST, particularly in northern hilly regions.

CONCLUSION

The research study explores the ongoing phenomena of climatology, the UHI effects in elevated topography: plateau and mountainous region. The findings suggested that from 2001 to 2019 a vast amount of heat sink landscapes (forest, agriculture, and water body) got transformed into heat source landscapes (built up area, current fallow land, barren land, sand). Thus, this alteration in land use and cover patterns has intensified the UHI effect and LST in the study area. The research study indicates the presence of the UHI effect in the study area and its tendency to exacerbate over time. Spatially, peripheral areas of Bangalore reported higher temperatures compared to the city center which indicates the presence of reverse UHI effect. On the other hand, in Dehradun, the southern region showed high LST values compare to northern areas due to the presence of compact human settlement towards the south. Thus, all these findings have various policy and managerial implications which will assist the policy-makers to formulate effective land use planning and minimize the UHI effect in the region. Therefore, the study is an example to depict the existence of the UHI effect in mountainous areas as well as plateau areas. Moreover, it highlights the utilization of remote sensing imageries with geographic information science (GIS) methods to explore the spatio-temporal dynamics of the weather phenomena, scrutinizes its increasing intensity over time and identifies the areas with higher values to priorities those areas during city planning or land use planning. The study can be further extended to evaluate different other factors responsible for the current UHI dynamic in the study area and its correlation with the land use pattern.

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Table No.1 Data used for the research study.

S.No.	STUDY AREA	SATELLITE IMAGERIES	SPATIAL RESOLU-TION	LULC	LST	PATH AND ROW
1.	BANGALORE	27-03-2001 (Landsat5 TM)	30M	**Band 3(RED) Band 4(NIR) Band 2(GREEN)**	**Band 6 (Thermal Band)**	**Path : 144 Row : 51**
		13-03-2019 (Landsat8 OLI)	10M	**Band 4(RED) Band 5(NIR) Band 3 (GREEN)**	**Band 10 (Thermal Band) Raster Calculator: Band 5 (NIR) Band 4 (RED)**	**Path : 146 Row : 39**
2.	DEHRADUN	01-03-2001 (Landsat 7 ETM+)	15M	**Band 3(RED) Band 4(NIR) Band 2 (GREEN)**	**Band 6 (Thermal Band)**	**Path : 144 Row : 51**
		28-04-2019 (Landsat 8 OLI)	10M	**Band 4(RED) Band 5(NIR) Band 3(GREEN)**	**Band 10 (Thermal Band) Raster Calculator: Band 5 (NIR) Band 4 (RED)**	**Path : 146 Row : 39**

Table No. 2 Land use land cover of Bangalore.

SL. NO.	LAND USE LAND COVER CLASSES	2001 (in %)	2019 (in %)
1	Agriculture	15.8	11.1
2	Barren	6.1	4.4
3	Current fallow	57.2	50.1
4	Sand	0.0	0.0
5	Built Up	11.8	22.0
6	Forest	7.6	11.3
7	Water body	1.5	1.1

Source: Author

Table No. 3 Land Use Land Cover Matrix of Bangalore.

2019 / ***2001***	Agriculture	Barren Land	Current Fallow	Sand	Built Up	Forest	Water Body	Total
Agriculture	82.99	8.83	162.72	0	44.38	39.98	6.63	345.99
Barren Land	7.01	28.93	32.13	0	43.67	21.47	0.17	133.61
Current Fallow	118.22	32.81	822.32	0	229.02	43.76	1.54	1249.62
Sand	0	0	0	0	0	0	0	0
Built Up	16.26	11.11	50.47	0	131.95	47.75	0.71	258.4
Forest	13.06	12.24	19.04	0	30.8	89.69	0.98	166.3
Water Body	3.05	2.26	7.56	0	1.5	3.47	13.9	31.82
Total	241.68	96.23	1094.42	0	481.34	247.18	24.96	2185.84

Source: Author

Note: The values represent area in Km^2

Table No. 4 Land use land cover of Dehradun.

SL. NO.	LAND USE LAND COVER CLASSES	2001 (in %)	2019 (in %)
1	Agriculture	5.7	7.1
2	Barren	9.8	11.4
3	Current fallow	20.2	19.9
4	Sand	3.4	4.3
5	Built Up	3.5	4.2
6	Vegetation	57.1	52.7
7	Water body	0.5	0.5

Source: Author

Table No. 5 Land Use Land Cover Matrix of Dehradun.

2019 / ***2001***	Agriculture	Barren Land	Current Fallow	Sand	Built Up	Forest	Water Body	Total
Agriculture	95.0	4.6	36.83	4.51	19.52	15.98	0.46	177.0
Barren Land	8.5	39.7	228.23	6.71	7.7	13.2	0.8	304.9
Current Fallow	68.2	144.6	276.94	25.27	24.13	89.7	1.11	630.0
Sand	7.9	2.8	20.85	51.26	7.24	11.22	3.82	105.2
Built Up	13.0	8.0	6.4	4.39	58.49	8.03	0.1	108.5
Forest	28.3	156.4	41.07	39.91	13.78	1502.85	2.99	1785.3
Water Body	0.5	0.7	0.36	1.27	0.38	7.35	4.77	15.4
Total	221.73	357.14	620.71	133.34	131.26	1648.37	14.08	3126.6

Source: Author, Note: The values represent area in Km^2

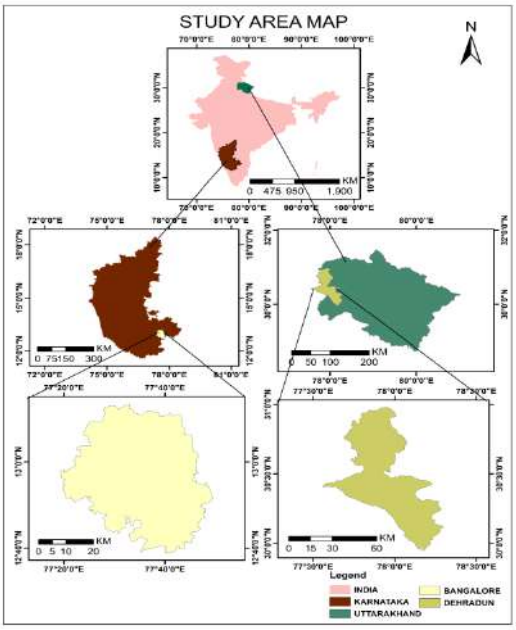

Figure 1. Study area map

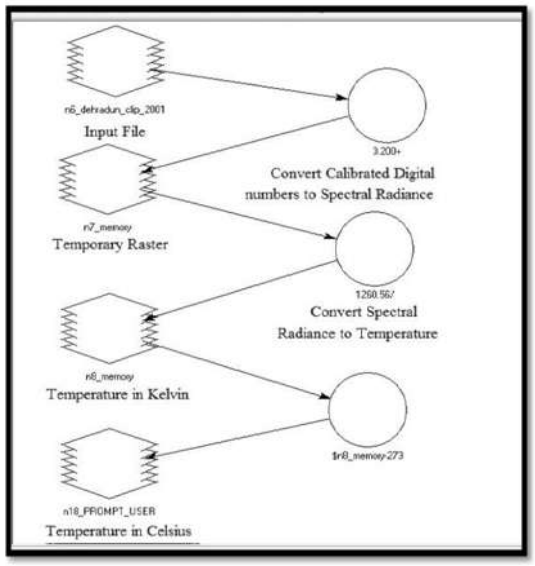

Figure 2. Model Builder for LST calculation

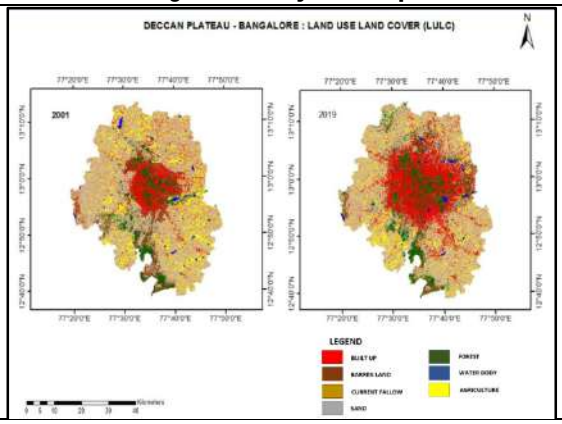

Figure 3. Land Use Land Cover Map of Bangalore (2001 and 2019). Source: Author

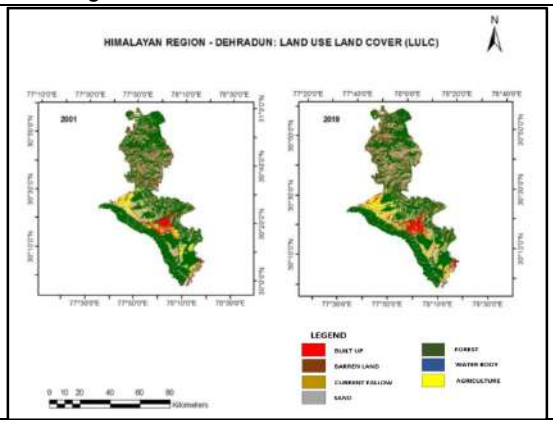

Figure 4. Land Use Land Cover Map of Dehradun (2001 and 2019). Source: Author

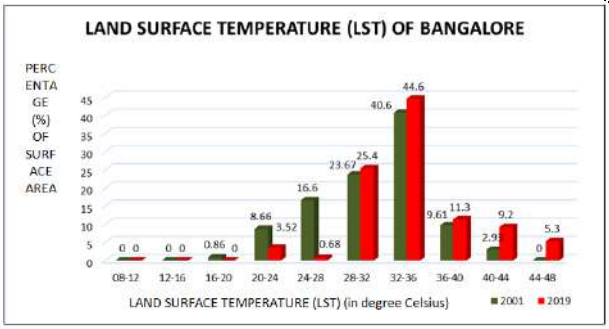

Figure 5. Percentage of surface area under different each category of LST (Bangalore). Source: Author

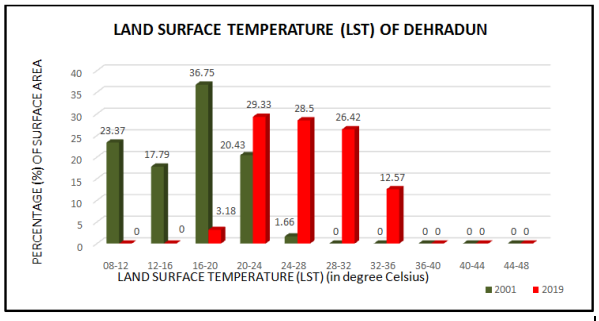

Figure 6. Percentage of surface area under different each categories of LST (Dehradun). Source: Author

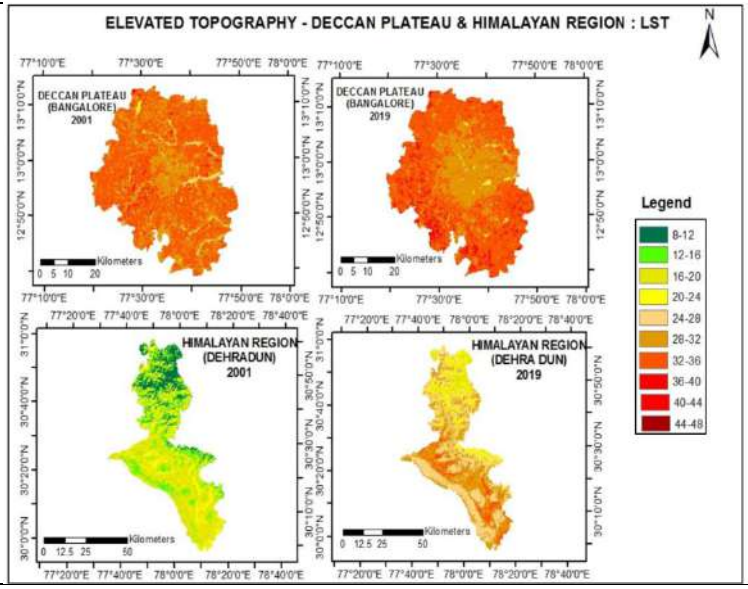

Figure 7. Map showing Land Surface Temperature of Bangalore and Dehradun (2001-2019). Source: Author

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RESEARCH ARTICLE

Cytotoxic Flavonoid from the Leaves of *Premna corymbosa* against MCF 7 Breast Cancer Cell

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ABSTRACT

The present study aimed to correlate the cytotoxic effect of isolated flavonoid compound from the leaves of *Premna corymbosa* against breast cancer cell lines MCF-7. During the study nearly 50% inhibition of viability of IC50 value 13.4 was noted in the 20 µL concentration of isolated compound flavonoid and tested human breast cancer cell MCF-7 exhibited maximum apoptotic cells of 82 ± 13%, and apoptosis or programmed cell death is recognized by a characteristic pattern of morphological, biochemical and molecular changes occurring in a cell. The isolated 100 flavonoid compound against human breast cancer cell MCF-7 significantly reduced the DNA content, making them appear in the sub-G0/G1 phase of apoptosis, with consequent loss of cells in the G1 phase (126.3 ± 1.52%). In RT PCR examination, cells mRNA expression increased more than twofold than the control and this suggesting cell cycle arrest. The range of fold regulation varied widely with ADP-ribosyltransferase (ADPRTL1) exhibiting the maximum up-regulation (29.25-fold) and Hyaluronan-mediated motility receptor (RHAMM) exhibiting the maximum down regulation (−15.41-fold). The present study may provide a pavement to use this isolated flavonoid from leaves of *P. corymbosa* as a source for drug designing against breast cancer.

Keywords: *Premna corymbosa*; bioactive compound; flavonoid; anticancer activity; breast cancer; MCF-7

INTRODUCTION

Natural products with supreme miscellany and functionality remain to instigate unique findings in both physiology and medication. Plants contain diverse types of bioactive compounds, phytochemicals that are proven effective in protecting life and environment, lowering the risks posed by the consequences of urbanization (Bernhoft, 2010). Among the important application of the phytochemicals, their contribution in the fields of food and medicine are noteworthy (Traka and Mithen, 2011). These naturally aborigine products, in course of evolution have transformed into molecules similar to drugs (Newman and Cragg 2016). The antiquity of the usage of phytochemicals as therapeutics dates a very long period back, in time. Until now, most of the global population are dependent upon such folklore therapies accounting for rigorous research activities that intent to lure out the medicinal constituents

for the treatment of various ailments (WHO, 2013). As a consequence, a voluminous number of conventional drugs, ingrained on folk medicinal products are under usage globally (Patra *et al*., 2020). In addition to their ability to cause no side effects, the abundance of availability, cultivability and economical production of plant based therapeutics are increasing their demand. Amid various life threatening diseases, cancer is the most widespread among all classes of people disregard of their age and demography (Kapinova *et al*., 2017). Recent technological advancements have shed more light on the origin and development of cancer at molecular level, helping us to phase a step up towards their treatment. Breast cancer (BC) or mammary carcinogenesis, is the most common type of cancer, instigating the most frequent cases, has attained an epidemic status (Uramova *et al*., 2018). Despite the fact that inborn genetic components are the pivotal reason for the development of BC, epigenetic factors such as changes in lifestyle and undesirable environmental modifications are also evolving as additional carcinogenic factors (Golubnitschaja *et al*., 2016). While chemo and endocrinal therapies are successful only in primary stages, surgical removal is the only effective treatment available for latter phases of BC. However, emerging young asymptomatic patient cohorts are posing serious challenges in managing BC. With the multipole increase in the rapid incidence of BC, management strategies are now focusing on rigorous and novel screening platforms, prognostic diagnosis, effective targeted anticipation, besides personalized treatments (Hortobagyi, 2002). One such approach suggests the involvement of phytochemicals in diet, for prevention or as medicines as treatment, against BC and other types of cancers (Abbasi *et al*., 2018).

The effectiveness of anti-oncogenic properties of plant based diet relies exclusively on the synergistic action of two or more phytochemicals rather than a single compound. These compounds are usually secondary metabolites that are synthesised by plants to mitigate biotic and abiotic stress (Kennedy and Wightman, 2011). Diverse phytochemicals are being reported with pro-apoptotic, anti-metastatic, anti-proliferative besides anti-angiogenic capabilities against BC (Kapinova *et al*., 2017). Phytochemicals such as curcumin, phycocyanin and quercetin were reported with anti-cancer potentials in BC cell lines (Ouhtit *et al*., 2013). Other noteworthy phyto-compounds with proven anti-oncogenic effects against BC include folate (Chen *et al.,* 2014), flavonoids and flavones (Hui *et a*l. 2013), Resveratrol (Zhu *et al*., 2012) besides Epigallocatechin-3-gallate (Zhang *et al*., 2012). Additionally several plant alkaloids such as paclitaxel,vincrstine and vinblastine, along with saponins such as pfaffosides A–G were also observed with anti BC capabilities (Levitsky and Dembitsky, 2015). Botanical compounds with cancer prevention and treatment properties are widely used in chemoprevention therapies against breast cancer. Such types of treatments are being standardised, and also economical besides being a readily pertinent approach to manage BC. *Premna corymbosa* Rottl. (Verbenaceae), is a large aboriginal shrub found in several parts of India and Bangladesh, encircling an extensive range of pharmacological as well as therapeutical possessions (Karthikeyan *et al*., 2011). It has been a part of traditional medicine involving the usage as whole plants or parts such as roots, bark, stem and foliage for the treatment of rheumatism, fever, cough, haemorrhoids and also tumors. Foliage of the plants is utilized to reconcile limb weakness, relieve headache and also to treat diabetes (Radhika *et al*., 2014). Several researches have also proved the radical scavenging potential of the plant (Chua *et al.,* 2015). Several bioactive compounds such as sterols, resin, tannins and botulin have been identified from *P. corymbosa* (Med, 2016). However there is only limited information pertaining to the bioactive compounds and their capabilities towards their applicability in the treatment of cancer. To this end, the current research was entitled to explore the anti-cancer potentials bioactive compound isolated from the leaves of *P. corymbosa* and to be developed for targeted use for the management of breast cancer.

MATERIALS AND METHODS

Extraction of Flavonoid

The leaves of *Premna corymbosa* ,was obtained from the Siddha garden at the Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, TamilNadu, India. Detailed methodology of extraction of flavonoid was already elaborated in the previous publication Immaculate and Umarani (2015).

Cell Line and Culture

MCF-7 cell lines were obtained from the National Centre for Cell Science (NCCS), Pune. These cells were kept in Minimal Essential Media supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cell lines were maintained in a 5% moistened CO2 incubator at a temperature of 37°C. The cells were cultured in a sterilized condition, and exponentially growing cells were used for experimental studies. All the reagents were purchased from HiMedia and Sigma Aldrich, Mumbai.

Isolation of DNA

DNA was isolated by the standard phenol chloroform method (Sambrook *et al*., 1989). The pellet was suspended in 0.5 mL of lysis buffer (10 mM Tris (Sigma Chem. Co, USA), 25 mM Ethylene diamine tetra acetic acid (EDTA) (Serva), 0.5% Sodium dodecyl sulphate (SDS) (Serva) pH 8.0, 100 mM Sodium chloride (NaCl), and 20 µL Proteinase K (20mg/mL) (Merck, Germany) and incubated for 3 hours at 56°C. The lysate was cooled to room temperature and subjected to RNase (10 µg/mL) treatment for 1 hour at 37°C. Subsequently, an equal volume of Tris saturated phenol, chloroform and isoamyl alcohol in the ratio of 25:24:1 was added. The contents were then centrifuged at 11000 x g for 15 minutes at 4°C. The aqueous phase containing DNA was transferred carefully to a fresh micro-centrifuge tube. To this, 1/10thvolume of 3M sodium acetate and two volumes of ice cold 100% ethanol was added. The contents were mixed gently by repeated inversion of the tube. DNA precipitation was enhanced by storing overnight at -20°C. DNA was pelleted and centrifuged at 11,000 x g for10 minutes, and washed twice with 70% ethanol and allowed to air dry to remove the ethanolcontent. The pellet was resuspended in 50 µL of the Tris (TE) buffer and stored at -20°C.

Cell Cycle Analysis by Flow Cytometry

Cell cycle analysis helped to measure the DNA content of the cells by flow cytometry using propidium iodide (PI). The cells were treated with 20 µg/mL of isolated compound for 48 hrs. After which, the adherent cells were harvested by trypsinization. The cells were centrifuged at 2000 x g for 5 minutes at room temperature and washed twice with Hank's balanced salt solution (HBSS). Finally, the cells were resuspended in 400 µL of HBSS. RNaseA (100 µg/mL) was added to the cells and incubated at 56°C for 3 hours. Finally, propidiumiodide (PI) (20 µg/mL) was added and incubated at room temperature for 15 minutes. After incubation, the DNA content was analyzed by using flow cytometry (MoFlo, Beckman Coulter, USA).

DNA Fragmentation Assay

MCF-7 cells were treated with 20 µg/mL of isolated compound for 48 hours (Gavrieli, 1992). At the end of treatment, both adherent cells and floating cells were harvested and left for 1 hour with 300 µL of lysis buffer cocktail (10% NP-40 + 200 mM Ethylene diamine tetra acetic acid (EDTA) + 0.2 M Tris-Hyrochloric acid (HCl) + 0.50 mg/mL proteinase K) and then incubated at 60 °C for 1 hour with RNAse (100 µg/mL). At the end of the process, samples were mixed with loading dye (xylene cyanol in 30% glycerol) and resolved in agarose gel (1.50% Agarose in 1X TBE buffer). Electrophoresis was carried out at 60 V for 90 minutes using 1X Tris Borate EDTA (TBE) buffer.

Isolation of RNA

To the cell pellet, 1 mL of the Trizol reagent was added, homogenized and was stored at room temperature for 5 minutes to permit complete dissociation of nucleoprotein complexes. To this, 0.2 mL of chloroform/mL of Trizol reagent was added, vortexed vigorously for 1 minute and centrifuged at 11000 x g for 15 minutes at 4°C. After centrifugation, the top clear aqueous phase containing RNA was transferred carefully to a fresh micro-centrifuge tube without disturbing the interphase. To the aqueous phase, an equal volume of isopropanol was added and centrifuged at 11000 x g at 4°C for 10 minutes. RNA was formed as a white pellet at the bottom of the tube. The supernatant was removed, and the pellet was washed twice with 70% ethanol. At the end of the experiment, the pellet was dried under vacuum centrifuge for 2 minutes. Care was taken not to allow the RNA pellet to dry completely, as it will greatly decrease its solubility. The RNA pellet was then suspended in 30 µL of nuclease-free

water and placed in a water bath for 10 minutes at 60°C to ensure complete solubility and then the RNA sample was stored at -80°C (Chomczynski and Sacchi 1987).

Quantification of RNA

RNA was quantified spectrophotometrically by measuring the absorbance (A) at 260 nm (Fourney *et al.*, 1988). An absorbance of 1 OD is 73 equivalent to RNA concentration of 40 μL/mL. Therefore, the yield can be calculated by multiplying the absorbance of the sample at 260 nm and 280 nm. A ratio of absorbance at 260/280 nm is generally considered as good quality of RNA (>1.8).

RNA Quality Assessment using Bioanalyzer

The integrity of the RNA is checked using 2100 Bioanalyzer instrument (Agilent Technologies). Micro-fluidics were used to analyze RNA sample-specific chips. 1 μL of the sample was mixed with a fluorescent dye and injected into the wells in the chip. When an electric field was applied, the samples move through a gel matrix in the micro-channels and are separated by electrophoresis. The quality of the RNA is measured using RNA integrity number (RIN). The RIN scale ranges from 0 to 10, with 10 being the maximum RNA integrity.

Real Time PCR (RT-PCR/qPCR)

cDNA Synthesis: The total RNA (1000ng) content was converted into cDNA using Affinity Script qPCR cDNA synthesis kit (Agilent Technologies, USA) as per the manufacturer`s protocol.

qPCR: The expression levels of the selected genes were analyzed using SYBR Green chemistry (Brilliant II SYBR Green qPCR master mix (Agilent Technologies, USA)) in Stratagenem x 3005P instrument (Agilent Technologies, USA).

Relative Quantification Analysis: The genes was relatively quantified using the standard 2^- ΔΔCt method as described by Pfaffl, (2001). The GAPDH gene was used as the reference gene to normalize the qPCR experiment.

In Vitro Assay for Cytotoxicity Activity

MTT assay helped to determine the anticancer activity of the samples on MCF-7. Cells were transferred to a 96-well plate at about 1 × 105 cells per well in 0.2 mL of medium. For the next 72 hours, cell plates were incubated in a 5% CO2 incubator. The isolated compound was added in varied concentrations in 0.1% DMSO (Dimethyl sulphoxide) andplaced in a 5% CO2 incubator for 24 hours. The sample solution was removed and 0.5% 3- (4,5-dimethyl-2-thiazolyl) - 2,5-diphenyl-tetrazolium bromide (MTT) in phosphate buffered saline was added at 20 μL/well (5mg/mL). After four hours of incubation, 1 mL of Dimethyl sulfoxide (DMSO) was added. The viable cells were obtained, and the absorbance was read at 540 nm. The Inhibitory Concentration (IC50) values of the compound was determined through a graph where increase in concentration reduced the cell viability percentage by 50%.
The percentage (%) of cell viability was calculated by the following formula:
Percentage of cell viability = A540 of treated cells /A540 of control cells × 100%

Apoptosis Detection

The 6-well tissue culture plate seeded with 4 x 10^5 cells was treated for 24 hours with the isolated compound at concentrations of 5, 10 15 and 20 μg/mL corresponding to their Inhibitory Concentration (IC50) standards for 48 hours. After 48 hours, through trypsinization, the adherent cells were harvested and pooled with floating cells. The cells were collected and centrifuged at 2000 rpm for 5 minutes at room temperature. The cell pellet was washed twice with Hank's balanced salt solution (HBSS). After washing the cell pellet, the cells were stained with Acridine Orange (AO)- Ethidium Bromide (EB) (2 μg/mL) mixture and was incubated for 10 minutes in a CO_2 incubator at 37°C. Then, the cells were placed on a glass slide with a cover slip and examined under a fluorescent microscope (Leica, Germany).

Cell Treatment Procedure for Isolation of DNA/RNA

MCF-7 cell Lines were seeded at a density of 2 x 10^6 in a T25 flask supplemented with Dulbecco's Modified Eagle Medium with 10% fetal calf serum and incubated overnight for cell attachment. Cells were then treated with 5, 10, 15 and 20 μg/mL of the isolated compound for 48 hours. Untreated control cells (50 μL of 100% DMSO) were included. At the end of the treatment, cells were trypsinized and collected after centrifugation at 1000 x g for 10 minutes. Subsequently, the cells were washed twice with phosphate buffer.and the cell pellet were proceeded further to isolate the DNA/RNA.

RESULT AND DISCUSSION

Medicinal and healing properties of plants are strongly interconnected to their chemical constituents and they are categorized into groups like alkaloids, polyphenols, flavonoids, anthocyanins, saponins, tannins etc. Among these, phenolics are broadly spread in the plant kingdom and are the richest phytomolecules of plants. Apart from being frequently associated with anti-oxidative properties, the presence of phenolic compounds in plants has been attributed to a number of significant pharmacological activities, for instance as a cancer cell growth and development inhibitor (Nurul *et al*., 2016). Thus, the present study made such attempt to screening anticancer activity against breast cancer cell lines using flavonoid compound which was isolated from the leaves of *P. corymbosa. P.corymbosa*is a huge shrub or a small tree found in India. The roots of the plant is used to treat cardiac disorders, neuralgia, asthma, bronchitis, inflammations, skin diseases, leprosy, diabetes and general disability (Karthikeyan and Deepa, 2010). Very less phytochemical and biological studies on leaves have been performed (Brankovic *et al*., 2011).

In Vitro assay for Cytotoxicity of isolated Compound on Breast Cancer Cell Lines

MCF-7

The cytotoxic effect of isolated flavonoid compound from the leaves of *P. corymbosa* was treated against breast cancer cell lines MCF-7. This was determined by a rapid colorimetric method using MTT (Methyl-thiazolyl-tetrazolium bromide) assay. The OD values were recorded and the concentration required for 50 percent inhibition of viability (IC50 value 13.4) was determined graphically (Fig. 1 and 2). The effect of the samples on the proliferation of MCF-7 cells was expressed as the percentage cell viability. The results indicated that 16% cell viability was observed against human breast cancer cell line MCF-7 in 20 μL concentration of isolated compound flavonoid from the leaves of *P. corymbosa*.

Hymavathi *et al*. (2009) compared the cytotoxic activity of isolated compound of *Premna tomentosa*, against A-549 (lung cancer), HT-29 (colon cancer) and MCF-7 (breast cancer) cell lines were exposed to a series of concentrations of either extract or isolated compounds for 48 hours and the percentage of viability was determined by MTT method with IC50 value of 204.7 and 129.3 μg/mL. The present study was also showed that the isolated flavonoid compound of *P. Corymbosa* indicated higher proliferation inhibition effect on MCF-7 cancer cells. Results from this study defer that isolated flavonoid compound of *P.corymbosa* possess stronger anticancer activity, which is in assurance with the finding of Kejuan (2016) who discovered that flavonoid extracts from medicinal plants were effective against cancer.

Effect of Isolated Compound on Human Breast Cancer Cell Line MCF-7 Inducing Apoptosis

To explore anticancer potential of isolated flavonoid compound from the leaves of *P. corymbosa*, human breast cancer cell line MCF-7was treated with different concentrations (5, 10, 15, 20 μg/mL) of flavonoid compound. After 24 hours of treatment, the apoptotic cell was induced by flavonoid compound. It was identified by typical nuclear condensation visualized with ethidium bromide staining. Fig. 3 showed a dose dependent pattern of flavonoid compound which induced apoptotic cell death in human breast cancer cell line. After treatment with 5, 10, 15 and 20 μg/mL concentrations of flavonoid compound, the human breast cancer cell MCF-7 exhibited maximum apoptotic cells of 83.13%, and apoptosis or programmed cell death is recognized by characteristic pattern of

morphological,biochemical and molecular changes occurring in a cell (Fig. 4). Aranya *et al*. (2016) emphasized that these compound may have other complementary and overlying mechanisms as well as regulation of gene expression in cell proliferation, induction of cell-cycle arrest and antioxidative activities. These results reveal that isolated flavonoid compound of *P. corymbosa* induced apoptosis in cells, which is further supported by an increase in the number of apoptotic bodies. Chen *etal*. (2009), stated that gallic acid, a major component of *Tenodera sinensis* leaf extracts, inhibited the growth of DU145 human PCA cells by activating pre existing apoptotic processes and cell cycle arrest machinery. Thus phenolics, such as epicatechin, luteolin and rutin have cytotoxicity against human cancer according to literature (De la Rosa *et al.,* 2014).

Effect of Isolated Flavonoid Compound on Cell Cycle Control of Human Breast Cancer Cell MCF-7

To examine whether the isolated flavonoid compound of *P. corymbosa* has affected cell cycle regulation, flow cytometry was executed. Incubation of isolated flavonoid compound of *P. corymbosa* with human breast cancer cell MCF-7 for 24 hours significantly reduced the DNA content, making them appear in the sub-G0/G1 phase of apoptosis, with consequent loss of cells in the G1 phase (126.3 ± 1.52%). After treatment with 20 μg/mL of isolated flavonoid compound of *P. corymbosa* for 48 hours, the apoptotic cancer cells decreased to 92.66 ± 2.08% significantly (Fig. 5). Specifically, the cell population in G0/G1 phase increased, comparatively in S and G2/M phases decreased ($P < 0.05$), suggesting a growth arrestin the G0/G1 phase of the cell cycle. Cells treated by the drug undergo inhibited spindle formation resulting in mitotic arrest and cell death. Flow cytometric analysis of isolated flavonoid compound of *P. corymbosa* on treated cell cycle exhibited increase in sub G0/G1 phase and decrease of cells at S phase in concentration dependent manner indicating induction of apoptosis and inhibition of DNA synthesis in S phase. Apoptotic cell death has typical characteristics, including chromatin condensation, membrane leakage, cell shrinkage and an increased population of sub-G1 phase hypodiploid cells (Yang *et al*., 2007). Flow cytometry was used to measure the induction of apoptosis and cell cycle distribution. The isolated flavonoid compound of *P. corymbosa* increased the proportion of the sub-G1 fraction (M1) cells in a time-dependent manner.

DNA Fragmentation Analysis of Isolated Flavonoid Compound on Human Breast Cancer Cell MCF-7

Deprivation of genomic DNA, due to activation of endogenous endonucleases, is one of the early events of apoptosis. For this reason it was tested for the ability of isolated flavonoid compound of *P.corymbosa* to induce apoptosis in whole cells by DNA fragmentation. Human breast cancer cell MCF-7 (inoculated at 105 cells/ dish) were exposed for 24 hours and 48 hours to isolated flavonoid compound of *P. corymbosa* at varying concentration (0,10,15,20 μg/mL). After exposing for 24 hours the cells showed marked DNA fragmentation pattern, which was not observed in untreated cells (Fig. 6). Although a typical DNA ladder due to the release of oligonucleosome associated DNA fragments was not observed, after 48 hours of incubation with isolated flavonoid compound of *P. corymbosa* a clear disintegration of genomic DNA with high molecular weight fragments was obtained. According to Kejuan *et al.* (2016), DNA fragmentation is one of the hallmarks of apoptotic cell death that is induced by most anticancer agents. *Avicennia marina*, the most abundant and common mangrove species used as a traditional medicine has been found to have its leaf extracts exerting cytotoxic effects on human cancer cell lines via apoptosis due the presence of high phenolic and flavonoid contents (Huang *et al.,* 2016).

Effect of Isolated Flavonoid Compound on mRNA Expressions of Human Breast Cancer Cells MCF-7

The mRNA expressions were determined by semi-quantitative RT-PCR. MCF -7 cells were treated for 16 hours with isolated flavonoid compound of *P. corymbosa* at 0 (control), 20 μg/mL. mRNA expression level was normalized to β-actin. For one experiment, 3 assays were carried out and only one set of gel is shown. The density of the band (normalized to β-actin) shown as mean ± SD is relative to that of the control (designated as 1.00). Statistical analysis using the ANOVA shows significant decrease in the mRNA expressions were in the isolated flavonoid compound of

P. corymbosa (n = 3) *, $P < 0.05$, significant difference. The results of RT-PCR revealed that the isolated compound of *P. corymbosa* on treated MCF-7cells mRNA expression increased more than two fold than the control, indicating cell cycle arrest. (Fig. 7).

Confirmation of the Representative Genes by RT-PCR Change of Gene Expression Profile

A powerful tool used to evaluate changes in gene expression is the reverse transcription quantitative-polymerase chain reaction (RT-qPCR) technique. Features including great accuracy, high sensitivity, reproducibility and high-throughput make RT-qPCR the most prevalent technique to assess mRNA expression (Boik, 2001). In this study, 0ut of 3360 genes studied, 170 differential genes were identified from oligonucleotide microarray in which gene expression was increased or decreased more than 2-fold in cells treated with 20 μg/mL of isolated flavonoid compound of *P. corymbosa* compared with control human breast cancer cell MCF-7 cultured under identical condition without treatment; 41 (1.22%) were up-regulated (Table 1) and 129 (3.84%) were downregulated (Table 2).Upregulated expression has been reported in cancer, vascular diseases and many different types of inflammatory diseases (Fanjul-Fernandez *et al.,* 2010) and down-regulated expression was reported in breast cancers (Freitas *et al.,* 2013) and gastric carcinoma carcinoma (Huang *et al*., 2018). Several research studies have been focused on the usage of traditional medicinal plants in the form of herbal extracts to treat specific diseases including cancer, and an effort to discover new therapeutic agents that lack the toxic side effects related to current chemotherapeutic agents (Adebayo *et al*., 2010). Prasad *et al*. (2007) studied the mechanism of the 'aflavins' action on cellular proliferation and cell death in the human prostate cancer cell line PC-3. They found that flavonoid compound aflavins, act as anti-proliferative agent by modulating cell growth regulators in prostate cancer cells.

CONCLUSION

Traditional Indian have been used in the treatment of different diseases in the country for centuries. There have been claims that some traditional healers can successfully treat cancer using herbal drugs. In this study, it is evident that the isolated flavonoid fraction of *P. corymbosa* possesses effective anticancer activities.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1 List of genes up-regulated more than 3-fold by isolated flavonoid compound of *P. corymbosa* in Human breast cancer cells MCF-7

Gene description	UniGene Symbol	UniGene ID	Fold increase
			Isolated flavonoid compound
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	Hs.179665	**3.02**
Superoxide dismutase 3 Extracellular	SOD3	Hs.2420	**3.13**
Cytochrome P450, subfamily I (aromatic compoundinducible), polypeptide 1	CYP1A1	Hs.72912	**7.07**
Insulin-like growth factor binding protein 6	IGFBP6	Hs.274313	**3.63**
Alanyl-tRNA synthetase	AARS	Hs.75102	**3.02**
Sterol regulatory element binding transcription factor 1	SREBF1	Hs.166	**3.16**
ATP synthase, H+ transporting, mitochondrial F1F0, subunit d	ATP5JD	Hs.64593	**3.12**
ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase) -like 1	**ADPRTL1**	**Hs.77225**	**27.25**

Table 2 List of genes down-regulated more than 3-fold by isolated flavonoid compound of *P. corymbosa* in Human breast cancer cells MCF-7

Gene description	UniGene Symbol	Uni Gene ID	Fold increase
			Isolated flavonoid compound
Gardner–Rasheed feline sarcoma viral (v-fgr) oncogene homolog	FGR	Hs.1422	**–5.06**
Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	PPP2CA	Hs.91773	**–3.12**
Transcription factor 12 (HTF4, helix loop–helix transcription factors 4)	TCF12	Hs.21704	**–3.25**
Interleukin 3 (colony stimulating factor, multiple)	IL3	Hs.694	**–6.31**
U4/U6-associated RNAs plicing factor	HPRP3P	Hs.11776	**–5.19**
General transcription factor IIA, 2 (12 kDa subunit)	GTF2A2	Hs.76362	**–6.31**
RNA binding motif, single stranded nteracting protein 1	RBMS1	Hs.241567	**–3.72**
Hyaluronan-mediated motility eceptor (RHAMM)A disintegrin and metalloproteinase domain 9 (meltrin gamma)	HMMR	Hs.72550	**–15.41**
ATP-binding cassette, subfamily E (OABP), member 1	ABCE1	Hs.12013	**–4.38**
A disintegrin and metalloproteinase domain 9 (meltrin gamma)	**ADAM9**	**Hs.2442**	**–5.12**

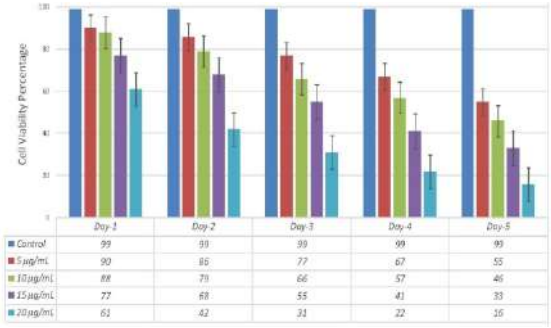

Fig. 1 Effect of flavonoid compound of *P. corymbosa* on cytotoxicity of human breast cancer cell MCF-7

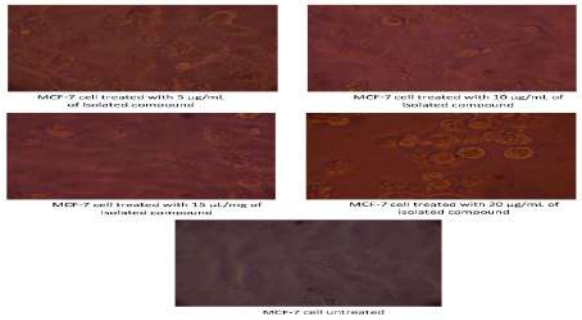

Fig. 2 Effect of cytotoxicity activity of isolated flavonoid compound of P. corymbosaon human breast cancer cell line MCF-7

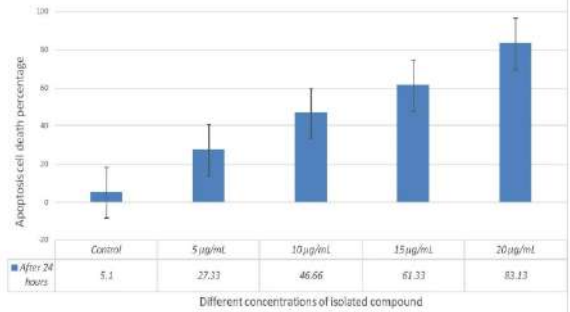

Fig. 3 Effect of isolated flavonoid compound on human breast cancer cell line MCF- 7 induced apoptosis

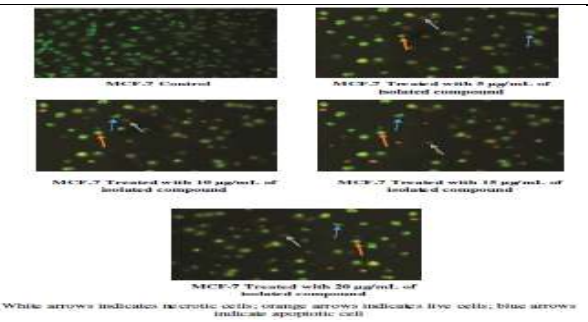

Fig. 4. Effect of flavonoid compound on human breast cancer cell line MCF- 7 induced Apoptosis

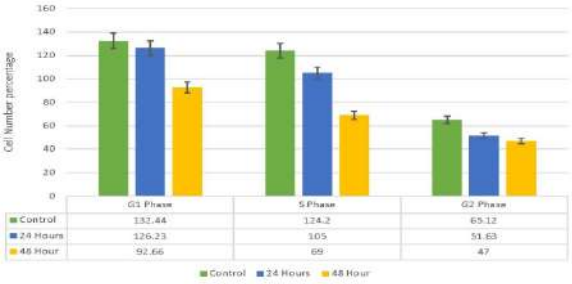

Fig. 5. Effect of flavonoid compound on cell cycle control of human breast cancer cell line MCF- 7

Fig. 6 Effect of flavonoid compound on human breast cancer cell line MCF- 7 of DNA fragmentation

Fig. 7 RT PCR detection

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RESEARCH ARTICLE

Common Indian Spices with Potential Anti-Diabetic Activity

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ABSTRACT

Plants and herbs have always been the sources of drugs in the treatment of various ailments. The Indian system of medicines is widely used for several years ago. This system involved the plants and its parts for the preparation of medicines. Diabetes is a serious condition that affects the production and uses of blood sugar (glucose) in the body. It is the major health problem in India. People with diabetes have increase risk of cardiac attack, Cerebrovascular disease. There are a variety of ways to treating diabetes, but the usage of herbal plants is favored since it has fewer side effects and is less expensive. Spices are commonly used in everyday life as food, and they are also employed in the treatment of diabetes due to their hypoglycemic properties. Many spices are also utilized in the treatment of various diseases as a source of vitamins and minerals. Spices are utilized in the therapy of diabetes mellitus as a functional food and adjuvant. The review mainly focused on the common Indian spices which are used in the management of diabetes.

Keywords: Herbs, spices, type of diabetes mellitus, Treatment.

INTRODUCTION

Diabetes mellitus could be a metabolic disease that influences insulin generation and activity within the body [1]. According to World Health Organization (WHO), Diabetes mellitus is mostly occurs in the developing countries. In 2020, the international Diabetes Federation (IDF) have reported that 463 million people have diabetes around the globe; 88 million of people in the Southeast Asia. Out of this 88 million, 77 million belong to India. The factors involved in the etiology of diabetes mellitus include genetics, environmental factors, lifestyle changes, obesity, age and other metabolic syndrome [2]. Hypoglycemia is the main symptoms of diabetes mellitus. It can leads to many complications such as micro vascular (nephropathy, retinopathy) which may also leads to the damage of blood vessels and macro vascular (coronary artery, periphery arteries and stroke) [3, 4].

Types of diabetes mellitus [5]

Type 1 is an autoimmune destruction of β-cells which result in the deficiency of insulin. It is also called as insulin dependent diabetes mellitus where the pancreas fails to produce enough insulin in the body. Therefore, the patients have to totally depend on the source of insulin (e.g., insulin therapy). Type 2 is caused by an inadequate insulin secretion, due to failure of the cells to response to insulin. Excess body weight and physical inactivity also caused type 2 diabetes. It is the most common form of diabetes which results from the unhealthy lifestyle. Therefore, the simple treatment is to engage in exercises and dietary changes.

Herbs and spices are extensively utilized in our everyday life as vital seasonings and flavorings for our meals. Spices are pungent or aromatic substances which derive from different parts of the plants. The seed, leaf, flower, bark, roots, flower and rhizome of some plants are used as additives, flavor and color [6].They are often widely used because of their health benefit properties such as antioxidant, anti-inflammatory, anticancer, anti-diabetic, and antimicrobial, neuroprotective, and cardiovascular impact [7, 8, 9, 10].Many plant spices such as fenugreek, cloves, turmeric and bay leaf have insulin enhancing activity [11].Certain plants possess medicinal properties that can manage the sugar levels in diabetics but they should not be substitutes for medications. Secondary metabolites found in medicinal plants, such as flavonoids, terpenoids, alkaloids, and polysaccharides, have been studied extensively for their anti-diabetic properties. [12, 13] .Plants have historically been an excellent source of drugs and many of the medications available today originate from them directly or indirectly[14].Currently there are a numbers of conventional Antihyperglycemic agents that helps in reducing blood glucose levels and promotes insulin secretion from β-cells or increasing the sensitivity of insulin. However, hypoglycemia, weight gain, and stomach pain have all been documented as adverse effects of these anti-diabetic drugs. As a result, herbal therapy has been utilized to treat diabetes mellitus for a long time [15, 16] .The aim of this study is to determine which Indian herbs and spices are most often utilized in the treatment of diabetes mellitus.

SPICES IN THE TREATMENT OF DIABETES MELLITUS

Herbs and spices have long been thought to be important sources of powerful anti-diabetic medicines. They also play a key part within the drug advancement program within the future are discussed in Table 1

Cinnamon (Sources: *Cinnamomum zeylanicum*; Family*: Lauraceae*)

Cinnamon is one of the most widely used spices on the planet. The US Food and Drug Administration has given it GRAS (Generally Recognized as Safe) status [17] .Cinnamon is commonly known as Dalchini in Hindi. The bioactive compounds found in cinnamon are Cinnamaldehyde, copane, cinnamyl acetate and camphor [18]. Cinnamaldehyde exhibit hypoglycemic activity and also enhances the activity of insulin. Cinnamaldehyde also showed a significant reduction of plasma glucose and hemoglobin (HbA1c) levels in STZ-induces diabetic rats [19]. Administration of cinnamon caused a significant reductions in weight, glucose and lipid levels in alloxan-induced diabetic rats [20].Daily consumption of 1-2grams of cinnamon in the form of tea or use in cooking and baking can help lower blood sugar level [21]. While a small amount of cinnamon might help reduce blood sugar, too much can cause it to

drop dangerously low. Hypoglycemia is the medical term for this condition. It can cause fatigue, dizziness, and even fainting.

Bay leaf (Source: *Cinnamomum tamala;* Family*: Lauraceae*)

In India dried leaves of bay leaf are mostly used as spices and flavors in soup, meat due to its aromatic properties. The leaves are commonly known as Tejpat. The leaves and bark have fragrant, stimulant and carminative properties. They are utilized in Indian framework of medication within the treatment of rheumatism, diarrhea, sickness and vomiting. Dried leaves and bark are moreover utilized for fever and iron deficiency [20].The leaves exhibit hypoglycemic effect due to the present of Phytochemical and essential oils such as quercetin, myrcene and kaempferol [21].Ethanolic extract of C.tamala leaves also restored blood glucose levels to near normal in SZ-induced diabetic rats [22].Diabetics can either use the whole leaves in their soup and curries or consume 1 to 3 grams of leave powder daily to improve insulin functions and regulate blood sugar level.

Black cumin (Source: *Nigella sativa*; Family*: Ranunculaceae*)

The seeds of black cumin are utilized as spice in pickle and curry. It is commonly known as Kalonji. The seeds have moreover been utilized as conventional drug within the treatment of different infections counting diabetes. It consists of Phytochemical and volatile oil such as linoleic acid, palmitic acid and thymoquinone (TQ). The oil of kalonji showed a significant reduction of Fasting Blood Glucose (FBG) and increased the levels of insulin when consumed with oral anti-diabetic drugs [23]. 2g/day of black cumin seeds powder taken with oral antidiabetic drugs improves diabetic control with T2DM [24]. Black cumin benefits the diabetic individual as it reduces appetite which reduces glucose absorption in intestine and consequently reduces blood glucose level. Diabetics can consume 0.5-2grams of black cumin seed powder daily for up to 12 weeks and mix half teaspoon of black seed oil in one cup of back tea to helps normal the glucose level [25].

Curry leaf (Source: *Murraya koenigii;* Family*: Rutaceae*)

Curry leaf is often used as spices due to its aromatic nature. It is commonly known as Curry patta. Murraya koenigii leaves are also utilized in Ayurvedic medicine as an herb. It consists of Cinnamaldehyde, mahanimbine as Phytochemicals constituents. The leaves are rich in minerals which help in maintaining blood sugar level by activating pancreas β-cells [21]. Consumption of curry leaves at an early diabetic state as dietary constituents helps in controlling hyperglycemia. [26] .Treatment with curry leaf also normalizes the body weight in alloxan-induced diabetic rats. [27]. Curry powder also has hypoglycemic properties which the fasting blood glucose levels in diabetic individuals. The leaves can be chew daily on an empty stomach daily and the leaf powder can be added to soup or curry and salads. Anti-diabetic, antioxidant, antibacterial, anti-inflammatory, hepatoprotective, anti-hypocholesterolemic, and other properties are highly valued. They also have iron in them.

Fenugreek (Source: *Trigonella foenum;* Family*: Leguminosae*)

Fenugreek seed is also known as Methi, which is used as spice and food. Fenugreek has also been shown to help reduce diabetic retinopathy and other ocular disorders. Fenugreek contains saponins that get gets converted into sapogenins in the gastrointestinal tract. Diosgenin is an important saponins [28]. The seeds contain soluble fiber (30%) and insoluble fiber (20%), these fibers slow down the rate of postprandial glucose absorption [29]. Including of seeds powder in diet helps reduces blood sugar and urine sugar with significant improvement of glucose tolerance and symptoms in type 2 diabetic patients [30]. The extract of fenugreek seeds also contains 4-hydroxyisoleucine which increased glucose-induced insulin release in human and rat pancreatic islet cells [31]. Seeds are soak overnight in water and drink the water in the next morning where as the seeds can be eaten on an empty stomach. Following this remedy daily will helps in maintaining blood glucose level in type 2 diabetic patients [32]. In alloxanized albino rat models, the seeds' alcoholic and aqueous extracts were found to exhibit anti-hyperglycaemic properties. In alloxan-induced diabetic rats, the seeds' aqueous extract was able to prevent cataract formation. The water soluble component of fenugreek seeds had an anti-diabetic impact by preventing carbohydrate digestion and absorption, resulting in an increase in peripheral insulin activity. In human subjects, raw and germinated fenugreek seeds

showed anti-diabetic benefits, but cooked seeds had no such effect. Regular consumption of the seeds might offer assistance within the management of diabetes beside anticipation of the atherosclerosis and coronary heart infection due to beneficial effects on dyslipidaemia and inhibition of platelet aggregation.

Ginger (Source: *Zingiber officinale*; Family: *Zingiberaceae*)

Ginger is widely cultivated throughout India and the rhizome of the plant is often used as spices in a variety of Indian dishes. The rhizomes of ginger have been utilized traditionally for the treatment of hypertension, diabetes, joint pain, sprain, strong hurts, sore throats, fever, cramps, gingivitis, toothache, asthma and infectious diseases. It is commonly known as Adarak. Dietary ginger has been considered to display hypoglycemic, anti-diabetic, anti-oxidant, hypocholesterolemic and hypolipidemic potential. Ginger contains a number of active components such as gingerols, shogaol and volatile oils including sesquiterpenes such as β-bisabolene and (-)-zingiberene [33]. Gingerol, shogaol, zingerone and paradol are the major components in the rhizome. Gingerol promotes glucose uptake by increasing GLUT4 in cultured L6 myotubes planta Medica [34]. Ethanolic extract of ginger has been reported to reduce the levels of blood sugar and also restored total cholesterol and total protein in kidney tissue in STZ-induced diabetic rats [35]. When combine with other herbs, ginger shown a significant reduction of body weight, serum triglyceride and cholesterol in diabetic patients [36].Daily consumption of 2-3 cups of ginger tea daily proved to be useful for diabetic patients. According to these research, ginger's anti-diabetic properties are due to its restorative actions on pancreatic -cells, which increase insulin sensitivity, action, and peripheral glucose consumption. Increased hepatic glycogen synthesis via enhanced glycogen regulating enzyme expression in the liver, inhibition of carbohydrate metabolic enzymes, stimulation of pancreatic insulin release, and inhibition of hepatic glucose production are among the other methods.

Black pepper (Source: *Piper nigrum;* Family*: Piperaceae*

Black pepper is commonly used as spices in various food preparations. It is commonly known as Pippali in India. Indonesia is one of the biggest pepper producers within the world, either white or black pepper. Traditionally black pepper used for diarrhea, dyspepsia, cholera and gastric ailments. Black pepper seeds have a strong aroma and spicy taste. Piperine is the major active compound, known for its active bioenhancing property. Combination of Piperine with metformin drug reduced glucose levels in alloxan-induced diabetic mice [37]. Ethanolic leaves extract of black pepper shows a significant reduction of blood glucose levels because of its antioxidant properties [38].Black pepper powder can be taken with warm water as tea [39].

Turmeric (Source: Curcuma longa; Family: Zingiberaceae)

Turmeric is commonly utilized as spices in food preparation in raw and powder form. It is known as Haldi in Hindi. This plant is characterized by orange tuberous rhizomes and is broadly known and developed in South East Asia. It is utilized as a normal helpful medication for various pathological conditions in these regions since ancient times. The rhizome is for the most part utilized as a normal cure within the medications of different sicknesses due to the nearness of dynamic constituents, Curcumin which posse's anti-bacterial, anti-diabetic andante-cancer activity. It is also responsible for decreasing the impact of chemical in changing over carbohydrates into glucose which leads to decreasing the levels of glucose in blood [40].Curcumin exhibit hypoglycemic effect, decreasing dyslipidemia and glycemia in STZ-induced rats fed with high-cholesterol diet (HCD) [41].Administration of Curcumin at 80mg/kg of body weight decreases blood glucose level and promotes antioxidants defense in diabetic rats [42] .In patients with T2DM, curcuminoids have been shown to improve insulin resistance, lower glucose and insulin levels, increase adiponectin release, and lower levels of leptin, resisting, interleukin (IL)-6, IL-1, and tumor necrosis factor. These data show that these substances may have an impact on glucose homeostasis, diabetes complications, and T2DM patients' vascular risk.

Onion (Source: *Allium cepa; Family:* Liliaceae

The bulb of onion is mostly used as spice in curry and salads. It is called as Pyaaj in Hindi. The components of onion such as S-methylcysteine and quercetin are responsible for hypoglycemic activity which helps in decreasing the

levels of glucose in blood [43]. Raw onion can be added to the diet of non-insulin dependent diabetic patients to decrease the dose of antidiabetic medication in order to controls the disease [44]. Oral administration of hydro alcoholic extract of onion produced hypoglycemic effect which probably improves pancreatic beta-cells [45]. Onion can also counteract the hypoglycemic effect of antidiabetic medication taken simultaneously as food supplements due to the present of cysteine compound which causes glucogenic effects [46]. Onions are of incredible value as a critical source of several phytonutrients as flavonoids, anthocyanins, phenolic acid and flavanols fructooligosaccharides (FOS), thiosulfinates, other organ sulfur compounds, vitamins and few minerals. The secondary metabolites found in onions, phenolic, have antioxidant activity in addition to having beneficial effects against various degenerative pathologies such as cardiovascular and neurological diseases, as well as dysfunctions caused by oxidative stress. They can scavenge radicals through three major mechanisms: hydrogen atom transfer, electron transfer, and a combination of both. In alloxan diabetic rats, S-methyl-L-cysteine sulfoxide, which is extracted from onions, exhibits anti-diabetic, antioxidant, and anti-hypolipidemic properties.

Garlic (Souce: *Allium sativum;* Family*: Liliaceae*)

Garlic bulbs are widely used as spices in various Indian dishes. It is called as Lahsun in Hindi. Garlic powder contains alliin and allinase enzyme and raw garlic also contain adenosine. It helps in maintain blood sugar level due to its anti-diabetic and hypolipidemic properties [47]. Garlic oils contain active components such as diallyl, allyl methyl and dimethyl mono to hexa sulfides [48] .Garlic has a rich proportion of sulfur-containing compounds which exhibit an antioxidant effect [49]. Methanolic extract of garlic decreased the oxidative stress in hepatic and intestinal tissues of diabetic rats [50].2-3 raw garlic can be eaten daily on an empty stomach also helps treat diabetes. Garlic (Allium sativum is an herb related to onion, Leeks and chives. It is commonly used for conditions related to the heart and blood system. Garlic might also increase the risk of bleeding and cause allergic reactions in some people.

Holy basil (Source: *Ocimum sanctum;* Family*: Lamiaceae*

The plant is commonly called as Tulsi in India. The leaves contain chemicals such as apigenin, luteolin, orientin and eugenol, an essential oil. Tulsi powder supplements exert a glycemic control on non-insulin dependent diabetic individuals [51] .Aqueous and ethanolic extract of tulsi showed a significant reduction in the levels of blood glucose and glycosylated hemoglobin (52, 53, 54).Oral administration of onion hydro alcoholic extract resulted in a hypoglycemic effect, indicating that pancreatic beta-cells are likely to improve [55] .The hypoglycemic effect of Methanolic extract is due to the presence of active Phytochemicals which has anti-diabetic in nature [56].Tulsi has hypolipidemic and hypoglycemic activity which significantly reduce blood glucose and cholesterol [57].The ethanolic extract of the leaves was detailed to have hypoglycaemic impact as early as in 1968. The hypoglycaemic effect of the ethanolic extracts had been found to be 91.55% and 70.43% in normal and diabetic rats, respectively, when compared with Tolbutamide. A clinical study reported that the leaf extract resulted in a significant reduction of the fasting and postprandial blood glucose levels as well as a mild reduction in cholesterol levels, with the authors suggesting that basil leaves might be used as an adjunct therapy in mild to moderate non-insulin dependent Diabeties mellitus. Tulsi leaves can be chew and also added in dishes.

Clove (Source: *Syzigium aromaticum;* Family*: Myrtaceae*

The flower buds of clove have aromatic properties which are utilized as spices. The spice has a strong flavor. Cloves or its powder form can be added to tea or dishes. It is also called as Laung in Hindi. The major components present in cloves are oleonic acid and eugenol [58].These two compounds posses antioxidant activity which lowers the levels of glucose [59]. Dietary cloves have hyperglycemic, hypolipidemic and antioxidant effect in high fed STZ-induced diabetic rats. [60] Cloves extract have also shown to improve insulin action and lower glucose, total cholesterol and triglycerides in type 2 diabetic patients [61].

Fennel (Source: Foeniculum Vulgare; Family: Apiaceae)

Fennels are an important spice and commonly called as Saunf. It is highly aromatic herb. Active constituent of fennel contains 90% trans anethole, 20% fenchone, it also has small amounts of limonene, camphor and alphapinene [62].

Some components of fennel such as triterpene and phenols have antioxidant properties which alleviate diabetes complications [63] .Essential oil of fennel showed anti-hyperglycemic effect in STZ treated rats [64].The seed extract of fennel posses anti-hyperglycemic, anti-hyperlipidemic activities in STZ-induced diabetes rats which showed potential to restore hepatic complications of diabetes [65, 66].Administration of fennel extracts showed Antihyperglycemic effect in diabetic rats which potentially restored cardiovascular, renal and hepatic complications [67].

Coriander (Source: *Coriandrum sativum*; Family: *Apiaceae)*

Coriander is a well known herb which is also called as Dhaniya in Hindi. Fresh leaves and seeds powder have been used as spice for its flavor. The main components of coriander are linalool, alpha pinene and geraniol. The seeds powder showed anti-diabetic activity which increases the glucose transport and secretion of insulin from pancreatic β-cells [68]. The seeds also posses hypoglycemic activity due to the increase utilization of glucose in the synthesis of liver glycogen and decreased glycogen degradation [69].Oral administration of coriander seeds (5g/day) to type 2 diabetic were also shown to exhibit anti-hyperglycemic and hypolipidemic effect in STZ-induced diabetic rats [70, 71].

Caraway (Source: *Carum carvi*; Family: *Apiaceae*)

Caraway is a unique spice used in cooking and it is slightly bitter and earthy flavor. Caraway is also called as Jeera in Hindi. The Phytochemicals present in the seeds are carvone, limonene and terpinene. The flavonoids show in caraway play a major part in decreasing oxidative stress and the critical movement of caraway is due to the nearness of flavonoids [72]. Oral administration of caraway seed oil restrained weight misfortune in diabetics. The hypoglycemic impact of caraway move forward levels of glucose [73].Oral administration of caraway appeared a critical diminish within the levels of blood glucose, body weight and total cholesterol of treated rats [74, 75] .The ethanolic extract of caraway seeds too applied anti-hyperglycemic movement in STZ-induced diabetic rats [76].

Saffron (Source: Crocus sativus; Family: Iridaceae)

Saffron is the dried stigma flower which mainly used for seasoning and coloring agent. Crocin (crocetin glycoside), crocetin and safranal are the major constituents of saffron [77]. Crocetin was found to increase the sensitivity of insulin and also ameliorated abnormalities such as impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension in high-fructose diet and dexamethasone injected rats [78, 79, 80]. The ethanolic and hydromethanolic extract of saffron showed anti-hyperglycemic and also increased insulin levels in alloxan-diabetic and non-diabetic rats. [81, 82].

CONCLUSION

Spices are herbs which are mainly used in Indian cooking as colouring, flavoring agent as well as preservatives from ancient times. These spices were also having certain phytochemical constituents which exhibit variety of medicinal properties. Spices are rich in antioxidant and anti-diabetic compounds which have potential pharmacological actions in the management of diabetes and its complications. Spices' diabetic effects are achieved by stimulating the pancreas to produce insulin, which interferes with glucose absorption. Small amounts of spices in the diet on a daily basis may aid in the treatment of diabetes mellitus. The daily intake and the bioavailability of their active components play a major role in understanding their pharmacological actions in the management of diabetes mellitus. On the other hand, spices are natural products which can complement the side effects of synthetic drugs and controls increased risk of hypoglycemia and heart failure. Overall, adding spices in our diet serve as a delicious and maintaining a healthy body. Most of the research work has been reported on animal models and cell culture. Therefore, human model is needed for further studies to confirm the effects and efficacy of spices in human trials.

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Table 1: The commonly used herbs and spices in the management of diabetes

Sl. No	Name	Local name	*Botanical name*	Family	Plant part	Phytochemical constituents
1	Cinnamon	Dalchini	*Cinnamomum zeylanicum*	Lauraceae	Leaves	Cinnamaldehyde, eugenol
2	Bay leaf	Tejpat	*Cinnamomum tamala*	Lauraceae	Leaves	Myrcene, camphene, p-cymene, limonene, eugenol
3	Black cumin	Kalonji	*Nigella sativa*	Ranunculaceae	Seeds	Linoleic acid, oleic acid, palmitic acid
4	Curry leaf	Curry patta	*Murraya koenigii*	Rutaceae	Leaves	Cinnamaldehyde, mahanimbine, girinimbine and mahanine
5	Fenugreek seed	Methi	*Trigonella foenum*	Leguminosae	Seed	Trimethylamine, histidine, leucine, quercetin
6	Ginger	Adarak	*Zingiber officinale*	Zingiberaceae	Rhizome	Gingerol, shogaol and zerumbone.
7	Black pepper	Pippali	*Piper nigrum*	Piperaceae	Seed	Piperine, Curcumin
8	Turmeric	Haldi	*Curcuma longa*	Zingiberaceae	Rhizome	Curcumin, demethoxy Curcumin and bisdemethoxycurcumin.
9	Onion	Pyaaj	*Allium cepa*	Liliaceae	Bulb	Quercetin, fructose, quercetin-3-glucoside
10	Garlic	Lahsun	*Allium sativum*	Liliaceae	Bulb	Allicin, ajoeni, allinase
11	Holy basil	Tulsi	*Ocimum sanctum*	Lamiaceae	leaves	Eugenol, linalool, β-caryophyllene
12	Clove	Laung	*Syzigium aromaticum*	Myrtaceae	Flower buds	Eugenol, β-caryophyllene, eugenyl acetate
13	Fennel seed	Saunf	*Foeniculum vulgare*	Apiaceae	Seed and leaves	Anethole, estragole, fenchone
14	Coriander	Dhaniya	*Coriandrum sativum*	Apiaceae	Seeds and leaves	Linalool, pinene, geraniol
16	Caraway	Jeera	*Carum carvi*	Apiaceae	Seeds/fruits	Carvone, limonene, terpinene
17	Saffron	Kesar	*Crocus sativus*	Iridaceae	Flower	Crocin, safranal, crocetin

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REVIEW ARTICLE

Glutathione and Vitamin D to Prevent COVID-19: A Review

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ABSTRACT

The alarming pandemic situation of corona virus disease (COVID-19) outbreak arises due to rapid spread of Novel Corona Virus (SARSCoV-2). Impaired redox homeostasis in association with oxidative stress is found to be important biological processes that may account for increased individual susceptibility to COVID-19 infection. Glutathione (GSH) is one of the main nonprotein antioxidants in the cell which, together with its related enzymes constitute the glutathione-system. The glutathione system plays an important role in the maintenance of good health and prevention of various diseases. Several approaches have been used to enhance cellular GSH availability. Restricted diet, drug administration and nutritional supplementation shows moderate success. Regular exercise has also evolved as a new approach. Some evidences suggest that GSH and vitamin D supplementation can reduce the risk of COVID-19 infections and deaths. Present review discusses the possible roles of Glutathione and Vitamin D in preventing and reducing the risk of COVID-19 associated acute infections and severity.

Keywords: SARSCoV-2, COVID-19, Glutathione, Vitamin D

INTRODUCTION

The novel corona virus SARSCoV-2 (COVID-19) continues to spread throughout the world, affecting more and more people; majority of people have asymptomatic, mild, or moderate disease, and only 14% patients developed severe

and 5% developed critical illness [1]. Higher rates of death and serious illness from COVID-19 infection among older people and those having comorbidities suggest the role of age and disease-related biological processes leads individuals to be more sensitive to environmental stress factors, including infectious agents like corona virus SARSCoV-2 [2]. SARSCoV-2, with its minute virion size (50-200 nm diameter) enters into the human body by using its spike protein (ACE2) and protease (TMPRSS2) and binds to cellular receptor (ACE2) and then starts rapid multiplication in the lung tissue leading to breathing difficulty and death [3]. For its rapid growth the virus attacks the immune system, overpowers the defense mechanism and creates the so called 'cytokine storm' [4]. Severe oxidative stress caused by various factors leads to destruction of the phase II detoxification pathway, the major component of body's Innate Immune System. The function of this detoxification system is governed by GSH (reduced form) and some related enzymes [5, 6]. The thiol compound GSH (γ-glutamyl cysteine glycine, a ubiquitous tripeptide) is the major antioxidant that controls various biological processes, e.g., removal of free radicals, mitochondrial activity, apoptosis, immune response and even antiviral action. The cellular GSH level also keeps varying with age, sex and other disease features of the body [7]. Several studies indicate that higher levels of GSH may improve an individual's responsiveness to viral infections [2]. Recent reports disclose that Vitamin D plays a role in reducing the risk of COVID-19 and other acute respiratory tract infections and severity. Studies also suggest that vitamin D plays important role in reducing the risk of acute pneumonia. These include direct inhibition with viral replication or with anti-inflammatory or immunomodulatory ways [8]. Herein we will discuss the crucial role of Glutathione and Vitamin D, their inter-relation and feasibility to prevent and treat COVID-19 pathogenesis.

Glutathione and COVID 19

Glutathione (GSH) plays the role of 'master antioxidant' in all tissues [9]. Extracellular GSH uptake, regeneration from the oxidized form (GSSG) and *de novo* synthesis control the intracellular GSH balance. In cytosol, GSH synthesis takes place in two ATP-dependent reactions which are catalyzed by glutamate-cysteine ligase (GCL) and glutathione synthase (GS). GSH exists in reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is capable of donating a reducing equivalent to other unstable molecules, for example ROS. After donating an electron, GSH itself becomes reactive, and then it readily reacts with another reactive GSH to form glutathione disulfide (GSSG). Such a reaction is possible due to the presence of relatively high concentration of glutathione in cells (up to 5 mM in the liver). Glutathione reductase (GR) catalyzes the regeneration reaction of GSH from GSSG in the GSH redox cycle [9]. Studies including ours indicate that cellular GSH deficiency which is caused by increased depletion or decreased biosynthesis, results into oxidative stress, viral attack, immune dysfunction and cancer [10-12]. Recent biomedical literature also emphasizes that GSH deficiency is the most accepted explanation of higher COVID-19 infection among aged population and in persons suffering from comorbidity (diseases like diabetes, cardiac or pulmonary diseases) [13]. GSH deficiency can also promote the increased activation of von Willebrand Factor and leads to coagulopathy in COVID-19 patients [2].

Endogenous GSH progressively decreases with age and thus cells in elderly people (particularly in lung tissue) are more susceptible to oxidative damage caused by environmental factors and viral attack. Evidences disclose that the effect of glutathione deficiency, as seen in many chronic diseases, causes severe oxidative damage in COVID-19 patients [13, 14]. Oxidative damage thereby exacerbates inflammation in lung and airways leading to acute respiratory distress syndrome (ARDS), multiorgan failure and death [15]. Levels of cellular GSH is higher in female population than in males that may be the reason of prevalence of COVID-19 attack more in males [16]. Recent clinical findings also disclose that patients with moderate-to-severe SARSCoV-2 infection have higher levels of ROS, greater ROS/GSH ratio and lower levels of GSH than patients with mild illness [17]. Thus corona virus (SARSCoV-2) cannot actively replicate in patients having higher levels of cellular GSH; the lower viral load is manifested by milder clinical symptoms [15, 17, 16]. Ample evidences thus support the proposition that GSH may be a promising drug for etiological treatment of SARSCoV-2 infections [14, 18, 19]. Evidences also disclose that GSH inhibits replication of viruses at different stages of the viral life cycle, and this antiviral property of GSH may prevent increased viral loads and the cytokine storm [2]. Studies disclosed that following a 6-month preventive administration of N-acetylcysteine (NAC, glutathione precursor), there was significant reduction in the incidence of clinically apparent influenza and

influenza-like episodes, especially in elderly high-risk individuals. COVID-19 patients have lower level of cellular GSH compared to healthy individuals and therefore, GSH replenishment may usher a new era of an effective therapy [20].

Vitamin D and COVID 19

Vitamin D is a steroid hormone which is produced endogenously with the effect of solar radiation on human skin. It can also be obtained from exogenous food sources or dietary supplements [21]. Studies substantiated a potential link between vitamin D deficiency and various diseases, including systemic infection [22, 23, 24]. Some recent reviews hypothesized that insufficient vitamin D level may hamper respiratory immune function which in turn increases the risk of COVID-19 severity and mortality [8]. Vitamin D may be involved in maintaining the cell junctions, and gap junctions, increasing cellular immunity by decreasing the cytokine storm; these are achieved by influencing interferon γ and tumor necrosis factor α [25] and regulating adaptive immunity through inhibiting T helper cell type 1 responses and stimulating of T cells induction [26]. Several *in vitro* studies substantiated that vitamin D plays an important role in local 'respiratory homeostasis' either by stimulating the exhibition of antimicrobial peptides or by directly inhibiting the replication of respiratory viruses [27]. Vitamin D insufficiency can, therefore, be involved in ARDS (Acute Respiratory Distress Syndrome) which is noticed in severely ill COVID-19 patients [8]. Studies also suggest that deficiency of vitamin D can promote the renin-angiotensin system (RAS), which may result into chronic cardiovascular disease (CVD) and reduced lung function [28]. People with such comorbidities possess higher risk of severe illness in case of COVID-19 [8].

Relation between Glutathione and Vitamin D

Several studies indicated that greater the GSH levels, greater the level of active vitamin D [2]. Reports disclose in T2D patients, lower levels of L-cysteine (a rate-limiting precursor of GSH) and GSH correlated with lower vitamin D binding protein (VDBP) and Vitamin D levels [29]. L-cysteine supplementation is known to improve GSH status through upregulation of the expression of VDBP, Vitamin D 25-hydroxylase, and vitamin D receptor, thereby increasing vitamin D (VD) levels and decreasing inflammatory biomarkers in diabetic rats [30]. Recent study [31] also suggests that the deficiency of GSH in association with increased oxidative stress epigenetically alters Vitamin D regulatory genes which lead to suppressed gene expression which in turn decreases VD biosynthesis, ultimately leading to a secondary deficiency of vitamin D. Replenishment of GSH by L-cysteine treatment beneficially altered epigenetic enzymes methyl transferases and increased the expression of vitamin D metabolism genes. Hence we may conclude GSH is essential for controlling endogenous vitamin D biosynthesis and can be used in the treatment of vitamin D deficiency [31].

CONCLUSION

Considering very high rate of serious illness and mortality due to COVID-19 in senior people and those with comorbidity, identification of effective drugs for the treatment and prevention is the need of the hour. Reduction in oxidative stress may be an effective approach to prevent and treat COVID-19 patients. Most of the cofactors (aging, diabetes, hypertension, cardiovascular disease) of COVID-19 are found to be associated with low levels of cellular GSH [20]. Glutathione is also involved in maintaining Vitamin D level as we already discussed. Hence, we propose that the increase of cellular GSH may prove to be a new approach to treat COVID-19. Patients should consume lot of pure vegetables, fruits, dairy products containing high amount of GSH. Examples of some GSH containing vegetables are mushrooms, asparagus, avocado, cabbage, brussel sprouts, spinach, broccoli, garlic, onions, tomatoes, cucumber, almonds, walnuts etc. Exercise should be included in the daily routine of COVID-19 patients as yoga and exercise reduces oxidative stress and increases cellular GSH level. N-acetyl cysteine *i.e.*, oral GSH precursor, may represent a novel treatment approach for reducing cytokine storm syndrome, oxidative stress and respiratory distress in severe COVID-19 cases.

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REVIEW ARTICLE

A Review: Microsponges for Topical Drug Delivery System

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ABSTRACT

A Microsponges Delivery System (MDS) is a polymeric system made up of porous microspheres that can entrap a diverse variety of actives before slowly releasing them into the skin in response to a trigger. The diameter is between 10 and 25 microns. A Porogen drug is entrapped with a one-step procedure and neither hinders nor activates the polymerization process. It is also stable to free radicals (liquid-liquid suspension polymerization). MDS technology is being used currently in cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products. One of the best feature of microsponge is it is self-sterilizing. This review is focused on method of preparation, characterization and application of microsponge. The aim of this article is to provide detailed about microsponges including method of preparation characterization, mechanism of the drug release from microsponges, different formulation and process factors, and a few application about microsponges which are either proven or under research.

Keywords: Microsponge; Topical Drug Delivery System; Porous Microsphere; Polymeric Delivery Devices.

INTRODUCTION

A novel drug delivery system (NDDS), also known as controlled drug delivery system (CDDS), is a combination of improved procedures and new dosage forms used to improve drug potency, control drug release, improve safety, and targets a drug to a specific tissue. The word "controlled release" has a broader definition than just "continuous release." In other words, the release kinetics of a regulated release must be repeatable and predictable. NDDSs allow for the efficient use of costly pharmaceuticals and excipients, as well as a reduction in manufacturing costs. By improving comfort drug delivery devices, NDDS improves therapy and raises the standard of living [1].

RECENT DEVELOPMENTS IN NOVEL DRUG DELIVERY SYSTEM [2]

- Liposome
- Microsponges
- Ethosome
- Solid lipid nanopartical
- Hydrogels
- Niosomes
- Nanoparticles
- Microsphere
- Phytosome
- Proniosomes
- Dendrimers

TRANSDERMAL DRUG DELIVERY SYSTEM [3]

Transdermal drug delivery system supplies the drug to systemic circulation through the help of number of penetration enhancers. The target of transdermal drug delivery is to supply maximum amount of drug in blood circulation by crossing skin layers. commonly there is needed to keep drug in skin layers for local action without entering in systemic circulation is a tough task. To achieve this goal carrier and micro particle technology is required, by using these technology one can maintain drug in skin only. Generally creams, pastes and ointments are the formulations applied to the skin for its local action. These formulations give some drawback such as stickiness, greasiness, and require high amount of drug in formulation, also evaporation of many drugs takes place. These formulations also give particular odors and sometimes may chance to allergy with skin. So elimination of such a drawbacks, By using new technology to make such formulation which can give more drug release on skin surface without entering in blood stream .

MICROSPONGES

A Microsponges Delivery System (MDS) is a polymeric system made up of porous microspheres that can entrap a diverse variety of actives before slowly releasing them into the skin in response to a trigger. The diameter is between 10 and 25 microns [4]. Micro-sponge polymers have the ability to carry a broad range of actives, affording them the benefit of enhanced product efficacy, mildness, tolerability, and extended wear to a wide range of skin therapies. Under the area of transdermal delivery system (TDS), which uses the skin as a portal of entry, several predictable and dependable systems for systemic medications have been created. Many medications' efficacy and safety have improved as a result of it. TDS, on the other hand, is ineffective for delivering materials to the skin's final destination. As a result, there is a need for a system that maximises the amount of time an active ingredient is available on the skin's surface or within the epidermis while decreasing transdermal penetration into the body [5,6,7]. Microsponges are porous microsphere-based polymeric delivery devices. They are spherical sponge-like particles with a porous surface. Furthermore, they may improve stability, minimize adverse effects, and alter drug release in a positive way. Microsponge technology has a number of advantages that make it a useful versatile delivery mechanism. Microsponge Systems are made up of microscopic polymer-based microspheres that can suspend or entrap a wide range of ingredients before being included into a manufactured product like a gel, cream, liquid or powder. MDS can effectively increase the efficacy of topically active compounds while also improving their safety, product stability, and aesthetic qualities [8,9,10].

Won invented the microsponge technique in 1987, and Advanced Polymer Systems, Inc. was given the original patents. This company created a variety of processes that are used in cosmetics, over-the-counter (OTC), and prescription pharmaceutical drugs. This fascinating technology has been licenced for the time being by Cardinal Health, Inc., for use in topical products. The internal structure of the microsponge particle is revealed by scanning electron microscopy as a "bag of marbles." The interstitial spaces between the marbles are responsible for the

porosity. Emollients, perfumes, essential oils, sunscreens, anti-infective and anti-inflammatory agents, and other active compounds can be trapped in the interstitial pores [11].

As a result, drug delivery methods are needed to optimise the quantity of active component that remains on the skin's surface or within the epidermis while decreasing transdermal penetration into the body, as in the case of sunscreens. A novel method like this might potentially boost the efficacy of topically active medicines while also improving product safety. Microsponges can be used to alleviate the issues that these traditional methods possess. The Microsponge polymeric system that can meet these requirements. These are tiny sponge-like (Porous) spherical particles that can entrap active chemicals and then gradually release them onto the skin in response to a trigger. They can also increase the product's aesthetic properties and extend the product's stability due to their unique arrangement. Microsponges also speed up the solubilization of weakly water soluble medications by trapping them in the microsponges' small pores. Because these pores are so small, the medication is effectively converted to minute particles, boosting surface area and solubilization rate substantially [12].

Benefits of microsponge drug delivery systems [13,14,15]

- Product performance has been improved.
- The duration of the release has been extended.
- Reduce irritability, resulting in increased patient compliance.
- Elegance of the product has improved.
- Improved oil control due to its ability to absorb up to 6 times its weight in oil without drying out.
- Allows for new product forms to be created.
- Enhances therapeutic efficacy.
- The ability to experiment with new product forms.
- Non-toxic, non-irritating, non-mutagenic, non-allergenic, and non-mutagenic.
- Stability, thermal, physical, and chemical stability are all improved.
- Improves material processing, such as converting liquids to powders.

Features of a microsponge drug delivery system that could be useful [16,17,18]

- Tolerable stability at pH levels ranging from 1 to 11 as well as high temperatures (up to 130°C).
- Demonstrate good compatibility with a wide range of vehicles and ingredients.
- High entrapment efficiency of up to 50%-60%.
- They have a free-flowing quality to them.
- Because the typical pore size of microsponges is small (0.25 m), germs cannot penetrate them, they do not require sterilisation or the addition of preservatives.
- Absorbs up to 6 times their weight in oil without drying out.

Characteristics of moieties that is entrapped in microsponges [19,20]

- Fully miscible in monomer or capable of being made miscible by a tiny amount of a water immiscible solvent.
- In contact with the polymerization catalyst and under polymerization conditions, it is stable.
- Microsponges' spherical structure should not collapse.
- Microsponge-entrapped active substances can then be used in a variety of products, including creams, gels, powders, lotions, and soaps.
- To avoid cosmetic concerns, the solubility of actives in the vehicle must be controlled; no more than 10 to 12 percent w/w microsponges must be included into the vehicle. Otherwise, before the application, the vehicle will deplete the microsponges.
- Immiscible in water or only slightly soluble in it.
- Monomers are inert to it.
- The payload and polymer design of the active microsponges must be tuned for the required release rate during a certain time period.

MICROSPONGE DRUG DELIVERY SYSTEM PREPARATION METHOD

A Porogen drug is entrapped with a one-step procedure and neither hinders nor activates the polymerization process. It is also stable to free radicals (liquid-liquid suspension polymerization). The following procedures are suitable for preparing microsponges:

Liquid-liquid suspension polymerization [21,22,23]

Suspension polymerization in liquid-liquid systems is used to make porous microspheres. In this process, immiscible monomers are first dissolved with active components in a suitable solvent monomer, and then dispersed in aqueous phases containing additives such as surfactants and suspending agents to aid suspension formation. The polymerization is then triggered by raising the temperature, irradiating it, or adding a catalyst. The polymerization process continues to create a reservoir-like system with a spherical shape. The solvent is evaporated after the polymerization process, leaving spherical structured porous microspheres.

The various steps involved in the preparation of microsponges are summarised as follows

Step 1: Choose a monomer as well as a monomer combination.
Step 2: As polymerization begins, chain monomers form.
Step 3: The formation of ladders as a result of chain monomer cross-linking.
Step 4: The monomer ladder is folded to form spherical particles.
Step 5: Agglomeration of microspheres results in the formation of microsphere bunches.
Step 6: Binding of bunches to produce microsponges.

Quasi-Emulsion Solvent Diffusion Method [24,25,26]

A quasi-emulsion solvent diffusion approach (two-step procedure) was also used to make porous microspheres (microsponges) utilising an internal phase containing polymer such as Eudragit RS 100 dissolved in ethyl alcohol. The drug is then slowly added to the polymer solution and dissolved under ultrasonication at 35°C, with the addition of a plasticizer such as triethylcitrate (TEC) to improve plasticity. After 2 hours of continuous stirring, the inner phase is poured into the external phase, which contains polyvinyl alcohol and distilled water. After that, the microsponges were separated by filtering the mixture. The product (microsponges) was washed and dried in an air heated oven at 40°C for 12 hrs.

MICROSPONGE RELEASE MECHANISM [27,28]

The active drug is freely flow in and out of the particles and into the vehicle until equilibrium is attained, when the vehicle becomes saturated, because the microsponge particles have an open structure (they do not have a continuous membrane enclosing them). When the completed product is applied to the skin, the active in the vehicle is absorbed into the skin, depleting the vehicle, causing it to become unsaturated and disrupting the balance. They could cause the active drug to flow from the microsponge particle into the vehicle, and then from the vehicle to the skin, until the vehicle is either dry or absorbed. Even after that, the active will be gradually released to the skin through the microsponge particles that remain on the stratum corneum's surface, providing a long-term release. The applicable of developing vehicles for usage with microsponge entrapments is highlighted by this proposed mechanism of action. The goods will not give the desired benefits of progressive release if the active is too soluble in the desired vehicle during compounding of the finished products.

Accelerated or Triggered by following mechanism

- ❖ Pressure triggered systems
- ❖ Temperature triggered systems
- ❖ pH triggered systems
- ❖ Solubility triggered system.

Pressure triggered system [29]

When pressed or squeezed, the microsponge system releases fluid or active ingredient, replenishing the quantity of entrapped active ingredient on the skin. The amount released may also be affected by the sponge's release and the Microsponges' resilience.

Temperature triggered system [30]

Temperature can trigger the release of active substances from microsponges. Few entrapped active substances are too viscous to flow quickly from microsponges onto the skin at normal temperature. As the temperature of the skin rises, so does the flow rate, and hence the rate of release.

pH triggered system [31]

Modifying the coating on the microsponge can be used to trigger the active's pH-based release. This has a wide range of uses in drug delivery.

Solubility triggered system [32]

In the presence of water, microsponges containing water miscible ingredients such as antiseptics and antiperspirants will release the component. Diffusion can also be used to activate the release, however this must take into account the ingredient's partition coefficient between the microsponges and the external system.

FACTORS AFFECTING DRUG RELEASE FROM MICROSPONGE DELIVERY SYSTEM [33]

- Microsponge system physical features such as pore diameter, pore volume, resilience, and so on. The characteristics of the vehicle in which the microsponges are disseminated.
- Pressure The active substance in microsponges can be released onto the skin by rubbing or applying pressure.
- Changes in temperature At normal temperature, certain entrapped actives are too viscous to flow spontaneously from microsponges onto the skin. A rise in skin temperature can lead to an increase in flow rate and, as a result, release.
- Solubility Microsponges containing water-soluble compounds such as antiperspirants and antiseptics release the chemical when they come into contact with water. Diffusion, which considers the ingredient's partition coefficient between the microsponges and the rest of the system, can also be used to trigger the release.

CHARACTERIZATION OF MICROSPONGE DRUG DELIVERY SYSTEM

Determination of particle size [34]

Laser light diffractometry or any other acceptable method can be used to determine the particle size of loaded and unloaded microsponges. For all formulations, the values (d50) can be represented as a mean size range.To investigate the effect of particle size on drug release, the cumulative percentage drug release from microsponges of various particle sizes will be plotted versus time. Because particles larger than 30 m might create a gritty sensation, the final topical formulation should have particles between 10 and 25 m. The most common methods for visualising microparticles are light microscopy (LM) and scanning electron microscopy (SEM) (SEM).Microparticles' shape and exterior structure can be determined using both methods. In the case of double-walled microparticles, LM allows you to modify the coating parameters.The structure of multiple walled microparticles is characterised using conflocal fluorescence microscopy. Other than instrumental approaches, laser light scattering and multi size coulter counter can be used to characterise the size, shape, and morphology of microparticles (microsponges).

Morphology and surface topography of microsponges [35]

SEM microscopy can be used to examine the surface morphology of prepared microsponges that have been coated with gold–palladium under an argon environment at room temperature (SEM). An SEM image of a shattered microsponge particle can be used to show its ultrastructure.

Compatibility studies [36]

Thin layer chromatography (TLC) and Fourier Transform Infrared spectroscopy can be used to investigate drug compatibility with reaction adjuncts (FT-IR). Powder X-ray diffraction (XRD) and Differential Scanning Colorimetry can be used to investigate the effect of polymerization on medication crystallinity (DSC). For DSC, about 5 mg samples can be properly weighed into aluminium pans, sealed, and heated at a rate of 15 C/min in a nitrogen environment across a temperature range of 25–430 C.

Resiliency [37]

Depending on the final formulation's needs, the resilience (viscoelastic properties) of microsponges can be adjusted to create softer or stiffer beadlets. Cross-linking tends to slow down the pace of release.

Drug release kinetics [38]

Various models were applied to the dissolution profile of each formulation to assess the kinetics of drug release, including Zero order kinetics (percentage drug release against time), First order kinetics (log percentage drug unreleased against time), Higuchi (percentage drug released against square root of time), and Korsemeyer-Peppas (percentage drug released against square root of time) (log percent drug released against log of time).

Dissolution tests [39]

The dissolution apparatus USP XXIII with a modified basket made of 5 millimetre stainless steel mesh can be used to examine the dissolution of microsponges. The rotational speed is 150 rpm. To guarantee sink conditions, the dissolving medium is chosen while considering the solubility of the actives. At various times, samples from the dissolving media can be analysed using an appropriate analytical technique.

Determination of true density [40]

The true density of micro particles and BPO was estimated from a mean of multiple measurements using an ultra-pycnometer and helium gas and was calculated from a mean of repeated determinations.

Characterization of pore structure [41]

Pore volume and diameter are critical in determining the intensity and duration of an active ingredient's efficacy. The migration of active substances from microsponges into the medium in which the material is distributed is also influenced by pore diameter. Mercury intrusion porosimetry can be used to investigate the relationship between pore width and volume and drug release rate from microsponges. Mercury intrusion porosimetry can be used to measure porosity characteristics of microsponges such as intrusion-extrusion isotherms, pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density.

APPLICATION OF MICROSPONGES [42,43,44]

Topical prescription, over-the-counter, and personal care products employ microsponge delivery methods to improve their safety, efficacy, and aesthetic quality.

- The Topical Microsponge systems are used in three ways in products in development or on the market:
- As reservoirs that release active chemicals over time.
- As receptacles for absorbing unwanted items like excess skin oils.
- As closed containers that keep ingredients away from the skin for superficial action.

MICROSPONGE IN PHARMACEUTICAL APPLICATIONS

LONG-LASTING COLORED COSMETICS [45,46]

Colors entrapped in microsponges can be used to extend the life of a range of coloured cosmetics such as rouge and lipsticks. Microsponges, as previously indicated, aid in consistent spreading and improved covering power. Colored cosmetics with microsponges would be extremely beautiful as a result.

For topical administration [47,48]

A single microsponge is as small as a talcum powder particle, with a diameter of less than one thousandth of an inch. Each microsphere, like a real sponge, is made up of a network of interconnected voids inside a non-collapsible structure that can hold a wide range of substances. The outer surface is usually porous, enabling things to flow in and out of the sphere in a regulated manner. During the manufacturing process, many key features, or parameters, of the microsponge system may be specified in order to produce spheres that are suited to certain product applications and vehicle compatibility. Polymers used in microsponge systems are physiologically inert.The polymers have been shown to be non-irritating, non-mutagenic, non-allergenic, non-toxic, and non-biodegradable in extensive safety investigations. As a result, the human body is unable to break them down or convert them into other chemicals. These systems are too big to penetrate through the stratum corneum when integrated into topical treatments, despite their tiny size. Skin irritation is a frequent adverse effect of benzoyl peroxide, which is often used in topical formulations for the treatment of acne.

For oral administration [49]

The microsponge system has been found to improve the rate of solubilisation of poorly water soluble drugs in oral applications by trapping them in the pores of the microsponge system. Because these pores are so tiny, the drug is effectively reduced to microscopic particles, resulting in a substantial increase in the rate of solubilization. The acrylic polymer Eudragit RS is used to control the oral administration of ibuprofen microsponges by altering their intraparticle density. The dry impact blending method is used to make a sustained release formulation of chlorpheniramine maleate utilising powder-coated microsponges for oral drug delivery.

For Bone and Tissue Engineering [50,51]

Prepolymerized polymethyl methacrylate powders and liquid methyl methacrylate monomer were mixed with two aqueous dispersions of tricalcium phosphate grains and calcium deficient hydroxyapatite powders to create compounds. The finished composites had a porous appearance and functioned as microsponges. Based on the biodegradation of the sponge matrix, basic fibroblast growth factor (bFGF) embedded in a collagen sponge sheet was maintained released in the mouse sub-cutis and demonstrated local angiogenic activity in a dose-dependent manner.

In cardiovascular engineering [52]

A biodegradable material with autologous cell seeding necessitates a time-consuming and intrusive technique that poses an infection risk. To avoid these issues, researchers created a biodegradable graft material with collagen microsponges that allows for the regeneration of autologous vascular tissue. Using and without precellularization, the capacity of this material to expedite in situ cellularization with autologous endothelium and smooth muscle cells was examined. To create a vascular patch material, poly (lactic-co-glycolic acid) was combined with collagen microsponge as a biodegradable scaffold.The canine pulmonary artery trunk was patched with poly (lacticco-glycolic acid)-collagen patches with (n = 10) or without (n = 10) autologous vascular cellularization. 2 and 6 months after implantation, histologic and biochemical evaluations were conducted. In both groups, there was no thrombus development, and the poly (lactic-co-glycolic acid) scaffold was virtually fully absorbed. The development of an endothelial cell monolayer, a parallel alignment of smooth muscle cells, and a rebuilt vascular wall with elastin and collagen fibres were all seen on histologic examination.the patch's cellular and extracellular components had reached levels comparable to those found in native tissue. In cardiovascular surgery, this patch shows potential as a bioengineered material for facilitating in situ cellularization and autologous tissue regeneration.

In reconstruction of vascular wall [53]

A collagen microsponge was combined with a biodegradable polymeric scaffold made of polyglycolic acid knitted mesh and strengthened on the exterior with woven polylactic acid to create the tissue-engineered patch. The porcine descending aorta (n = 5), the porcine pulmonary arterial trunk (n = 8), and the canine right ventricular outflow tract (as the larger graft model; n = 4) were all grafted with tissue-engineered patches that were not precellularized. Histologic and biochemical assessments were performed 1, 2, and 6 mo after the implantation. No thrombus formed

in any of the animals. All of the grafts exhibited excellent in situ cellularization by hematoxylineosin and immunostaining two months after grafting. Two months after implantation, a polymerase chain reaction investigation of the cell population revealed a significant number of endothelium and smooth muscle cells. The architecture of the patch in the massive graft model was comparable to that of native tissue 6 months after implantation, indicating that this patch may be employed as a novel surgical material for circulatory system repair.

Microsponges for Biopharmaceuticals Delivery [54]

The microsponge delivery system (MDS) is used in both drug delivery and tissue engineering. The hybrid 3D scaffolds combine the benefits of natural type I collagen with synthetic PLGA knitted mesh. Collagen microsponges aided cell seeding and tissue development, while a skeleton made of mechanically robust PLGA mesh acted as a support. Three groups of scaffolds were created:

- Thin: collagen microsponge formed in interstices of PLGA mesh;
- Semi: collagen microsponge formed on one side of PLGA mesh;
- Sandwich: PLGA mesh with collagen sponge on both sides.

Recent advances in micro sponge drug delivery system

Modifying the ways to create Nan sponges, nanoferrosponges, and porous micro beads led to a number of advances. - In contrast to polymeric micro or nanosponges, CD nanosponges have been created that can be utilised for both hydrophobic and hydrophilic drugs. As a model drug, dexamethasone, flurbiprofen, doxorubicin hydrochloride, itraconazole, and serum albumin were examined using these sophisticated systems.These nanosponges were created by treating the CD molecule with biphenyl carbonate to crosslink it. Nanosponges have also been recognized by certain researchers to be an excellent vehicle for the delivery of gases. Researchers also discovered that including a cytotoxic in a nanosponge carrier system can double the drug's effectiveness, implying that these carriers could be utilised to target malignant cells.

CONCULSION

The Microsponge delivery system is a unique technology for the controlled release of macro porous beads, loaded with active agent, offering a potential reduction in side effects, while maintaining their therapeutic efficacy. The Microsponge drug delivery system offers entrapment of its ingredients and is believed to contribute toward reduced side effects, improved stability, increased elegance, and enhanced formulation flexibility. In addition, numerous studies have confirmed that Microsponge systems are nonirritating, no mutagenic, no allergenic, and nontoxic. This technology is being used currently in cosmetics, over the counter skin care, sunscreens, and prescription products. This kind of drug delivery technology may lead to a better understanding of the healing of several diseases. Hence, the Microsponge based drug delivery technology is likely to become a valuable drug delivery matrix substance for various therapeutic applications in the future.

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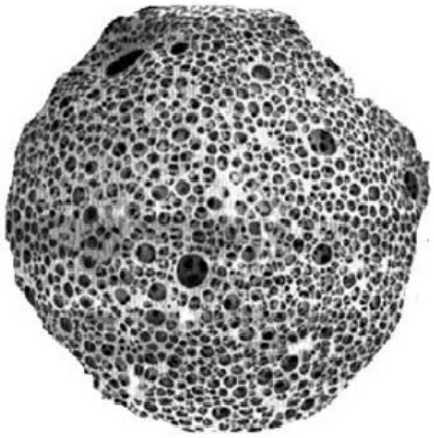

Figure 1: microsponges

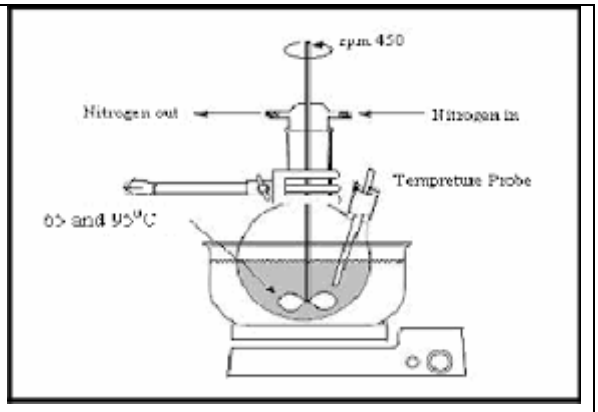

Figure 2: Microsponges preparations by liquid-liquid Suspension polymerization

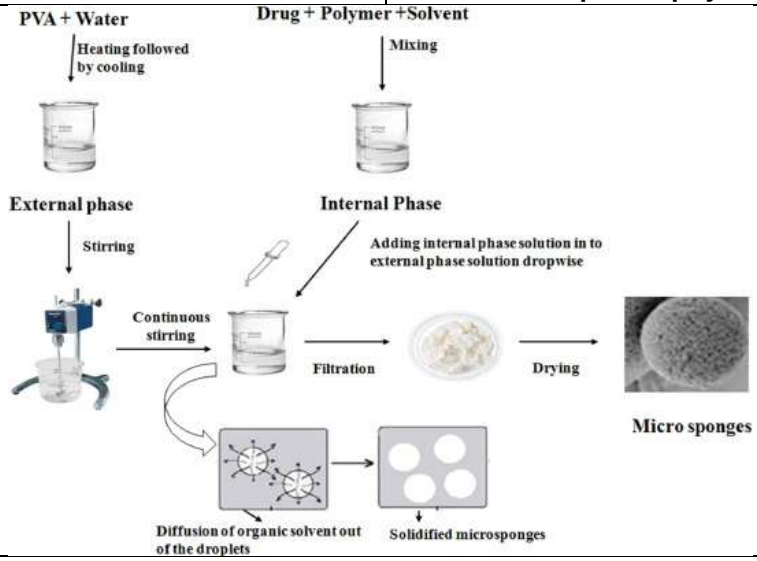

Figure 3: Preparation of microsponges by quasi emulsion solvent diffusion method

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RESEARCH ARTICLE

Survey, Pathogenicity, and Characterization of Different *Colletotrichum* spp. Associated with Chilli Anthracnose Disease

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ABSTRACT

A survey was conducted in March 2021 to study the incidence of Chilli anthracnose in major chilli growing areas of Andhra Pradesh and Tamil Nadu and also to study the cultural, morphological and pathogenicity of different isolates associated with the disease. Among the surveyed regions, the disease severity ranged between 8.13 to 38.66%. Highest disease incidence was recorded from Ramakoodal village of Dharmapuri district in Tamil Nadu whereas lowest disease incidence was recorded from Palur village of Cuddalore district in Tamil Nadu. Among the different inoculation methods tested, Pin pricking was found to be most effective with average lesion size of 6.90mm and PDI of 58.81%. Different isolates were tested for the development of disease under pot culture and highest disease incidence was reported from C 7 (65.3%) which was isolated from Kamapatti village of Tamil Nadu. The majority of the isolates exhibited light brown color of their mycelia and with cottony and fluffy growth. The highest colony diameter was recorded from C 11 (89.9mm). The colour of acervuli in all the isolates was found to be black.

Keywords: Chilli anthracnose, morphological characterization, pathogenicity, survey, *Collectotrichum* spp., inoculation methods.

INTRODUCTION

Chilli (*Capsicum annum L.*) is one of the largest cultivated spice crops from the Solanaceae family. There are more than 400 different chili varieties that are grown all over the world. It is well known for its pungency and color. It was introduced by the Portuguese into India in the 17th century. India is the largest producer, exporter, and consumer of

chillies in the world. In the year 2019 – 2020, Indian Chilli was grown in an area of about 18.11 lakh acres (7.33 lakh hectares) with an overall production of 17.64 lakh tonnes while the productivity remained at 2400 kg per hectare(Chilli Outlook). Major Chilli growing states in India are Andhra Pradesh with a production of 7 lakh tonnes, Telangana with a production of 3.06 Lakh tons, Madhya Pradesh with a production of 2.18 lakh tonnes, and Karnataka with a production of 1.80 lakh tons. Tamil Nadu ranks in 7th position in terms of area and production (22900Tons) (www.horticulturalstatistics.com, 2019-2020).

The production of Chillies is constrained by several biotic and abiotic factors which include diseases caused by various fungi and bacteria. Some of the important diseases of chilli are Alternaria leaf spot, *Cercospora* leaf spot, powdery mildew, anthracnose, chilli mosaic, and leaf curl. Among them, chilli anthracnose caused by *Colletotrichum* spp.is most destructive as it not only causes damage in the field but also to the harvested produce by bringing down their quality (2). *Colletotrichum* spp. has been recorded as one of the ten most destructive plant pathogens in the world (3). Even a small lesion on the fruit will reduce the marketability of the produce (4).

Economic Importance: Crop losses of more than 50% are reported throughout different parts of the country and post-harvest losses of up to 80% have been recorded (5). This disease occurs on all the aerial parts of the plant and chiefly it causes fruit rot to both green and ripe fruits which are of economic significance. The magnitude of this disease is more on the ripe fruit; hence it is also called as ripe fruit rot of chilli. Important environmental factors leading to the disease development are high humidity, high rainfall intensity and duration which prolong leaf wetness which has been directly related to the severity of the disease (6).

Symptomatology: The characteristic symptom of this disease appears as multiple sunken circulars or angular lesions which often coalesce to form severe fruit rot. These lesions are characterized by the presence of black-colored spots in concentric rings when matured which are initially orange or pink in color (6). These are called acervuli containing setae entrapping conidia. The symptoms also appear on stems and leaves which results in defoliation. The infection of the growing tip results in necrosis of branches which proceeds backward and killing it (Die backstage). This dieback may kill the whole plant. The objective of the present study is to survey different chilli growing areas from Tamil Nadu and Andhra Pradesh to know about the present status and severity of chilli anthracnose disease. Doing so will helps in understanding the distribution of pathogen populations and different factors which contribute to the severity of the disease.In order to understand the ecology, virulence, and evolutionary aspects of *Colletotrichum* spp. causing chilli anthracnose, it is essential to study the cultural, morphological and pathogenic variability among different isolates of *Colletotrichum.* These studies help in identifying different biotypes of pathogens present across different geographic locations. Method of inoculation plays a major role in early establishment of the pathogen. So, different inoculation methods were tested to see their influence on the virulence of the isolates. So far, very few studies have been conducted with respect to *Colletotrichum* spp. and most of them are related to the control and management aspects of chilli anthracnose disease.This study aims at understanding the occurrence and severity of chilli anthracnose disease present across major chilli growing areas of Tamil Nadu and Andhra Pradesh and to study about the cultural, morphological and pathogenic variability among different isolates responsible for the disease.Studying these aspects helps in understanding the ecology and evolutionary aspects of the pathogens which in turn is very useful for planning management strategies under field conditions.

MATERIALS AND METHODS

Disease Survey

A roving survey was conducted in major chilli growing districts of Tamil Nadu and Andhra Pradesh. From each district, one or two villages were selected at random based on the occurrence and severity of the disease. Two fields were selected from each village and from each field five plots were selected at random with an area of 5 m^2 from which one plot was plotted at the center of the field and the remaining plots were plotted at random locations away

from the border rows of the field. Infected chilli plants were identified based on the symptoms of leaf and fruit infection which were collected separately in polythene bags and neatly labeled. They are brought to the laboratory and stored under proper conditions (4^0C) for further studies. The assessment of disease incidence was done by counting the number of infected plants (leaf infection and fruit rot) out of the total number of plants present in each plot ($5m^2$). Mean disease incidence was calculated from two fields in each village. The percent disease incidence was calculated by using the formula (7).

$$\text{Percent Disease incidence} = \frac{Number\ of\ plants\ affected}{Total\ number\ of\ plants\ observed} \times 100$$

The magnitude of infection on the chilli fruits is also calculated and expressed as percent disease indexas per the grade chart (Table:1)and using the formula given by Thaveedu S, 2019 (8).

The formula for calculating percent disease index (PDI)

$$\text{PDI} = \frac{Sum\ of\ numerical\ rating}{total\ no.of\ fruits\ observed} \times \frac{100}{Maximum\ grade\ observed}$$

Isolation of Pathogen

The plant samples were brought to the lab and washed thoroughly in tap water for removing all the debris from it. A small portion of the infected tissue along with a healthy portion was exercised by using a sterile scalpel (2×2 mm). These bits are now surface sterilized in 70% alcohol for 20 seconds followed by 1% sodium hypochlorite for 1 min. Then they are washed thrice with sterile distilled water to get rid of the surface sterilizing agents and dried on a sterilized filter paper in the air of the laminar airflow chamber. After drying, these bits were aseptically transferred to Petri-plates containing potato dextrose agar medium (PDA) in such a way that each plate containing three bits towards the periphery of the plates. These plates are now incubated in biological oxygen demand (BOD) incubator at 28 ± 1^0C for 3 days. Purification is done by the single spore isolation method and the pure cultures thus obtained are maintained on PDA slants and stored in the refrigerator at 4^0C for future studies. The same procedure is adopted for all the isolates of the pathogen collected from different areas of Tamil Nadu and Andhra Pradesh.

Cultural and Morphological characterization

Nine mm discs of the 15 days old culture of pathogens were placed at the center of Petri plates containing 20 ml of PDA aseptically and incubated at 28 ± 2^0C for 20 days in BOD incubator. The mycelial and morphological characters like mycelial growth, color, the shape of conidia, and a number of septa per setae of isolates were observed. The radial growth of mycelia was measured eight days after inoculation along with the colony color, growth pattern on culture media.

Effect of different methods of inoculation for the development of fruit rot

Healthy semi-ripen chilli fruits are selected as they contain less phenolics and wax contents as compared to green fruits and also the incidence of chilli anthracnose disease is more on semi-ripen to ripe fruits (9). They are brought to the lab and are washed in running tap water. Now they are surface sterilized using sodium hypochlorite solution (1%) for 1 min. Now they are allowed to dry under aseptic conditions in Laminar airflow. The spore suspension was prepared from 15 days old culture of isolated pathogens and their concentrations were adjusted to 10^6 conidia per ml. Then these fruits were inoculated with isolates of *Colletotrichum* spp. using different inoculation techniques and then the size of the lesions was measured. The techniques used were pin pricking followed by placing a disc of mycelia, spore suspension spray, injection of spore suspension, dipping of fruits in spore suspension, and placing a mycelial disc on the fruits. Separate controls were maintained for each inoculation method where the sterile distilled water is used instead of spore suspension. The fruits are observed for 7 – 15 days for the development of symptoms and the size of the lesion was recorded and percent disease index was calculated.

Pathogenicity test

In this experiment, the virulence of the isolates will be determined. All the 20 isolates collected were evaluated for their virulence by pathogenicity test. Five kilograms (KG) of topsoil collected from the chilli field was collected and was sterilized using discontinuous heating (tyndallization) for 3 days and filled in cement pots (30cm × 60cm). All the isolates were cultured on PDA at 28^{0}C in a BOD incubator. The inoculum of each isolate is multiplied on corn sand (1:9) based medium and mixed with the soil in the pots at the rate of 20grams per kg of soil. Chilli seedlings of variety K-2 which are one month old were transplanted into the pots and the whole experiment is replicated thrice. Soil moisture was maintained at 25% by regularly irrigating the pots with sterilized water. The plants are observed for the development of symptoms and the percent disease incidences on both fruits and foliage is calculated as per the formula mentioned above and mean disease incidence is recorded. Healthy chilli fruits are selected and while they are attached to the plants inoculated with the pathogen by the pinprick method. Chilli fruits which are inoculated with sterile distilled water served as control. The fruits are observed for 7 – 15 days for the development of symptoms. The intensity of the fruit rot is calculated as per the grade chart and formula mentioned above and expressed as the percent disease index.

Experimental Design and Statistical analysis

All the laboratory experiments were laid out in a completely randomized design (CRD) the pot trails were laid out in Randomized Block Design with 3 replications. Statistical analyses were carried out using SPSS 16.0 (SPSS Inc). The level of significance between the data was taken at $P< 0.05$.

RESULTS AND DISCUSSION

Survey

A survey was conducted during March 2021 in the major chilli growing areas of Tamil Nadu and Andhra Pradesh to assess the incidence of anthracnose disease. Results revealed that the disease was present in all the surveyed districts of Tamil Nadu and Andhra Pradesh with varying magnitude. The highest disease incidence was recorded from Ramarkoodal village of Dharmapuri district in Tamil Nadu (38.66%)It was followed by Ponnaluru village in Prakasham district of Andhra Pradesh with disease incidence of 36.69%. The lowest incidence of chilli anthracnose in Tamil Nadu was recorded from Palur village (8.13%) of Cuddalore district followed by Dondapadu village (10.7%) of Guntur district in Andhra Pradesh. Similarly, villages from four districts; Theni (14.76%) and Namakkal (14%) from Tamil Nadu, West Godavari (14.66%), and East Godavari (14.06%) from Andhra Pradesh recorded significantly similar incidence of chilli anthracnose disease (Table 2). The highest percent disease index on chilli fruits was reported from Ponnaluru village of Prakasham district in Andhra Pradesh (35.53%). This was followed by Siripuram village (32.83%) from Guntur district in Andhra Pradesh. Whereas, lowest percent disease index was recorded from Palur village of Cuddalore district in Tamil Nadu.

The survey conducted in order to assess the disease incidence of chilli anthracnose in major chilli growing areas of Andhra Pradesh revealed that disease incidence ranged between 10% to 36.69% and percent disease index ranged between 12.8% to 35.53%. Whereas in Tamil Nadu the disease incidence ranged from 8.13% to 38.86% and percent disease index ranged from 9.86% to 30.76%. This variation in the disease incidence and percent disease index among different places might be due to the presence of isolates with varying virulence and also due to resistance expressed by different host crop varieties (10). It was earlier reported that the maximum incidence of chilli fruit rot was observed in Perumalpatti in the Theni district and a lower incidence at Virudhunagar district in Tamil Nadu (8). Similarly, the maximum disease incidence was reported at Theni at 19% and lower disease incidence at Sathanur of Perambalur district in Tamil Nadu (11). 60.33% of fruits were found to be infected in the surveyed 4 locations in Jaipur district where as the maximum infection was 66.70% (12). A survey was conducted in three taluks of Gujarat and reported that the disease incidence ranges in between 45 – 55% (13). Similarly (14) conducted a survey for chilli

fruit rot and reported maximum disease incidence from Kovilpatti and Sivapuri villages and stated that variation in the virulence of the isolates is due to difference in their virulence and susceptibility of the host.

Effect of different methods of inoculation for the development of fruit rot

Among the different methods of inoculation, the Pin Prick method was found to be most effective with an average lesion size of 6.90mm and PDI of 58.81%.This was followed by the Spore suspension injection method in which the average lesion size was 6.37mm and PDI of 51.41% (Table 3)which proves that the damaged skin of the fruit is favorable for fungi to penetrate and establish itself, whereas on the unpierced fruit, the pathogen has to break the skin of the fruit using mechanical force or enzymatic digestion which takes a considerable amount of time during which the pathogen may either die due to lack of nutrition. Fruit dip method produced small lesions with an average lesion size of 4.63mm and PDI of 36.88% followed by placing of mycelial discs with an average lesion size of 5.45mm and PDI of 43.3%. Control fruits in all the methods did not produce any symptoms. Experiments were conducted earlier by inoculating chilli fruits of different ages by using different inoculation methods revealed that pin pricking method was most effective and semi ripen to ripen fruits produced severe symptoms. (9) (15). Similar experiment was conducted earlier by inoculating the chilli fruits while they were still attached to the plants using different methods and reported that the pinprick method of inoculation produced the largest lesions on the fruits (10).

Pathogenicity test

Pathogenicity test was carried out on K-2 variety of chilli with the 20 fungal isolates under pot culture conditions. All the isolates are found to be virulent and produced fruit rot symptoms of varying degrees. The symptoms manifested were sunken necrotic lesions with acervuli initially pink which turned black in later stages in concentric rings and the presence of conidial masses. Severe conditions resulted in enlargement of lesions in elliptical shape which was spread across the fruit and turns the skin of infected portion to straw or white-colored. On leaves and stems, the symptoms appeared as water-soaked lesions which eventually enlarged and turned dark. Under extreme conditions, the whole branch died due to die-back. Among all the isolates tested C 7 from Kammapatti resulted in the highest incidence of fruit rot (percent disease index) which is 65.3%. This was followed by C 11 (59.1%) from Siripuram. Whereas, C 5 (53.64%) from Kodangipatty, C 14 (52%) from Tadepalli, C 3 (51.23%) from Thirumangalam, C 8 (51%) from Palur and C 6 (50%) from Swaminatham produced significantly similar percent disease index. The lowest incidence of percent disease index was reported from C 12 (27.86%) from Bellamkonda followed by C 19 (30.46%) from Chikkala, C 13 (31.5%) from Dondapadu and C 4 (33.06%) from Thondamuthur which are significantly similar. Highest leaf infection was produced in C 7 from Kamapatti which is 66.3% followed by C 11 (61.8%) and C 6 (58.6%). The isolates C 14 (49%), C 5 (49.6%) C 3 (50.3%), C 8 (51.3%) and C 9 (51.5%), produced significantly similar percent leaf infection on inoculation in the host plants.Lowest leaf infection was produced from C 12 from Bellamkonda which was 25.9%, C 19 (27.9%) from Chikkala and C 4 (28.8%) from Thondamuthur which are significantly similar. There was a significant difference in the virulence of the isolates as they are from different locations, presence of different biotypes, and also due to change in environmental conditions. The virulence of a pathogen changes from place to place, and it may be due to differences in temperature, humidity, rainfall etc. (11). Similarly, 15 isolates of *Colletotrichum capsici* were collected from Krishnagiri and Cuddalore districts of Tamil Naduproduced disease which varied between 15.32% to 36.15% (16).

Cultural and Morphological characterization

The majority of the isolates exhibited the light brown color of their mycelia. While some exhibited creamy white color, very few exhibited greyish color. The growth pattern of 11 isolates was found to be cottony while the remaining 9 isolates exhibited fluffy growth of mycelia (Table 5). The highest colony diameter was recorded from C 11 (87.9 mm). This was followed by C 7 with a diameter of 85.2 mm. The lowest colony diameter was recorded from C 9 (64.1 mm). Conidia produced by most of the isolates are falcate while some isolates produced spindle and cylindrical conidia (Table 5). The color of acervuli produced by all the isolates was dark brown. Number of septa ranged in between 2 – 6. In the majority of the areas, the pathogen responsible for chilli anthracnose was *Colletotrichum capsici* whereas at other locations the disease was caused by *C.gloeosporioides*, *C. acutatum, C. truncatum*

*and C. scovilei.*The cultural and morphological characters of the different isolates differed significantly. The results on culture variability in the present experiment are in line with the finding of several earlier researchers. 60 isolates of *Colletotrichum* spp. were collected and among them *Colletotrichum capsici* isolates produced whitish-grey colony with irregular margin and flat texture (17). The variation in the cultures of *Colletotrichum* may be due to the variation in environment and biotypes of the pathogen (18). Similarly, the results coincide with the earlier works of (19, 20).

CONCLUSION

The incidence of chilli anthracnose revealed significant variations from different parts of Andhra Pradesh and Tamil Nadu. The disease incidence was higher in areas where monocropping of chilli was in practice. It may be due to the buildup of pathogen inoculum in larger amounts over time.So, it's always better to practice crop rotation with non-host crops in order to reduce the magnitude of disease. The pathogenicity test revealed that among the 20 isolates tested, all the isolates produced disease of varying magnitude and C 7 from Kammapatti village recorded highest fruit rot incidence and highest percent leaf infection and these variations could be due to environmental conditions, and isolates with varying virulence. Among the different methods of inoculation tested, pin pricking method resulted in larger lesions and this was followed by spore suspension injection. It clearly states that the method which wounds the skin of fruit resulted into larger lesions as it will be easy for the pathogen to enter and establish as discussed earlier. The variation in cultural and morphological characters may be due to presence of different strains and biotypes of the pathogen. Although one major drawback with these kinds of studies is that the virulence of the isolates changes from place to place and also from one variety of crop to another. While majority of the studies aimed at management aspects of chilli anthracnose, this study focuses on variability and pathogenicity in pathogen population across different locations. Molecular studies have to be done to see the molecular variability among the virulent and non-virulent isolates. Different botanicals, bio-control agents and chemical fungicides can be tested against the virulent strains to develop an integrated approach for the management of chilli anthracnose.

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Table: 1. Grade Chart

Category Value	Percent fruit area infected
0	0
1	1 – 10
3	11 – 15
5	16 – 25
7	26 – 50
9	51 and above

Table: 2. Survey for the occurrence of chilli anthracnose in the districts of Tamil Nadu and Andhra Pradesh

S. No	Districts	Locality	Variety	Crop Stage	Disease Incidence (%)	Percent Disease Index
1	Theni	Pemuralpatti	K - 1	Fruiting	14.76	13.06
2	Dharmapuri	Ramarkoodal	C0 - 1	Fruiting	38.86	25.93
3	Madurai	Thirumangalam	K – 2	Fruiting	12.46	13.53
4	Coimbatore	Thondamuthur	K - 1	Vegetative	25.66	26.00
5	Dindigul	Kodangipatti	K - 1	Fruiting	29.83	30.76
6	Virudhunagar	Saminatham	IK - 1	Vegetative	15.33	15.26
7	Virudhunagar	Kammapatti	K – 1	Fruiting	12.73	13.96
8	Cuddalore	Palur	PLR – 1	Fruiting	8.13	9.86
9	Cuddalore	Sivapuri	CO - 1	Fruiting	20.16	24.66
10	Namakkal	Namakkal	CO - 1	Fruiting	14	15.56
11	Guntur	Siripuram	Guntur Sannam	Vegetative	25.82	32.83
12	Guntur	Bellamkonda	334	Fruiting	15.2	16.73
13	Guntur	Dondapadu	Teja	Fruiting	10.7	12.8
14	Krishna	Tadepalli	Ankur	Fruiting	23.82	24.7
15	Krishna	Krishnapuram	Teja	Fruiting	15.87	17.96
16	Prakasham	Parchur	273	Fruiting	24.12	26.1
17	Prakasham	Ponnaluru	Teja	Fruiting	36.69	35.53
18	West Godavari	Pallantla	Jwala	Fruiting	29.1	28.23
19	West Godavari	Chikkala	Bhagya lakshmi	Vegetative	14.66	16.43
20	East Godavari	Katravulapalli	Aparna	Fruiting	14.06	16.33

Table: 3. Effect of different methods of inoculation for the development of fruit rot

Isolate	Pin Prick		Spore Suspension Spray		Spore Suspension Injection		Fruit Dip		Mycelial Discs	
	Lesion Size (mm)	PDI	Lesion size (mm)	PDI	Lesion size (mm)	PDI	Lesion size (mm)	PDI	Lesion size (mm)	PDI
C1	8.86	73.53	7.93	66.33	8.63	69.66	7.1	57.33	7.73	67.66
C 2	7.23	65.26	6.86	56.33	7.06	58.33	6.3	50.33	6.9	58.33
C 3	5.43	44.1	4.5	33.66	4.83	39.66	3.43	31	4.16	31.66
C 4	7.26	64.3	6.63	53.66	6.93	55.33	5.2	38	4.96	39.66
C 5	6.36	58	5.3	44	5.73	47	4.13	30.33	4.9	37.33
C 6	6.4	53.66	5.06	41.66	5.5	45.33	4.5	31	5.26	41

C 7	10.6	86	9.36	82	9.56	82.33	7	54	8.13	67.33
C 8	9.43	80.66	8.53	74	9.06	76.33	6.5	52	7.16	59.33
C 9	5.26	45.66	4.13	36	4.26	37	2.23	13.66	3.26	20.33
C10	5	41.33	3.86	30	4.46	33	3.2	24	4	31.66
C11	8.66	76.33	7.7	63.33	8.5	66.33	5.7	45.66	6.03	49.33
C 12	4.23	36.66	3.23	25.33	3.7	29.66	2.26	16	2.63	22
C 13	5.33	46	4.26	32	4.53	35.33	2.86	20.33	3.6	24.66
C 14	8.66	71.33	7.46	61	8.03	66.66	6.46	52	7.3	58.66
C 15	6.7	52.66	5.73	47	6.2	49.66	4.5	34	5.1	38
C 16	6.16	51.33	5.13	42.33	5.76	44.66	4.8	36.33	5.26	40
C 17	5.63	47.66	4.73	37.33	5.43	40.33	4.36	28.66	5.03	38
C 18	6.53	60	5.433	44.33	5.93	46	5.I23	41	5.8	48.33
C19	7.56	68.33	6.13	52.33	6.73	53.66	5.6	44	6.16	50
C 20	6.66	53.33	5.9	46.33	6.53	52	4.96	38	5.6	42.66
Average	6.90	58.81	5.90	48.45	6.37	51.41	4.63	36.88	5.45	43.3
C.D.	0.659	2.525	0.650	3.313	0.0739	2.747	0.493	2.812	0.438	2.273
SE(m)	.0230	0.880	0.227	1.155	0.258	0.957	0.172	0.980	0.153	0.792
SE(d)	.325	1.245	0.321	1.633	0.364	1.354	0.243	1.386	0.216	1.121
C.V.	5.762	2.592	6.659	4.128	7.004	3.225	6.174	4.604	4.853	3.169

Table: 4. Virulence of different isolates (Pathogenicity test)

Isolate	Locality	Fruit Rot Incidence (PDI)	Percent Leaves infected
C1	Perumal patty	45.73	46.1
C 2	Ramarkodal	33.86	31.4
C 3	Thirumangalam	51.23	50.3
C 4	Thondamuthur	33.06	28.8
C 5	Kodangipatty	53.64	49.6
C 6	Saminatham	50	58.6
C 7	Kammapatti	65.3	66.3
C 8	Palur	51	51.3
C 9	Sivapuri	48.86	51.5
C10	Namakkal	44.23	43
C11	Siripuram	59.1	61.8
C 12	Bellamkonda	27.86	25.9
C 13	Dondapadu	31.5	32.9
C 14	Tadepalli	52	49
C 15	Krishnapuram	40.53	38.9
C 16	Parchur	45.8	45.9
C 17	Ponnaluru	33.83	35.7
C 18	Pallantla	48	44
C19	Chikkala	30.46	27.9
C 20	Katravulapalli	47.1	47.9
C.D.		5.682	6.704
SE(m)		1.981	2.337
SE(d)		2.801	3.305
C.V.		7.513	8.979

Table: 5.Cultural and Morphological Characterizations

S. No	Colony Character		Colony Diameter (mm)	Conidial Morphology	Acervuli colour	No of septa	*Colletotrichum* species
	Mycelia Color	Mycelial Growth					
C 1	Light brown	Cottony	84.9	Falcate	Dark Brown	2 – 5	*Colletotrichum capsici*
C 2	Dull grey	Fluffy	83.9	Falcate and spindle	Dark Brown	3 – 6	*Colletotrichum capsici*
C 3	Light brown	Fluffy	79.3	Falcate	Dark Brown	2 – 5	*Colletotrichum scovillei*
C4	Light brown	Fluffy	68.32	Falcate	Dark Brown	3 – 5	*Colletotrichum capsici*
C 5	Creamy white	Cottony	74.8	Falcate	Dark Brown	4 – 6	*Colletotrichum acutatum*
C 6	Grey	Fluffy	79.2	Falcate and cylindrical	Dark Brown	4 – 5	*Colletotrichum gloeosporioides*
C 7	Whitish grey	Fluffy	85.2	Falcate	Dark Brown	3 – 5	*Colletotrichum acutatum*
C 8	Light brown	Cottony	70.6	Falcate and spindle	Dark Brown	2 – 5	*Colletotrichum truncatum*
C 9	Creamy white	Cottony	64.1	Falcate	Dark Brown	3 – 4	*Colletotrichum scovillei*
C 10	Light brown	Cottony	82.6	Falcate	Dark Brown	2 – 5	*Colletotrichum capsici*
C 11	Light brown	Fluffy	87.9	Falcate and spindle	Dark Brown	4 – 5	*Colletotrichum scovillei*
C 12	Creamy white	Cottony	72.4	Falcate	Dark Brown	3 – 4	*Colletotrichum capsici*
C 13	Dull grey	Cottony	79.8	Falcate and spindle	Dark Brown	3 – 5	*Colletotrichum capsici*
C 14	Light brown	Fluffy	78.3	Falcate	Dark Brown	4 – 5	*Colletotrichums covillei*
C 15	Light brown	Fluffy	73.4	Falcate	Dark Brown	2 – 4	*Colletotrichum capsici*
C 16	Creamy white	Cottony	65.4	Falcate and cylindrical	Dark Brown	2 – 5	*Colletotrichum acutatum*
C 17	Creamy white	Fluffy	84.2	Falcate	Dark Brown	3 – 4	*Colletotrichum capsici*
C 18	Dark grey	Cottony	79.3	Falcate and spindle	Dark Brown	2 – 5	*Colletotrichum scovillei*
C 19	Blackish	Cottony	73.6	Falcate	Dark Brown	4 – 5	*Colletotrichum gloeosporioides*
C 20	Light brown	Cottony	83.2	Falcate	Dark Brown	3 - 5	*Colletotrichum truncatum*
C.D.			1.929				
SE(m)			0.672				
SE(d)			0.951				
C.V.			1.487				

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RESEARCH ARTICLE

Foliar Applied Salicylic Acid and Ascorbic Acid Induced Physiological Changes Enhancing Salt Stress Tolerance and thereby Harvest Attributes in Cowpea Grown under NaCl Stress

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ABSTRACT

Among abiotic stresses soil salinity has become one of the major environmental constraints results in reduction of growth and yield of important crops around the world. Thus, to reduce the negative impact of NaCl stress on crops some alternative approaches like use of growth regulators and vitamins could serve as potential weapons to mitigate the deleterious effects of NaCl stress on agricultural crops. In this view, present study was conducted to find out the efficacy of exogenous applied salicylic acid (SA) (0.25mM) and ascorbic acid (AsA) (0.5mM) on physiological and yield traits of cowpea under NaCl stress (200mM) in pot culture. For determination of physiological traits fresh plant leaves were plucked from each treatment randomly on 30th, 40th and 50th DAS respectively, and seed harvest was done at maturity of seeds. The salt stress results decline in relative water content and membrane stability index and a tremendous increase in ion leakage. Similarly, NaCl stress also caused highly reduction in grain yield. However, foliar application of SA and AsA results increased membrane stability index (MSI) and relative water content (RWC) of plants and thereby declining electrolyte leakage (EL). Which helps plant to regain osmoticum and provides NaCl stress tolerance that aid in improved yield and yield attributes of cowpea plants when treated with NaCl stress.

Keywords: NaCl stress, physiological changes, yield traits, *Vigna unguiculata* L.

INTRODUCTION

Soil salinity has become a major agricultural problem of the modern world [1]. More than 45 million hectares of irrigated lands have been damaged by salt globally, and an estimation of 1.5 million hectares are taken out of production each year from high salinity levels in the soil [2-3]. An estimation of around $14-19 million US dollars lost due to crop research has mostly centered on soil salinity that results huge global economic losses [4,2,5]. The reason being low rainfall, high evaporation rate, poor irrigation water, and poor water management induces declining in soil fertility and increasing soil salinization and also extensive use of mineral fertilizers, which are considered the main problems faced by farmers all over the world [6]. The excess amounts of soluble salts cause osmotic stress, while exchangeable sodium (Na+) causes ion toxicity under salinity stress [7-8]. The results would be oxidative stress led by excess generation of ROS that disrupts or halts the essential metabolic activities of plant due to increased amounts of salt ions in the soil medium. Thus, results decreased plant growth and productivity [9-10]. Furthermore, the imbalance between the ROS production and antioxidant defense systems triggers excessive ROS accumulation and induces oxidative stress in plants and become one of the most significant consequences of salt stress [11-12].

Therefore, improving plant tolerance to salinity stress is one of the current major objectives in achieving sustainable agriculture. This requires an understanding of the inherent mechanisms in plants that enable continued survival and growth when subjected to changes in soil salinity [13]. Therefore, it is evident that tolerance to salinity stress is a complex trait, and no single strategy can be used in obtaining salinity stress tolerance**.** Ascorbic acid (AsA), commonly known as vitamin C, is a major non-enzymatic antioxidant in plants. Which plays an important role in mediating certain oxidative stresses caused by abiotic stress including salt stress [14,1]. AsA can enhance the growth performance of a plant and enhance its capacity to withstand stress [15]. Moreover, AsA is the first line of plant defense against oxidative stress by removing a number of free radicals, such as $O2^{\bullet-}$, HO•, and H_2O_2, mostly as a substrate of APX, an essential enzyme of the ascorbate–glutathione pathway [15-14]. Vitamin-C plays a significant role in growth of maize plants by enhanced activation of antioxidant defence system [16], increasing crop production in millet [17], maintaining growth, pigment and ion homeostasis in barley plants [1].

Salicylic acid (SA) is a phenolic compound and a gaseous signaling phytohormone, which ameliorates effects of salinity on growth and development of plants [18]. As a phytohormone, SA vitally regulates growth and multiple developmental events like photosynthesis, ion uptake, antioxidant defense activity, and inducing salt stress tolerance [19-21]. *Vigna unguiculata* L. Walp (Cowpea) is an economically essential legume crop belongs to family Fabaceae. Due to its high nutrition values, it is of great importance in under-developed and developed countries as well [22]. Cowpea is a valuable source of income for farmers and grain traders in many African countries [23]. Cowpea is a drought tolerant, thrives well in poor soil conditions. However, it shows less resistance to salt stress. Since, its yield is greatly affected by saline conditions [22]. In this regard, present work was assessed to find foliar applied SA and AsA selected could alter morphological and physiological changes of cowpea and thereby enhances salt tolerance to increases crop growth and yield productivity.

MATERIALS AND METHODS

Plant material

The Cowpea variety (vamban-1) seeds were collected from National Pulses Research Centre, Vamban, Tamil Nadu, India.

Growth regulators and NaCl Salt

The growth regulators-salicylic acid and ascorbic acid, and analytical reagent grade NaCl as well were purchased from HI-Media company Mumbai Ltd.

Experimental setup

The seeds selected were planted in plastic pots filled with 8 kg of homogenous mixture of red soil, sand and farmyard manure in the ratio (1:1:1) for sowing purpose. Subsequently, NaCl salt treatment 500ml L^{-1} was imposed on 15th days after sowing (DAS). The salt stress was given in increments to achieve the desired concentration (200mM) and avoid salt shock. Thereafter salt concentration required was maintained through soil EC and last up to end of the experiment. In addition, foliar supply of SA and AsA solutions were made ready. However, spraying was done twice a week and uniformly sprayed on both the surfaces of leaves, using Tween-20 (0.05%) as a wetting agent. Generally, spraying was done manually using normal spraying bottle and soil surface was covered by a polyethylene sheet during spraying. However, control plants were irrigated with tap water. The pot culture arranged was in a Completely Randomized Block (CRB) manner. Plants were taken into six groups (T1-T6) with three replicates(n=3) each group. The samples were collected for observations on 30th, 40th and 50th DAS respectively. The treatments used in the experiment are as follows

Estimation of relative leaf water content (RLWC)

The relative water content (RWC) was determined in fresh leaf discs of 2cm^2 diameter, excluding midrib. Discs were weighed quickly and immediately floated on deionized distilled water (DDW) in Petri dishes to saturate them with water for the next 4h, in dark. The adhering water of the discs was blotted and turgor mass was noted. The dry mass of the discs was recorded after dehydrating them at 80°C for 24 h in a hot air oven. RWC was calculated according to the formula [24].

$$\text{RWC (\%)} = \frac{[\text{FW} - \text{DW}]}{[\text{TW} - \text{DW}]} \times 100$$

Membrane stability index (MSI)

Membrane stability index (MSI) was estimated by taking 100 mg leaf tissue to 10 ml of DDW in two separate sets each. One set was boiled at 40°C for 30 min in a water bath and its electrical conductivity bridge C1 was measured with a conductivity meter (LABTRONICS-Model LT-23). The second set was heated at 100°C in a water bath for 10minutes and the electrical conductivity C2 was also measured using a conductivity meter. MSI was calculated using the formula [25].

MSI = [1 – (C1/C2)] × 100

Electrolyte leakage determination (EL)

The total inorganic ions leaked out in the leaves were estimated by the method [26]. leaf discs 20 in number were taken in a boiling test tube of 10 ml deionized water and electrical conductivity (ECa) was measured. The content was heated at 45°C and 55°C for 30 min each in a water bath and electrical conductivity (ECb) was measured. Later the content was again boiled at 100 °C for 10 min and electrical conductivity (ECc) recorded. The electrolyte leakage was calculated by using the formula:

$$Electrolyte\ leakage\ (\%) = \frac{EC_b - EC_a}{EC_C} \times 100$$

Yield and yield traits

For determination of grain yield and other related characters fully matured pods were harvested from each plant separately. Afterwards, pod length, pod number and number of seeds per pod were determined and recorded. Subsequently, the seeds were separated from each pod and dried in order to quantify the 100-seed weight and yield per plant.

Statistical Analysis

The data were analyzed statistically using SPSS software (version 22.0) followed by one-way ANOVA. The obtained data represented in bars are mean values of three replicates (n=3), and (±) standard error (SE). The 0.05 % was chosen as significance using Duncan's Multiple Range Test (DMRT).

RESULTS

Leaf relative water content (LRWC)

The NaCl stress given at 200mM level significantly declined relative water content (RWC) in the leaves of cowpea plant displayed in Figure 1. The decreased percentage noted at 200mM NaCl treatment were 59 % (RWC) than that of unstressed plants (control) which were recorded 91% on 50DAS. On other hand, cowpea plants when treated with foliar applications of SA and AsA either alone (non-stressed) or in presence of NaCl showed maximum enhancement in leaf relative water content. This rose provides better surveillance to cowpea under continuous exposure of NaCl salt stress. The increase in RWC recorded in cowpea plants after sprayed with SA alone was 96 % and under salt treatment was 86.6%. Similarly, AsA supplied shows positive trend both alone and when applied to salt treated cowpea plants and values recorded were 93% and 86.86% respectively on 50DAS.

Membrane stability index (MSI)

The reduction in membrane stability index of cowpea plants treated with NaCl stress was noticed 64% percent than control one% as represented in figure 2. The increase in MSI values after NaCl treated plants combined with SA and AsA were 83% and 80% of respectively. It represents that external application of SA and AsA have made tolerance in cowpea plant against NaCl stress and displays maximum enhancement of growth of cowpea plant. Moreover, SA and AsA treated plants under non-stressed conditions provides further increase in membrane stability of cowpea on 50 DAS compared to control one.

Electrolyte leakage (EL)

The EL of cowpea plants treated with 200mM NaCl shown a significant rose in membrane leakage compared to control (Figure 3). The increased percentage of electrolyte leakage observed in NaCl stressed plants was 67% and in control it was 44 % on 50th DAS. Besides, application of SA and vitamin C showed a great reduction of EL content of cowpea leaves when treated against NaCl stressed plants. The reduction noted was 52.55 % and 46.8 percent respectively, on 50th DAS. It indicates a positive role of SA and AsA in relieving stress of NaCl treated cowpea plants. Moreover, cowpea plants treated alone with SA and AsA also have significant effect on ion-leakage control and values recorded are 41.5% and 37.45% respectively on 50DAS.

Yield and Yield attributes

The results of the pot culture experiment showed that NaCl stress (200mM) significantly reduced the grain yield and reduction noted was 45.10%, and also found variations in other yield parameters of cowpea compared to control (Fig.4). With regard to yield parameters a significant reduction was also noted especially in case of pod number per plant 37.73% and 100-grain weight 34.70% compared to control. However, exogenous applied SA and AsA both under non-saline as well as saline conditions showed increased percent yield attributes i.e. pod length, seed no. per pod, pod no. per plant, hundred grain weight and yield per plant respectively. The values noted under stressed conditions were 67.73%, 72%, 69.7%, 73%, & 63% (NaCl +SA) and 71.7%, 74%, 73.24%, 76% & 64.56% (NaCl +AsA) respectively compared to Saline stress (200mM).

DISCUSSION

The rapid explosion of population and demand for food production has become a challenge for plant biologists and agriculturists due to changing environmental conditions. Among abiotic environmental stresses, salt stress plays a immense role in limiting the plant growth performance and yield of economically important agricultural crops around world [1]. However, by employing short term approaches to lessen the negative effects of salinity and to enhance the stress tolerance potential in plants could be a positive way to improve crop growth and production at global level [27]. One of these approaches is the application of some agronomic measures, i.e., the exogenous application of different growth promoters including vitamins, micronutrients and hormones [28].

Relative Water Content (RWC) and Membrane Stability Index(MSI) (%)

The NaCl-stress not only affects the growth and productivity of cowpea but also extends to hurt the membrane integrity by increasing the electrolyte leakage (EL) while reducing the MSI and RWC (Fig. 1&2). The present work show that NaCl stress significantly decreased RWC and MSI in cowpea plants when exposed to increased salt stress. while the exogenous application of SA and AsA retained back the membrane properties through improving high values of MSI and RWC. Thus, provides salt stress tolerance to the plants by maintaining water status of the cell. Similar findings for maize [29], wheat [30], choysum plants [31] and Faba Bean plants [32] have been reported earlier. The reduction in leaf RWC in salt-stressed plants might be associated with salt-induced water imbalance and decreased osmotic potential [31].

Electrolyte Leakage (%)

The presence of excess NaCl to plants contributes a significant increase in electrolyte leakage, compared to the control one. Our results show higher EL% in the leaf mainly due to the injuries in cell membrane generated from high NaCl-stress (200mM) led ROS-oxidative stress (Fig. 3). Several authors worked on various crops [33, 29, 34] reported increase in the amount of EL when plants are treated with increased NaCl concentrations and would be related to chain reactions initialized by free radicals [35]. However, treatment of the NaCl stressed plants with AsA and SA neutralized the ion toxicity and caused a significant improvement in the membrane stability by maintaining osmotic potential of cell. Our results are agreed with the results observed on *V. angularis* L. [36] and *Hordeum vulgare* L. [33], who reported soil salinity stress tolerance in plants would be reduction in the EL (%) and thereby enhancing RWC and MSI.

Yield

The yield characters such as pod length, number of pods, seed no. per pod, yield and 100 seed weight of cowpea plants were greatly reduced under salt stress (figure 4). The decrease in yield per plant rely on no. of pods. Under salt stress pod no. was significantly reduced as shown in the figure (4). This decrease might be due to stress-induced embryo abortion during flowering and pod setting [37]. The decrease in hundred-grain weight, observed in our study, reflects that the seed filling duration is decreased under salt stress, that results to small sized seeds [22]. Our results of decreased yield and yield traits are in line with those of [38,22] in cowpea and [1] in barley reported tremendous decrease of yield when plants are subjected to increased NaCl salt stress concentrations. On the other hand, foliar spray of SA and AsA improved yield attributes under non-saline and saline conditions as well. Similar results of exogenous application of SA and AsA improved yield attributes has been previously reported by several authors in barley [1,39], sweet pepper [21], and olive trees [40].

CONCLUSION

The yield of agricultural crops is severely affected by various environmental abiotic stresses, particularly salinity. The results showed that foliar application of SA and AsA can actively regulate the physiological changes such as RWC, MSI in order to ameliorate the negative effect of salinity by minimizing the ROS induced EL. These adjustments are the key mechanisms for cowpea to improve NaCl stress tolerance and maintain yield of the plant. Thus, it can be concluded that exogenous applied SA and AsA have positive effect on cowpea in improving yield of crop plants. Thus, exogenous supply of SA and AsA can be used to enhance salt stress tolerance of crop plants cultivated under saline soils.

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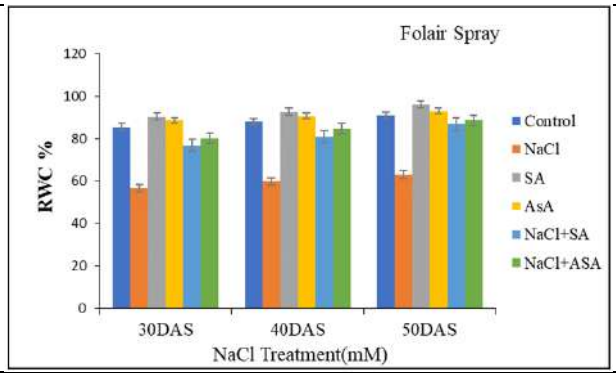

Fig. 1. Effect of foliar application of SA and AsA on RWC of cowpea (vamban-1 variety) under 200mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

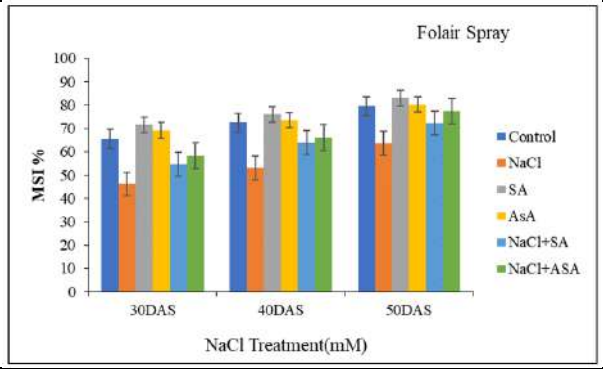

Fig. 2. Effect of foliar application of SA and AsA on MSI of cowpea (vamban-1 variety) under 200mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

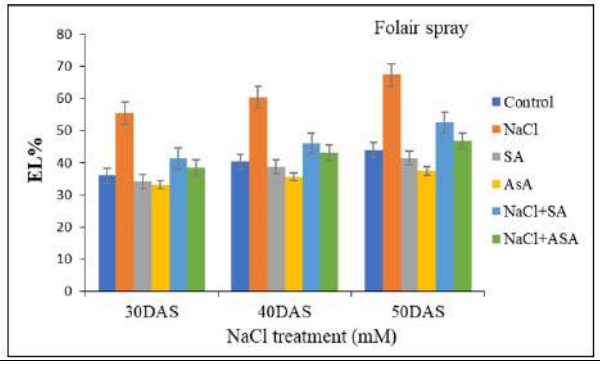

Fig. 3. Effect of foliar application of SA and AsA on EL of cowpea (vamban-1 variety) under 200mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

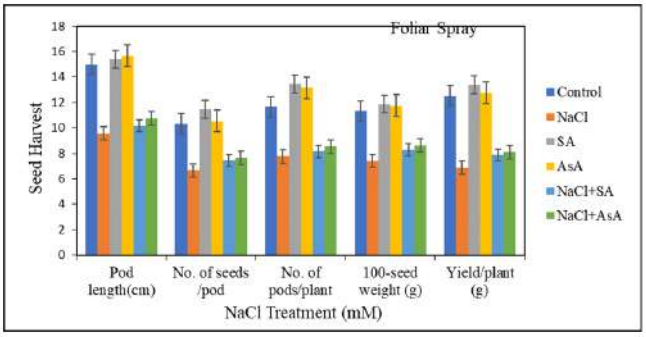

Fig. 4. Effect of foliar application of SA and AsA on yield traits of cowpea (vamban-1 variety) under 200mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

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RESEARCH ARTICLE

Formulation and Evaluation of Fast Dissolving Tablets of Nateglinide

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ABSTRACT

Nateglinide is an amino-acid derivative that lowers blood glucose levels by stimulating insulin secretion from the pancreas. The concept of Fast Dissolving Drug Delivery System emerged from the desire to provide patient with conventional mean of taking their medication. The quality of the drug and excipients was evaluated by various analytical techniques including FT-IR spectroscopy, Organoleptic techniques and angle of repose. The fast dissolving tablets were prepared by using direct compression method. 7 formulation batches were manufactured using this methodology. The prepared batches were evaluated for various qualitative and quantitative analytical techniques like disintegration time, dissolution rate and water absorption ratio. F6 had the least disintegration time and good water absorption ratio. The selected batch was subjected to stability studies as per ICH guidelines. This experimental study concludes that the enhancement of concentration of super-disintegrants can cause stability issues and needs to be protected. In this study, it was not an issue since the concentration was very less.

Keywords: Nateglinide, Disintegration and Fast dissolving tablet, Croscaramellose and wicking

INTRODUCTION

Nateglinide is an amino-acid derivative that lowers blood glucose levels by stimulating insulin secretion from the pancreas. It is mainly used for treatment of diabetes. Following oral administration immediately prior to a meal, naglinideabsorbwith mean peak plasma drug concentrations (Cmax) generally occurring within 1 hour (Tmax) after dosing.Plasma profiles are characterized by multiple plasma concentration peaks when nateglinide is administered under fasting conditions. This effect is diminished when nateglinide is taken prior to a meal. Hence, there is a need for the drug to be converted into fast dissolving tablets for providing room for improvement in absorption[1,2].

Fast dissolving tablet is an innovative tablet technology where the dosage form containing active pharmaceutical ingredients disintegrates rapidly, usually in a matter of seconds, without the need for water, providing optimal convenience to the patient. The concept of Fast Dissolving Drug Delivery System emerged from the desire to provide patient with conventional mean of taking their medication. Difficulty in swallowing (Dysphagia) is a common problem of all age groups, especially elderly and pediatrics, because of physiological changes associated with these groups of patients[3-6]. Ease of administration, quick absorption and dosage accuracy are some of the merits of fast dissolving drug delivery system[7]. The different types of preparation for fast dissolving tablets are as follows [8-11]: In this work, we discuss about the formulation and evaluation of nateglinide fast dissolving tablets.

METHODOLOGY

FT-IR Spectroscopy

IR spectra of drug in KBr pellets at moderate scanning speed between 4000-400 cm-1 was carried out using FTIR (Jasco FTIR 6100 type A). The peak values (wave number) and the possibility of functional group shown in spectra which compare with standard value. The comparison of these results with nateglinide chemical structure shows that the sample was pure nateglinide.

Solubility

It determined by dissolving drug substance in freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in chloroform and in ether.

Bulk Density

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weight powder (passed through standard sieve # 20) into a measuring cylinder and initial weight was noted. This initial volume is called the bulk volume. From this the bulk density is calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$D_b = M / V_b$$

Where,

M is the mass of powder

V_b is the bulk volume of the powder.

Angle of Repose

The friction forces in a loose powder can be measured by the angle of repose (θ). It is an indicative of the flow properties of the powder.It is defined as maximum angle possible between the surface of the pile of powder and the horizontal plane.It is evaluated by using fixed funnel method.

$$\theta = \tan^{-1}(h / r)$$

Where, θ is the angle of repose.h is the height in r is the radius in cms.

METHODOLOGY

The fast dissolving tablets are prepared using direct compression method. Nateglinide, and superdisintegrants, mannitol, micro crystalline cellulose, and aspartame were weighed accurately. Sieving through #40 mesh is done prior addition of other excipients like talc and magnesium stearate.The mixture was subjected to direct compression by rotary tablet machine post sieving through #80 mesh. The formulation table for the fast dissolving tablets was designed as follows:

Wetting Time

Five circular tissue papers of 10 cm diameter are placed in a Petri-dish with a 10 cm diameter. Ten millimeters of water-containing Eosin, a water-soluble dye, is added to petri-dish. A tablet is carefully placed on the surface of the tissue paper. The time required for water to reach upper surface of the tablet is noted as a wetting time.

Weight Variation

20 tablets were selected randomly from the lot and weighted individually to check for weight variation. Weight variation specification as per I.P. is shown in table No. 10.

Water Absorption Ratio

A piece of tissue paper folded twice was placed in a small Petri dish containing 6 ml of water. A tablet was put on the paper & the time required for complete wetting was measured. The wetted tablet was then weighed. Water absorption ratio, R, was determined using following equation,

R=100 (Wb-Wa/Wa)

Where, wb is weight of tablet before water absorption

wa is weight of tablet after water absorption.

Friability Testing

The crushing test may not be the best measure of potential behavior during handling and packaging. The resistance to surface abrasion may be a more relevant parameter. Friability of each batch was measure in "Electro lab friabilator". Ten preweighed tablets were rotated at 25 rpm for 4 min, the tablets were then re weighed and the percentage of weight loss was calculated**.**

F = (1 – W/Wo) 100

***In-vitro* Drug Release**

Release of the drug *in vitro*, was determined by estimating the dissolution profile**.**USP 2 Paddle apparatus was used and paddle was allowed to rotate at 50 rpm.0.01 N HCl (900 ml) was used as a dissolution medium. Determination of amount of drug dissolved form tablets was carried by Schimadzu UV 1601 spectrophotometer at 210 nm.

Stability Studies

Selected formulations were subjected to stability studies as per I.C.H. Guidelines.

Following conditions were used for stability studies

30°C/65 % RH analyzed at a time interval of 10 days till a period of 30 days

40°C/75 % RH analyzed at a time interval of 10 days till a period of 30 days

RESULTS AND DISCUSSION

FT-IR Spectroscopy

The drug was subjected to FT-IR spectroscopy by Potassium Bromide pellet technique. The FT-IR spectra of the drug was as follows:

The characteristic absorption peaks of nateglinide obtained at 3775cm^{-1}, 1714 cm^{-1}, 1280cm^{-1}, 680 cm^{-1}, were obtained in the given spectrum.

Solubility

It was found that the drug (Nateglinide) was freely soluble in methanol and other organic solvents like chloroform. But, it was practically insoluble in water and other hydrophilic solvents. This proves that nateglinide is lipophilic and hydrophobic in nature. It matches the criteria as per pharmacopeia.

Bulk Density

Bulk densities of the 7 batches are calculated and illustrated as follows:

Both the batches (F4 & F7) exhibit the highest bulk density of all formulation batches. The minimum bulk density was exhibited by the first batch (F1).

Angle of Repose

Angle of repose of the 7 formulation batches was calculated and represented graphically as follows:

It is observed that third batch (F3) has the maximum angle of repose of all formulation batches. Meanwhile, the most acute angle.i.e. lowest angle of repose has been exhibited by F5.

Wetting Time

The wetting time helps to estimate the disintegration rate of the fast dissolving tablet. The graphical representation of 7 batches for the wetting time is as follows:

It was found that F6 has the best dissolving capacity since it needed the least wetting time. But, F2 needed the maximum time of all formulation batches (F1-F7). So, F2 had the most inferior dissolving capacity of all batches.

Weight Variation

The average weight of the tablets of various formulation batches are illustrated as follows:

The F6 batch has the closest dose to 300 mg, which is the optimal dose for tablet. The other batches fluctuate with higher range when compared to F6.

Water Absorption Ratio

Water absorption ratio of 7 batches are evaluated and graphically represented as follows:

It was determined that F6 has the highest water absorption ratio, while the first batch (F1) had the least water absorption ratio.

Friability Test

7 batches are evaluated for friability and was graphically represented as follows:

The last batch (F7) showed the least friability, thereby having highest tablet strength of all batches. But, F5 had the least tablet strength due to highest friability of al 7 batches.

***In-vitro* Assay**

Dissolution studies was performed as per the protocol effectively and was determined to be as follows:

The sixth batch exhibited the best in-vitro drug release of all formulation batches with 100.5% in just 10 minutes. So, this batch was considered to be having the best fast dissolving tablets of all formulation batches.

Stability Studies

The stability studies of formulated tablets were carried out at 30◎C/65 % RH and 40◎C/75 % RH for two months. The effects of temperature and time on the physical characteristics of the tablets were evaluated. The stability study was carried out when the room temperature was 20 to 25ºC. The different parameters which were studied are Drug content (%), In vitro Disint. Time (secs), Wetting Time (secs), in vitro dissolution rate. Out of the all the batches (F1-F7), it was determined that F6 had the best stability of all batches. So, it was as follows:

At room temperature

CONCLUSION

It was found that nature of Disintegrant can modulate the disintegration time in fast dissolving tablets accordingly. It was found property of disintegration in the order as follows:

Crospovidone<Croscarmellose< Sodium Starch Glycollate

This feature concludes that the wicking type of Disintegrants can improve the disintegration rate thereby, diminishing the disintegration time. By comparing the various formulation batches of Nateglinide fast dissolving tablets, it was found that F6 was the best, since it revealed less disintegration time with high water absorption ratio. When tablets kept at real time (30/60oRH) and accelerated (40/75o RH) storage conditions, both disintegration time and hardness values decreased significantly. This may be due to loss of mechanical integrity. This experimental study concludes that the enhancement of concentration of super-disintegrants can cause stability issues and needs to be protected. In this study, it was not an issue since the concentration was very less.

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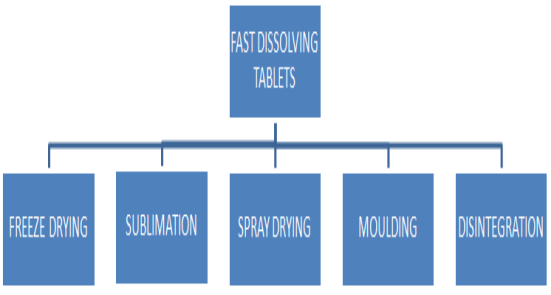

Fig.1: Fast dissolving tablets

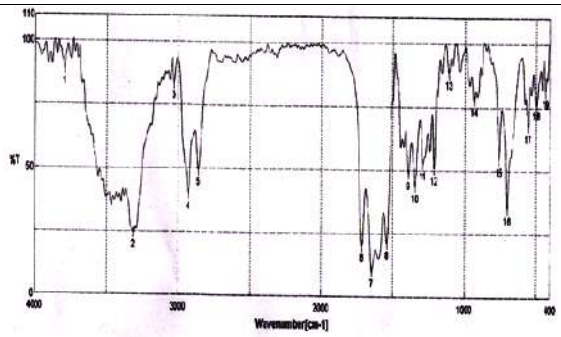

Fig.2 : FT-IR spectra of the drug

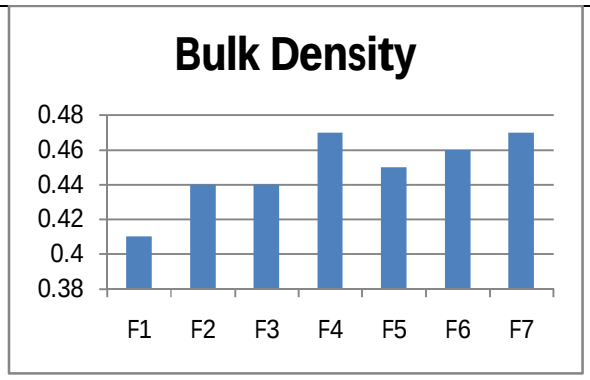

Fig.3 : Bulk Density

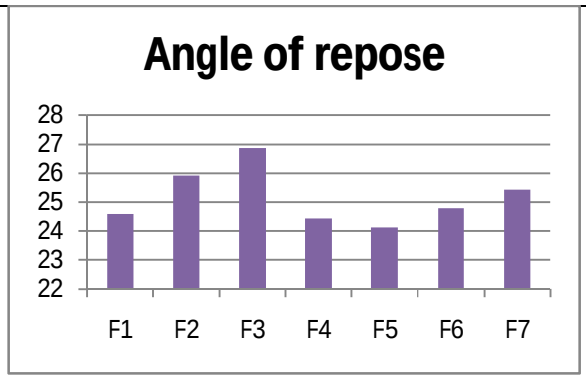

Fig.4 : Angle of repose

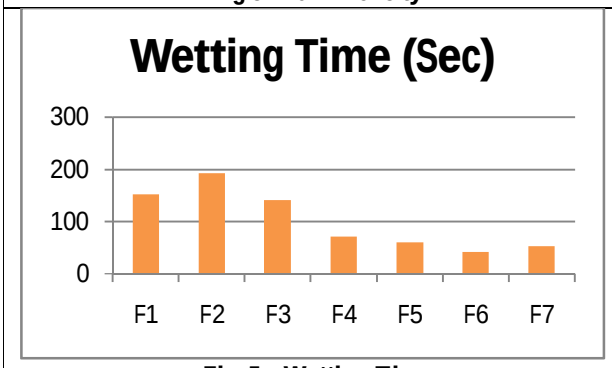

Fig. 5 : Wetting Time

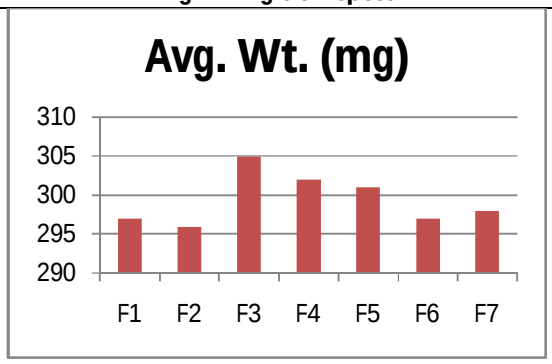

Fig. 6 : Average Weight

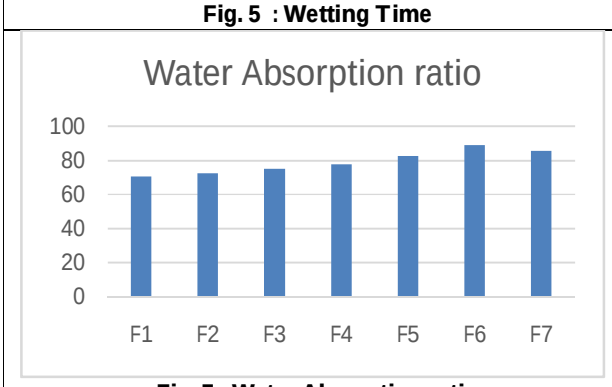

Fig. 7 : Water Absorption ratio

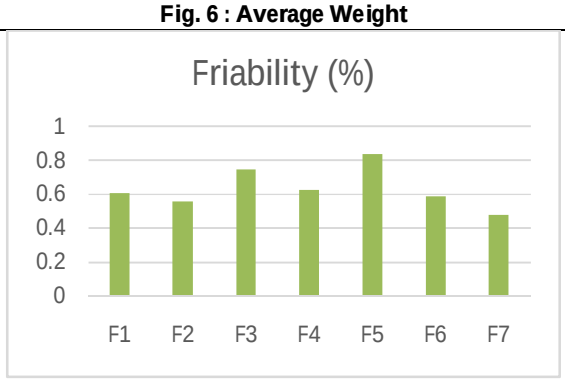

Fig. 8 : Friablity (%)

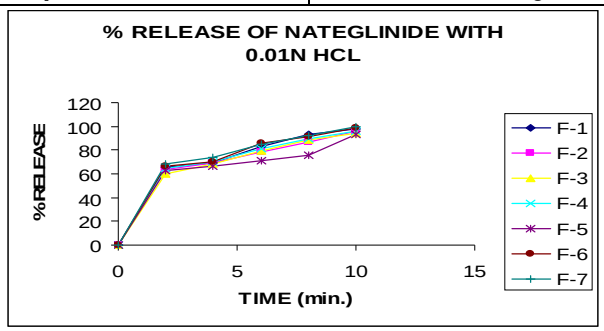

Fig. 9 : Dissolution studies was performed as per the protocol effectively and was determined

Table No.1 : The formulation table for the fast dissolving tablets was designed

SR.NO.	INGREDIENT	F1	F2	F3	F4	F5	F6	F7
1	Nateglinide	60	60	60	60	60	60	60
2	Croscarmellose Na	9	---	---	4.5	---	4.5	3
3	Na. Starch Glycolate	---	9	---	4.5	4.5	---	3
4	Crospovidone	---	---	9	---	4.5	4.5	3
5	Amberlite	9	9	9	9	9	9	9
6	Povidone	12	12	12	12	12	12	12
7	Aspartame	6	6	6	6	6	6	6
9	Mg Stearate	6	6	6	6	6	6	6
10	Mannitol	138	138	138	138	138	138	138
11	MCC	60	60	60	60	60	60	60

Table No.2 : Weight variation specification as per I.P.

Average Weight of Tablet	% Deviation
80 mg or less	±10
More than 80 mg but less than 250 mg	±7.5
250 mg or more	±5

Table No.3 : At room temperature in vitro dissolution rate.

Parameters	Controlled	After 15 days	After 1 month
Drug content (%)	100.23	99.98	99.91
Disint. Time (sec.)	30	30.5	31.4
Wetting Time(sec.)	42	43	44
Hardness(kg/cm²)	3.5	3.5	3.4
Friability (%)	0.48	0.48	0.46

Table No. 4 : At 40 degree Celsius in vitro dissolution rate.

Parameters	Controlled	After 15 days	After 1 month
Drug content (%)	100.23	99.94	99.90
Disint. Time(sec.)	30	31	31.5
Wetting Time(sec.)	42	43.2	44.5
Hardness(kg/cm²)	3.5	3.4	3.3
Friability(%)	0.48	0.47	0.45

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RESEARCH ARTICLE

Shock Induced Mixing of Fuel and Air in the Scramjet Combustor

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ABSTRACT

Combustion of Fuel in the Air environment is completely governed by the mixing efficiency of fuel with air in SCRAMJET engine. The mixing of fuel with air enhances the flame stabilization by increasing the flame speed within the combustor. The mixing of fuel with air is numerically simulated with modified DLR SCRAMJET engine combustor in order to study its mixing characteristics and efficiencies. The numerical simulation of hydrogen fuelled SCRAMJET combustor has been carried out by steady, compressible, and two-dimensional Navier-Stokes equation with SST k- turbulence model using commercial CFD software package. From the reported results, it has been identified that the formation of oblique shock waves is helpful to increase the mixing efficiency of the air and fuel. The oblique shock helps in modifying the velocity vector direction of the air in the combustor. The turbulent kinetic energy, velocity contour and static pressure clearly illustrate the role played by the oblique shock in enhancing the mixing of fuel with air.

Keywords: Combustion, Scramjet, Numerical simulation, Kinetic energy, Velocity

INTRODUCTION AND DESIGN PRINCIPLE

Sometimes engines also include a region which acts as a flame holder, although the high stagnation temperatures mean that an area of focused waves may be used, rather than a discrete engine part as seen in turbine engines. Other engines use pyrophoric fuel additives, such as silane, to avoid flameout. A scramjet is reminiscent of a ramjet. For a scramjet, the kinetic energy of the free stream air entering the scramjet engine is largely comparable to the energy released by the reaction of the oxygen content of the air with a fuel (e.g., hydrogen). Thus, the heat

released from combustion at Mach 2.5 is around 10% of the total enthalpy of the working fluid. Since the flow is supersonic, no downstream influence propagates within the free stream of the combustion chamber. Forcing the speed of air flow in the combustion chamber under Mach 1 in this way is called "thermal choking". It is clear that a pure scramjet can operate at Mach numbers of 6–8 but in the lower limit, it depends on the definition of a scramjet. There are engine designs where a ramjet transforms into a scramjet over the Mach 3–6 range, known as dual-mode scramjets. Further, vitiated facilities (with the ability to control air impurities, storage heated facilities, arc facilities and the various types of shock tunnels each have limitations which have prevented perfect simulation of scramjet operation. Computational fluid dynamics has only recently reached a position to make reasonable computations in solving scramjet operation problems. Much of scramjet experimentation remains classified. Several groups, including the US Navy with the SCRAM engine between 1968 and 1974, and the Hyper- X program with the X-43A, have claimed successful demonstrations of scramjet technology. Since these results have not been published openly, they remain unverified and a final design method of scramjet engines still does not exist. The final application of a scramjet engine is likely to be in conjunction with engines which can operate outside the scramjet's operating range.

GEOMETRICAL DESIGN & MESH GENERATION

DLR scramjet combustor model is modified for the cavity combustion as indicated in the figure-1. Here the wedge used to produce shock waves to increase the pressure and the rearward facing step used to create circulation to achieve the efficient mixing. The domain separated from the solid domain and Discretized as a structured grid with quadrilateral elements for the numerical simulation. The boundary conditions are applied as indicated in the figure 2. Here the inlet air assumed to be at the Mach number of 1.5 and the outlet is assigned as a velocity outlet. The hydrogen fuel is supplied at 100 m/s through the fuel inlet. The numerical equations are solved using the boundary conditions as indicated in the table-1. The walls of the combustor are defined as a solid wall with no slip boundary conditions. The stability of the solution in controlled by keeping the courant number as 0.5 for the analysis.

RESULT AND DISCUSSION

The mixing efficiency of air and fuel greatly affect the combustor performance. The resident time of air in the combustor is in milliseconds, within this short duration the air and fuel must get mixed properly. The turbulence in the supersonic combustor is not sufficient enough to increase the mixing efficiency. By creating more oblique shocks will help to increase the mixing efficiency. Here a wedge and sudden expansion in the combustor will be helpful to create more turbulence and oblique shocks to improve the mixing efficiency. DLR scramjet combustor geometry modified for the effective mixing and the fuel injected for the velocity of 100 m/s, 150 m/s and 200 m/s to understand the mixing characteristics. The Discretized computational model applied boundary conditions as listed in the table-1 and iterated for the solver conditions indicated in the table-2. The residuals of continuity, x-velocity, y-velocity, energy, specific rate of dissipation, and turbulent kinetic energy were monitored for solution convergence in the order of 10^{-5}.

From the wall static pressure distribution, it is observed that static pressure increases with the fuel injection velocity initially then no change in the static pressure after crossing the expansion fan the pressure further increases with fuel injection velocity both in the upper and lower wall of the combustor. The wall static temperature distribution on the upper and lower wall of the combustor. In the bottom wall, the temperature reduces with respect to increase in fuel injection speed. The wall static temperature not subjected to notable changes in the upper wall. The bottom wall temperature decreases mainly due to the fuel supplied at low temperature and the mass flow rate increases with velocity. High mass flow reduces the wall temperature of the combustor. The velocity profile at X=108 mm indicates the drop in the velocity will increase the air resident time in the combustor to ensure the proper mixing of air and fuel. Similar trend also absorbed at X=275 mm as indicated in the figure-5.4. At X=450mm the velocity almost constant for all the cases and it is for away from the combustion zone. The pressure distribution indicate the favorable condition for the effective mixing in the combustor. The turbulence kinetic energy, X- velocity and Y-

velocity distribution indicated in the figure-5.7, figure-5.8 and figure-5.9 are all having favorable results for the efficient mixing of the fuel and air.

CONCLUSION AND SUGGESTIONS FOR FUTURE WORK

The numerical simulation of hydrogen fuel scramjet combustor for basic DLR scramjet model and innovated models implies slightly modified versions of basic DLR scramjet model has been completed and discussed the results. From all the above reported contours and graphs it has been identified that the formation of oblique shock waves are helpful to increase the mixing efficiency of the air and fuel. The oblique shock helps to increase the resistant time of the air in the combustor. Turbulence model plays an important role in the modeling of combustion simulation; hence three main turbulence models has been considered and from the analysis of these results it is to be identified that k-ω model is the accurate turbulence model since its results have very much close approximation with experimental results. From all the above contours, graphs and results and discussion it is to be concluded that DLR scramjet model with additional wedge increases the mixing efficiency of fuel and air along with efficiency of high combustion.

ACKNOWLEDGEMENTS

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Table 1. Properties of air and hydrogen fuel

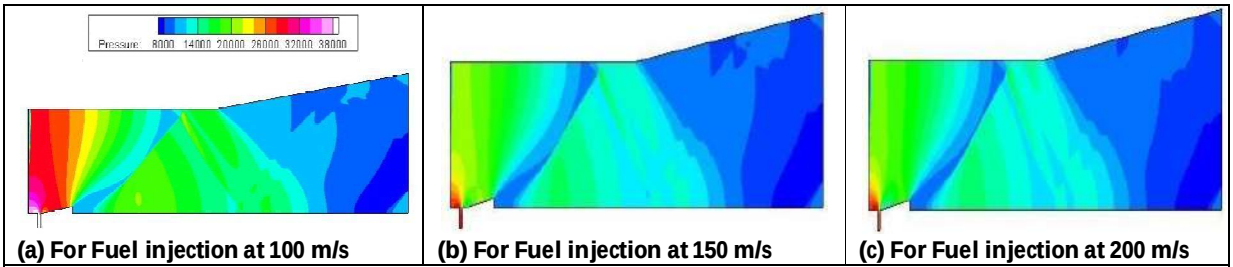
Property	Ambient air	Hydrogen fuel
Mach Number	1.5	0.3
Pressure Pa	12000	12000
Temperature K	1200	750
Density kg/m^3	1.002	0.097

Table 2. solver Type

Solver Type	Pressure- Based Solver
Turbulence Model	K-ω SST

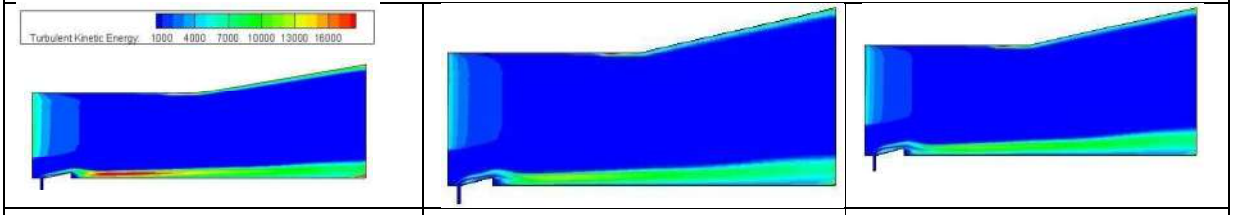
Table 3. solver Properties

Material property	**Method of calculation or value**
Density	Ideal gas law (kg/m^3)
Thermal conductivity	0.0242 W/m-K
Molecular weight	28.966 kg/kg mol
Specific heat	1006.43 J/kg-K
Viscosity	Sutherland's formula
Numerical scheme information	
Pressure velocity coupling scheme	Coupled
Gradient evaluation	Least squares cell-based
Flow spatial discretization	2ndorder upwind
Specific dissipation rate	2ndorder upwind
Solution control scheme information	
Courant number	0.5
Under relaxation factors	Default



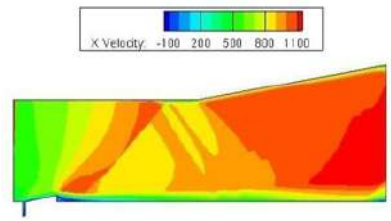
(a) For Fuel injection at 100 m/s **(b) For Fuel injection at 150 m/s** **(c) For Fuel injection at 200 m/s**

Figure 1. Pressure distribution in the combustor

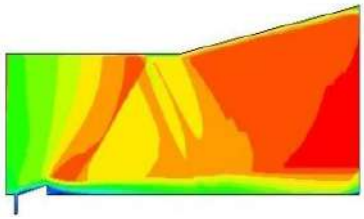


(a) For Fuel injection at 100 m/s **(b) For Fuel injection at 150 m/s** **(b) For Fuel injection at 200 m/s**

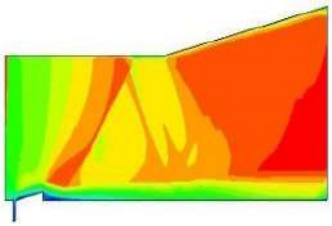
Figure 2. Turbulent kinetic energy distribution in the combustor



(a) Fuel injection at 100 m/s

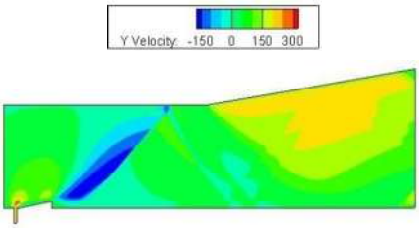


(b) Fuel injection at 150 m/s

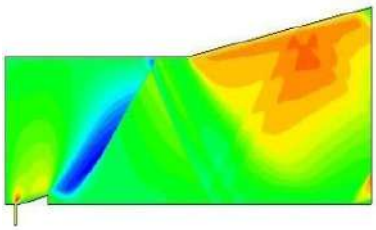


(c) Fuel injection at 200 m/s

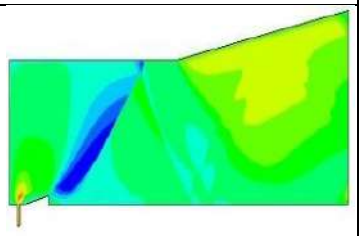
Figure 3. X- Velocity distribution in the combustor



(a) **Fuel injection at 100 m/s**



(b) Fuel injection at 150 m/s



(c) Fuel injection at 200 m/s

Figure 4. Y-Velocity distribution in the combustor

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RESEARCH ARTICLE

Synthesis of Substituted 1,2,4 Triazole Derivatives using Microwave

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ABSTRACT

A simple method have been developed for the synthesis of substituted 1,2,4-triazoles from hydrazines and formamide under microwave irradiation. Microwave-used organic synthesis provides an alteranative to conventional synthesis and reduction in reaction time. Triazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson's, antidiabetic, antiobesity and immunomodulatory agents, etc.

Keywords: Substituted 1,2,4-triazoles, Microwave-used, hydrazines, formamide

INTRODUCTION

This article deals with the investigation carried out by the laboratory synthesis of 1,2,4 triazole**.** The triazole nucleus is one of the most important and well known heterocycles which is a common and integral feature of a variety of natural products and medicinal agents. Triazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson's, antidiabetic, antiobesity and immunomodulatory agents, etc. The broad and potent activity of triazole and their derivatives has established them as pharmacologically significant scaffolds. The basic heterocyclic rings present in the various medicinal agents are 1,2,3-triazole and 1,2,4-triazole. A large volume of research has been carried out on triazole and their derivatives, which has proved the pharmacological importance of this heterocyclic nucleus. Literature reveals 1,2,4 Triazoles can be prepared by Pellizzari synthesis, Einhorn Burnner synthesis, Amidines, Acylhydrazines, Hydrazines etc.

MATERIALS AND METHODS

Microwave induced organic reaction enhancing the non-conventional technique for rapid organic synthesis. It is simple, inexpensive instrument, lesser quantity of solvents and eco- friendly technology. The oneequivalent Phenyl hydrazine and twenty equivalents formamide irradiated at 170'C for 8 minutes which yield 1-Phenyl-1,2,4-Triazole.

SCHEME: 1 Synthesis of 1-Phenyl-1,2,4- triazole

The synthesis were done by several trials by changing the concentration of hydrazine and formamide

The 22% of yield were obtained by using 5 moles of formamide at 170'C for 8 minutes, 46 % of yield were obtained by using 10 moles of formamide at 170'C for 10 minutes, 57% of yield were obtained by using 15 moles of formamide at 170'C for 10 minutes and 75% of yield were obtained by using 20 moles of formamide at 170'C for 8 minutes.

SCHEME: 2 Synthesis of 1-Methyl-1,2,4- triazole

SCHEME: 3 Synthesis of 1,2,4- triazole

When methyl hydrazine reacts with formamide at 140'C for 10 minutes it forms 1-methyl-1,2,4 triazole and hydrazine reacts with formamide at 170'C for 8 minutes it forms 1,2,4 –triazole. The synthesized product were extracted using ethyl acetate and the product were filtered and evaporated.

CONCLUSION

The 1,2,4-triazole substituted derivatives were synthesized using microwaveby using phenyl hydrazine, methyl hydrazine, hydrazine and formamide. It was simple, effective and optimised method for the synthesis of 1,2,4-triazole derivatives. 1,2,4 triazole,1-Phenyl-1,2,4 triazole and

1-Methyl-1,2,4 triazole were synthesized without catalyst by reduced time. It have more advantage than conventional method.

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Table 1. SCHEME: 1 Synthesis of 1-Phenyl-1,2,4- triazole

S.No	Formamide (equilvalent)	Temperature'C	Time (Min)	Yield%
1.	5 moles	170	8	22
2.	10 moles	170	10	46
3.	15 moles	170	10	57
4.	20 moles	170	8	75

Table 2. FTIR Spectra of 1,2,4 triazole derivatives (1-phenyl-1,2,4-trizole)

S.NO	Functional group	Absorption (cm^{-1})	Absorption Intensity
1.	Amines (double bond)	3308.98	Strong
2.	Aromatic	3068.24	Medium
3.	C=N	1598.66	Strong

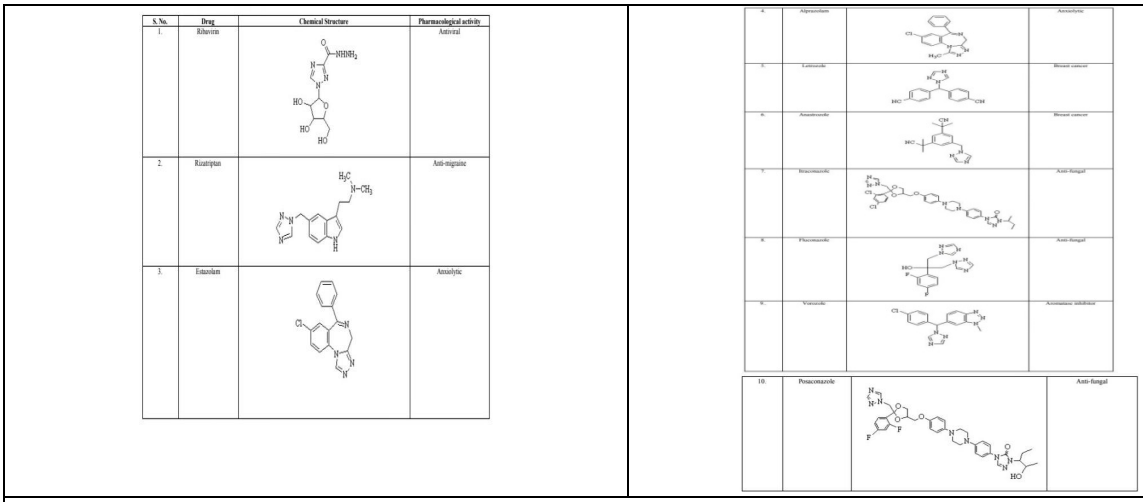

Fig.1. Chemical Structure

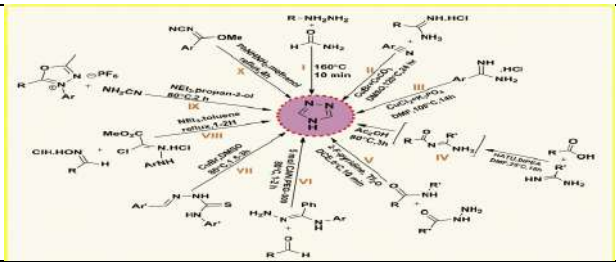

Fig.2. Literature reveals Various Methods synthesis of 1,2,4-Triazole

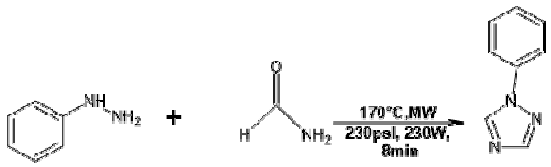

Fig.3. SCHEME: 1 Synthesis of 1-Phenyl-1,2,4-triazole

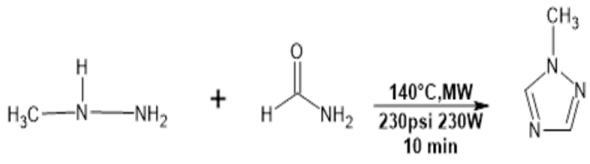

Fig.4. SCHEME: 2 Synthesis of 1-Methyl-1,2,4- triazole

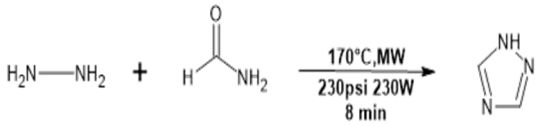

Fig.5. SCHEME: 3 Synthesis of 1,2,4- triazole

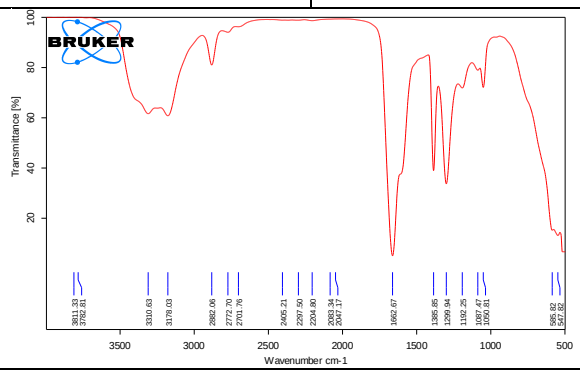

Fig.6. FTIR Spectra of 1,2,4 triazole derivatives (1-phenyl-1,2,4-trizole)

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REVIEW ARTICLE

A Review on Pharmaceutical Dosage Forms in Personalized Medicine

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ABSTRACT

Personalized or precision is a new paradigm that holds promise for individual diagnosis, treatment and care. Personalized evidence based medicine aims to use stored health data to prevent future illness. PM is difficult for patients with their genes, lifestyle, behaviours and environmental factors. The holistic approach to patient care is done by Combining the history, clinical examination, diagnosis of individuals. The PM must require the data of disease from where it is started and need some advances regarding the phenotype categories of disorders, population size and statistical analysis. The techniques based on disease categories and tumors which is followed by different test in Patients diagnostic methods. Inspite PM has its own benefits it also has drawbacks for patient in risk on genetic variants of traits in chronic disease.PM found its application in various fields. Clinical, ethical trials, diagnosis, infrastructure, market opportunities also maintained for better prevention of heath care.

Keywords: Medicine, genomic, prognosis, phenotype, precision, disease, patients, diagnosis.

INTRODUCTION [1-5]

The practice of medicine remains largely empirical; physicians generally rely on patterns matching to establish a diagnosis based on a combination of the patients' medical history, physical examination, and laboratory data. accordingly, a given treatment is often based on physicians past experience with similar patients. One consequence of this is that a blockbuster receives prescribed for a "typical patient" with a specific disease. Integration of molecular research with clinical data from individual patients to develop a more accurate molecular taxonomy of diseases that enhances diagnosis and treatment and tailors disease management to the individual characteristics of each patient Personalised evidence-based-medicine (EbM) uses stored health data, namely of patient diagnoses, laboratory work, insure claims, and demographic

information. This data allows to move past the reactive technique of treating contamination, permitting healthcare carriers to are expecting and save you destiny infection there's large agreement that PM is a therapeutic method targeted to person sufferers'or organizations of sufferers' needs, ensuring that patients get the proper treatment on the right time, the usage of a combination of diagnostic and healing equipment. The definition is intended to delineate a specific set of remedy procedures – inclusive of technology had to determine treatments (in particular diagnostic checks) as well as the treatments themselves which are differentiated from other untargeted remedy techniques or therapeutic

HISTORY OF PERSONALIZED MEDICINE [6-10]

Customized medicinal drug refers to tactics tailored to the particular variables that make each affected person an individual. The specific genes, lifestyle and behaviors, and environmental elements can have an effect on these variables. Thus, a success medicine in a positive character might not paintings for some other individual, affecting traits in areas together with therapeutic drug tracking. New methods are arising to house those variables and make the maximum particular treatments to be had to as many different sufferers as feasible. Through the 20th century, clinicians had evolved a form of personalized technique to the treatments of sufferers. For example, after the upward push of blood transfusion, knowledge gathered that indicated that individuals range in blood corporations. It became also cited that grouping such human beings resulted in a hit blood transfusions. The doctors later superior inside the documentation of individuals' family members to sicknesses depending on their households' histories. This changed into accomplished in sicknesses that appeared to be exceeded from generation to generation. The personalized remedy became more concrete at the beginning of the twenty first century with the solidification of the Human Genome challenge.

HISTORICAL PERSPECTIVES [11,12]

Combining history, clinical examination, diagnostics – individualized treatment plan Holistic technique to affected person care. There are 3 stages of present day medicine,

1. Intuitive medicine -1950-1980s
2. Evidence based totally remedy -1980-2010
3. Precision/customized medication -2010- present

NEED FOR PERSONALIZATION [13-15]

Confusion over the limits of a new methodical paradigm shouldn't surprise every person, however even the fundamental terminology isn't clear in this case. What's the relationship of precision remedy to personalized remedy, What difference, if any, is being made with evidence-based totally medication, Haven't clinicians always striven to offer specific suggestions, As a systematic survey lately concluded, whether or not referred to as precision remedy or personalised medicinal drug, the phrase has come to refer to the way non-public statistics and biomarkers specifically genetic biomarkers might be used to tailor treatments for character sufferers. Others skeptical of orthodox eugenics' emphasis on individual version, like the biometrician Raymond Pearl at Johns Hopkins, nevertheless tried to expose and degree the interplay of "constitutional" and environmental factors inside the distributions of ailment. Although particularly little-remembered today, Werner Kalow's 1962 textbook Pharmacogenetics had already set out the program of linking therapeutic reaction to each the biochemistry of drug retailers and to the position of genetics and evolution in shaping individual distinction.

ADVANCES REQUIRED TO IMPLEMENT PERSONALIZEDMEDICINE [16]

Ideally, we would like to have complete knowledge about all existing phenotype categories, including disorders, and for each of these phenotype categories, we would have a population of patients of unlimited size. A few remarks to improve upon these three factors

Population Size [17]

Enrolling more patients can improve visibility and facilitate the organization of large clinical trials (community-based/open consortia to allow anybody to contribute; strict regulation still required to avoid garbage-in/garbage-out). A particular problem in this respect is posed by orphan diseases because these would not allow the recruitment of large populations to ensure a sound statistical analysis. Importantly, personalized medicine does not provide solutions for this problem but faces the same problems as traditional medicine.

Phenotype categories [18]

Biomedicine is an excessive studies subject with many laboratories competing and taking part to higher recognize organic mechanisms underlying human sicknesses and their molecular characteristics (genotypes) and phenotypes. Higher phenotype categorization may be achieved by means of better sharing of published and unpublished information/code and effects (ontologies, MeSH phrases, and so on).

Statistical analysis [19,20]

Statistical analysis is probably the vicinity in which the advances is probably the maximum difficult to put in force for 2 reasons. First, computational genomics or similar regions are distinctly young research fields where primary improvements are still required to acquire a more efficient analysis. Especially, due to the distinctiveness of the statistics traits of recent technologies, e.g., next-era sequencing, the software of proprietary software appears inappropriate at instances because such programs are not at the leading edge of the statistical evaluation tendencies and are falling at the back of. This is as an instance because of the need for the time-ingesting establishment of graphical-consumer-interfaces (GUIs) so that you can make the methods on hand for "non-computational biologists". As a high-quality aspect-impact, R comes without license expenses and, subsequently, permits the unfastened distribution of software packages within the community to make analysis results fully reproducible.

EXAMPLE FOR PERSONALISED MEDICINE [21,22]

An excellent example of the fruits of customized medicinal drug is Herceptin that is a drug used in the remedy of cancer. It became approved in 1998. The drug is used to treat breast cancer patients with HER2 tumors. The research implies over 30% of patients with breast cancer take a look at positive for a HER2 protein, that's a tumor that doesn't respond to traditional therapy.

TECHNIQUES FOR PERSONALISATION[23]

There are various techniques involved in personalization medicine

Deep phenotyping linking physiological abnormalities and molecular states from beside to bench [24,25]

The evaluation of phenotype plays a key function in scientific research and scientific exercise in the direction of better diagnosis, affected person stratification, and selection of quality remedy strategies. In biology "phenotype" is described as the physical appearance or biochemical characteristic of an organism due to the interaction among its genotype and the environment. "Deep phenotyping" is defined as the right and comprehensive evaluation of phenotypic abnormalities in which the character components of the phenotype (taking a medical records or a physical exam, diagnostic imaging, blood checks, mental
Check, etc.

Computational biology and bioinformatics to aid biomarker development [26-28]

There is a need to develop novel computer-aided algorithms and methodologies for pattern recognition, visualization, and classification of distribution metrics for interpreting large sets of data coming from high throughput molecular profiling studies. Computational biology uses computational tools and machine learning for data mining, whereas bioinformatics applies computing and mathematics to the analysis of biological datasets to support the solution of biological problems.

Bioinformatics plays a key role in analysing data generated from different 'omics' platforms annotating and classifying genes/pathways for target identification and disease association. The goal of bioinformaticians is to use computational methods to predict factors (genes and their products) using,

1. A combination of mathematical modeling and search techniques
2. Mathematical modeling to match and analyze high-level functions and
3. Computational search and alignment techniques to compare new biomolecules (DNA, RNA, protein, metabolite, etc.)

Within each functional 'omics' platform. Combination of this and patient datasets are then used to generate hypotheses. Bioinformatics and computational biology enable fine tuning of hypotheses. These fields often require specialized tools and skills for data exploration, clustering, regression and supervised classification, pattern recognition and selection, and development of statistical filtering or modeling strategies and classifiers including neural networks or support vector machines.

Novel sensor platforms for Pocd [29-30]

Percent may be described as a ''close to affected person'' clinical-laboratory checking out instrument or tool, that can perform quantitative, semi quantitative and or rapid measurement, all without any pipetting, and most effective the use of the pre-mix reagents to provide result-oriented healing action. Percent will dominate the market inside the course of the following 15–20 years thru miniaturization, parallel analysis and networking. Microfluidics chips and electrochemical detection have already enabled the development of miniaturized devices wherein nL and pL variety liquid are handled, reducing the full time and required sample reagents, etc.

Sensor specificity probe attachment and design [31]

Accurate detection rests on sensor specificity. Therefore the transduction material must specifically probe the target(s) of interest, which often involves attaching functional molecules, e.g. ssDNA probes, antibodies, aptamers or any other surface attachable molecular probes that possess selective interactions to the biomarker of interest, to a transducer material, creating an inorganic–organic platform. Bioreceptors exist in a variety of formats, but the integration of these molecules with a transduction material involves careful design of surface and attachment chemistries to both ensure the functional surface's stability and protect the transducer's activity. Simple electrostatic attachment of protein or nucleic acid probes can produce strongly bound probes, although covalent attachment chemistry is typically employed to produce ordered probe layers with reproducible densities. Traditional covalent attachment chemistries exploit binding through common functional groups including carboxylic acid, amine, thiol or hydroxyl groups.

Electrical transduction mechanism [32]

Electrochemical biosensors exploit the maturity of electrochemistry as well as the instrumental simplicity, relatively low cost and portability that characterize the technique. Depending on the detected signal, amperometric, voltammetric, potentiometric or impedimetric, electrochemical biosensors all operate based on the sensing event occurring in close proximity to an electrode surface. Many examples of electrochemical nucleic acid sensors exist in the literature wherein oligonucleotide probes are immobilized to the electrode surface and the current, voltammogram, potential or impedance variation upon hybridization is collected for quantitative detection, often, but not always, in conjunction with PCR amplification and target labeling

Optical transduction mechanisms [33]

Fluorescence commonly used in current biosensing technologies, fluorescence detection involves optically tagging target analytes and observing the modification of their fluorescent signal. This usually occurs through the direct observation of increased fluorescent signal resulting from the formation of a probe–analyte duplex. SSDNA, thus producing no fluorescence while the quencher and fluorophore are proximal to one another. When a matching

analyte interacts with the molecular beacon, the stem-loop structure unfolds to hybridize and induce duplex formation, spatially separating the quencher from the fluorophore allowing the optical signal to increase.

Kras test [34-36]

Constitutively activated KRAS mutations occur in multiple human malignancies, including approximately 90% of pancreatic, approximately 30% of lung, approximately 60% of thyroid, and approximately 43% of colorectal carcinomas (CRC). Currently, within the United States, clinical KRAS mutation testing is performed on CRC, thyroid, endometrial, pancreatic, and non–small cell lung cancers. Research on KRAS began in 1964 when it was first identified as the cause of leukemia virus–induced rat sarcoma. Later, the Kirsten rat sarcoma retroviral oncogene sequence was cloned and used to identify the human homolog gene, now known as KRAS or KRAS2 (Kirsten rat sarcoma virus 2 homolog).

Kras normal testing methods

The clinician typically initiates KRAS testing requests for a patient. Usually, the testing is performed on tumor tissue removed from the patient during a previous surgery or biopsy procedure. Typically, patients undergoing KRAS testing are those with high-stage tumors that require adjuvant therapy. If metastatic disease is present, it is important to clarify that the sample needed for testing is not the primary tumor but rather a representative tissue sample from the metastatic lesion. Typically, tumorenriched areas will have relatively easily identified histology and can be dissected away from benign tissue. This process is often aided by having the fixed tissue cut and placed on an unstained slide and having a standard H&E stained slide of the same tissue cut available for comparison.

Kras mutation testing methods

Extra than 60 strategies were hired in KRAS checking out, most of which fall under the kinds of sequencing, excessive-resolution melting analysis (HRM), unmarried-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), denaturing excessive-performance liquid chromatography (DHPLC), array/strip evaluation, and allele-precise PCR. All of those strategies had been successfully carried out to scientific KRAS testing, and each has it's of the tumor.

Braf and Nras testing[41-44]

CRC may also exhibit BRAF and NRAS mutations in about 4.7% and 2.6% of cases, respectively.

a. Activating mutation of these genes is also associated with a resistance to panitumumab or cetuximab therapies
b. For this reason, the National Comprehensive Cancer Network recommends that all patients with CRC being considered for anti-EGFR therapy be tested for BRAF mutations.

The testing methods reviewed here are also commonly used for BRAF testing. For example, applied the same analysis comparing Sanger sequencing with standard versus locked nucleic acid primer amplification and found that the latter amplification method also gave better BRAF mutation detection.

Genomics, epigenetics, and micro-rnas emerging biomarkers on cancer, diabetes, autoimmune and inflammatory diseases [45,46]

Biomarkers with the potential to identify early stages of disease for example pre-neoplastic disease or very early stages of cancer are of great promise to improve patient survival. The concept of liquid biopsy refers to a minimally invasive collection and analysis of molecules that can be isolated from body fluids, primarily whole blood, serum, plasma, urine and saliva, and others. Regulated by way of DNA methylation and targeted with the aid of miRNAs had been of capability use as clinical markers. The have a look at determined that several genes which includes Stat4 and TRAF1-C5 were recognized as danger elements contributing to RA and different autoimmune sicknesses along with SLE. RA is also strongly related to the inherited tissue type MHC antigen HLA-DR4 and the genes PTPN22 and PAD14.

DNA methylation screening identified genes undergoing DNA methylation-mediated silencing which include IL6R, CAPN8 and DPP4, in addition to several HOX genes; and a panel of miRNAs which are controlled by using DNA methylation, and genes which are regulated by way of DNA methylation and are targeted by miRNAs.

Theranostics and pm – pocd or poc particles[47-50]

The new term ‘‘theranostic’’ is increasingly more linked to PM describing the conceptual integration of therapeutics and diagnostics to be applied into a platform. To be specific, it's far a diagnostic check designed especially to help healing choices of a physician for a specific affected person and at a selected time. There is debate about the independence of theranostics from PM, i.e. whether or no longer they're conceptually wonderful.

For example

The identical nanoparticle or tool used to treat inflammation can also be used to detect changes in infection. Most effective a small variety of theranostic products are available on the market, but they have got already led to a success treatment for the patient with cancer and HIV. A few specific examples are,

1. DAKO’s Herceptin Hercep genotyping test,
2. The BRCA/BRCA2 check for breast and ovarian most cancers,
3. Monogram’s Trofile for HIV tropsim,
4. Roche’s Amplichip to are expecting the healing reaction of affected person and
5. Bayer’s Trugene HIV take a look at.

It is now diagnosed that the huge implementation of theranostics may also take away useless treatment of patients in which the treatment itself can be inefficient or in fact dangerous. But, a great deal more attention is wanted from pharma agencies and the biotechnology industry for future development and marketplace growth. Many nano-carbons, including CNTs and derivatives of graphene, quantum dots and nanoparticles possess inherent optical homes including fluorescence permitting them to be beneficial evaluation marketers for optical sensing and imaging for the development of % particles instead of gadgets. The high floor areas of nano-carbons and their biocompatibility are Promising for bioconjugation and drug loading and transport. Graphene derivatives and CNT with their robust optical absorbance in the close to-infrared area also are applicable for cancer photothermal ablation. Theranostics technique the use of MNP have these days received full-size attention and offer precise advantages over traditional detection techniques.

ADVANTAGES

1. Delivering better treatments to patients. Delivering benefits to healthcare systems and society. More efficient development of new medicines. Selection of optimal therapy and reduce trial-and-error. Increase patient adherence to treatment (Inherited forms of hypercholesterolemia) [51]
2. The benefits to the healthcare system and society are evident from improvements in patient management and in terms of offsetting costs through reduced use of ineffective treatment, reduced cost of chronic conditions and reduced hospital stays [52]
3. Shift the emphasis in medicine from reaction to prevention (Breast Cancer) Prescribing (Herceptin and Colon Cancer). Help avoid adverse drug reactions (Warfarin and Clopidogril). Reduced healthcare costs (Warfarin, Heart diseases and Cancer). Better Quality of life (Heart transplant follow-up) [53]

DISADVANTAGES

1. Infrastructure requirements. Environmental population-level prevention measures are shown to be more cost-effective and equitable than efforts directed to individuals [54]
2. Limitations in Applying Genetics for Primordial, Primary, and Secondary Prevention of Chronic Diseases. The drawbacks could also result from factors that impact precision treatment variability such as gender, weight, ethnicity, and renal and hepatic function [55]

3. Designing choices in ways that affect decision making is termed "choice architecture." In workplace environments, designs that reduce obstacles toward healthful choices may be promising for promoting beneficial eating behaviour [56]
4. Predicting risk based on genetic variants is that traits for chronic diseases are complex. Interventions that target non conscious processes have been proposed to be more effective in changing behavior than those that engage conscious deliberative processes [57]

APPLICATIONS [58]

A growing issue is that the term "personalised medicine" (also called "predictive" or "precision" remedy) has become a proxy for DNA-targeted strategies to prevention and treatment. Personalized remedy is the individualizing of health care primarily based on a person's susceptibility to disorder and reaction to remedy. Environmental and behavioral factors are shown to make contributions extra to premature dying than genetic factors. But their contribution to complicated persistent diseases, which include diabetes mellitus, coronary arterial sickness, and most cancers, is over shadowed by means of a gene-targeted view.

Biomarkers and decision making[59]

BMs have been used to improve patient's stratification and/or increase centered cures facilitating the decision-making system for the duration of the new drug development technique. BMs constitute a rational technique which, at its most highest quality, displays both the biology of the disorder and the effectiveness of the drug candidate. Also, adding the right BMs to a drug-development approach enables the idea of 'fail rapid, fail early'; for this reason, allowing early identification of the high proportion of compounds that fail in the course of drug improvement.

Biomarker multiplexing [60]

More than one biomarkers are used to empower extra correct patient stratification. to improve patient stratification for immunotherapy, the evaluation of immuno-oncology biomarkers, like PD-L1, as well as a more complete evaluation of the immune and tumor-related pathways(the "cancer Immunogram) has for use for a better patient stratification in destiny immunotherapy trials. The "most cancers Immunogram" integrates each tumor- and immune-related traits assessed with both molecular and image-primarily based methods for individualized prediction of immunotherapy reaction. The authors suggest that anti-cancer immunity can be histologically segregated into 3 primary phenotypes

1. The inflamed phenotype ("hot" tumors)
2. The immune-excluded phenotype ("cold" tumors) and
3. The immune-wilderness phenotype ("cold" tumors).

Biomarkers used in diseases [61,62]

1. Type 1 diabetes, miR-342, miR-191, miR-375 and miR-21 and miR-510 and others
2. Kind 2 diabetes, miR-30, miR- 34a, miR-145 and miR-29c, miR-138, -192, -195, -320b, and allow-7a
3. Prediabetes (miR-7, miR-152 and miR- 192) and insulin resistance (miR-24, miR-30d, miR-146a), obesity and metabolic sicknesses
4. Multiple sclerosis (MS), miR-326, miR-17-5p.
5. Rheumatoid Arthritis (RA), miR-146a, miR-155 and miR-sixteen.
6. Primary biliary cirrhosis, miR- 122a, miR-26a, miR-328, miR-299-5p
7. Sjögren's syndrome, miR-17-92.
8. SLE, miR-146a.
9. MiR-516-5p, miR-637 and
10. Psoriasis, miR-203, miR-146a, miR125b, miR21.

In risk prediction [63-66]

Genome-primarily based data is changing the scientific landscape by using offering equipment that shift practice in the direction of hazard prediction and prevention. as an instance, women who deliver mutations in the high-penetrance BRCA1 and BRCA2 genes have an accelerated risk for susceptibility to breast cancer. Clinical screening for these genetic. Mutations has led to conventional preventive measures including surgical or radio healing interventions to assist patients reduce their risk. In colorectal cancer, scientific methods to discover microsatellite instability in mismatch repair genes MLH1 (mult homolog 1) and MSH2 (must homolog 2) become aware of at-risk patients and allow physicians to closely monitor them with frequent screening for the early detection of colon cancer. Clinically usable genetic risk elements have additionally emerged for long QT syndrome (LQTS) and quick QT syndrome.

For example,

β- Blockers are an effective remedy for patients with LQTS1 (long QT syndrome) (KCNQ1) (potassium voltage-gated channel subfamily Q member 1) but not for people with LQTS2 (KCNH2) or LQTS3 (SCN5A) (sodium voltage-gated channel alpha subunit 5), demonstrating how genotyping these patients can assist manual treatment. Using trying out for genetic version using genotyping and sequencing blended with interventional techniques may also put off disease development or avoid disorder altogether.

Pharmacogenomics [67,68]

Pharmacogenomics is the use of genomic information to determine or predict response to therapy. In oncology, the somatic genome from the tumor has been used to identify biomarkers that predict and guide therapy for an optimal response.

For example,

In acute lymphoblastic leukemia, approximately 20% of patients will become refractory to treatment. Two studies using differential gene expression found that genes involved in chemoresistance were also associated with a worse prognosis, laying the foundation for future studies of the mechanisms of resistance. Targeted therapy is often synonymous with pharmacogenomics. The growth factor receptor gene, human epithelial growth factor receptor (HER2), was shown to be amplified in 25%–30% of breast cancers, and its overexpression correlated with a worse prognosis. Trastuzumab, a monoclonal antibody that targets HER2, is effective in reducing tumor burden, and screening patients for HER2/ne.

Disease diagnosis and Molecular characterization [69]

An early utility of genome generation to clinical medicine is within the classification of sickness states. Genomic and molecular analyses have revealed wonderful subtypes of Ailment, which have been historically defined via vast medical or descriptive phenotypes. Because therapeutic response can vary among each subtype, this has clear implications for guiding clinical treatment.

Disease prognosis [70,71]

Include survival results, offer one of the clearest examples of the usage of genomic records clinically as prognostic equipment. MammaPrint R_ and Oncotype DXR_ are such prognostic checks which might be now clinically to be had for use in breast cancer. Oncotype DX is a 21-gene signature derived from a real-time polymerase chain response of paraffin-embedded samples used to predict remote recurrence over 10 years. MammaPrint, authorised by means of the U.S. meals and Drug administration (FDA) in 2007, changed into advanced on the Netherlands most cancers Institute, which created a 70-gene signature to discriminate patients with excessive and occasional hazard of metastatic recurrence inside five years.

Disease response monitoring to therapy [72]

Peripheral blood mononuclear cell (PBMC) gene expression profiling is now used routinely In some centers to monitor the status of grafts following solid organ transplantation, with Much of the effort focused on developing better tests for assessing graft rejection. Research in cardiac transplantation, perhaps more than any other organ, led the way in developing novel blood-based tests for distinguishing between quiescence and acute rejection.

CONCLUSION

It is without a doubt that the principle behind personalized medicine holds a great potential for translational medicine by improving diagnostic, prognostic, and the therapeutic approaches for patient care and the genomic science will undoubtedly change the future of medicine. The human genome project (shift), tailoring the individualized treatments of each patient, the success of rHGH (Replacement therapy, molecular diagnostics in personalized medicine plays a vital role for patients. The national policy to ensure prioritisation of personalized medicine should work hand in hand with existing health strategic plans. Eg, (National cancer plans) and the resources funding needs to be aligned to aspiration. The EQA (external quality assessment) are need to provide evidence development for the better testing quality. More important think that the major problem is a lack of a precise definition of personalized medicine that would an efficient experiment design translating the paradigm into practice.

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RESEARCH ARTICLE

Classification of Bioplastics Based on Sources

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ABSTRACT

Plastic is an amazing invention that has changed the world. Plastics, due to their excellent technical properties, are widely used in various fields by mankind. Despite diverse applications, its disposal poses a threat to the environment. Increasing concerns about the accumulating plastic waste pollution and the associated health hazard have stimulated rapid development in bioplastic synthesis, especially in biodegradable bioplastics derived from renewable resources. Bioplastics have the ability to replace fossil-based plastics and the current focus is on their development based on biodegradability. If we have to replace single use plastics with bioplastics and avoid further plastic waste accumulation, it is also imperative that we understand what waste management options are for these materials. The behaviors of different biodegradable plastics in a range of environments have to be hence assessed, and it must be determined under which conditions bioplastics show complete biodegradation. The current review focuses on the nature and performance analysis of various sources of bioplastics and their ability to reduce plastic pollution through various applications. This review also emphasizes the need to systematically classify bioplastics on the basis of biodegradability and not just the source and composition.

Keywords: Bioplastics, biodegradable, cellulose, PHA, PLA, plastics, zein.

INTRODUCTION

Plastics are polymers of synthetic or semi-synthetic origin and are popular for being lightweight, durable, strong and yet affordable. These key characteristics have made plastics to be indispensable part of human day to day life for the last 50 years. With growing human population, the excessive accumulation of plastics as wastes in environment has become a major concern worldwide. Conventional plastics like polypropylene (PP), polyethylene (PE), polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC) are produced from non-renewable petroleum. The

decomposition of plastics takes several centuries, and in the process release toxins that harm the ecosystem [1]. The plastics industry is constantly evolving, with technology simultaneously evolving to respond to ever-changing needs. Although plastics have multifarious uses with many advantages, plastic pollution is currently an environmental issue for every nation [2]. Plastics pollution, especially the microplastics, in addition to direct natural problems to ecosystems can also cause serious problems for organism and pose a risk to human health [2,3]. Collection and proper management of plastics waste is not currently appropriate, and about 5-13 million tons of plastics end up in the ocean each year [4]. Plastics which is viewed as an attractive material is becoming a problem because they are more resistant to degradation in the open environment [5]. Bioplastics have gained importance from all quarters owing to the harmful impact of plastics and need for an alternative to the ubiquitous plastic. A lot of people think that bioplastics are a great alternative to plastics as they are projected as just green materials. Bioplastics are a type of plastic that can be made from natural resources such as vegetable oils and starches, instead of the conventional source of fossil fuels. There is no single definition for the bio based plastics. A number of characteristics that a product can possess – both independently of each other and together, which will determine a stability of an object. The following terms are listed for better understanding the potential of a product that is reported to be biodegradable [6].

Bioplastics are a family of materials with different properties and application. If a material has biological, biodegradable or both properties it is define as a bioplastic (greendotbioplastics.com).
Bio-based products are carbon-based materials containing whole or partial biological carbon. The percentage of renewable materials required to meet this requirement will vary according to the product (greendotbioplastics.com).
Biocomposite material combines wood, starch, flex, hemp and other similar materials with traditional plastics. These products are used to promote natural aesthetics and improve physical properties, while reducing the amount of non-renewable petroleum based plastics, by replacing upto 70 percent with organic fillers up to 70 percent (greendotbioplastics.com).
Biodegradability is a biochemical process during which micro-organisms in the environment convert material into natural substance such as water, carbon dioxide and fertilizer. The biodegradable process depends on the surrounding environmental condition (greendotbioplastics.com).
Compostable plastic products are defined by American Society for Testing and Materials (ASTM) International standards 6400 and 6868. These standards state that the material should be biodegradable in a certain period of time and leave no toxic residue in the soil (greendotbioplastics.com).
Bioplastics is viewed as the most innovative material that are biological and biodegradable. They are believed to reduce the carbon emissions by 30 to 70% when compared to the conventional plastics. Bioplastics are made from a wide range of raw materials such as banana skin, organic waste, agricultural waste, newspaper waste, oil palm, sugarcane, corn flour, potato starch, rice straw, cotton, mulberry and a variety of cellulose and starch sources from plants [7]. Bioplastics are degraded by microorganisms present in soil and water such as bacteria, algae and fungi. This article reviews the classification of bioplastics including implementations, challenges and finally about future opportunities.

Environment impact of plastics

Production of plastics on an industrial scale began to flourish in the 1940s and 1950s. The overall production of plastics has surpassed 311 million tonnes annually and is expected to grow another 33 billion tonnes by 2050 [8]. Worldwide plastic demand is dominated by thermoplastic types of polypropylene (PP) (21%), low -and linear low-density polyethylene (LDPE and LLDPE) (18%), polyvinyl chloride (PVC) (17%), and high-density polyethylene, (HDPE) (15%), followed by other types like polystyrene, polyethylene terephthalate and polyurethane. Plastic polymers are not only used for consumer products but also to make synthetic fibers, foams, coatings, adhesives and sealants, which are used in wide range of sectors such as packaging, building and construction, automotive, electrical and electronics. [9]. It also contributes to conservation of food and fuel resources from wastage and is an integral part of many medical equipment such as tubing, disposable syringes, prosthetics thereby protecting human health [10]. This kind of diverse application, on the other hand, can also lead to diverse waste generation. Large volumes of

plastic wastes are produced, mainly due to the short utility value of several plastic products, with many of them being single use plastics. It is estimated that almost 40% of plastic products have a service life of less than a month. This enormous waste creates serious environmental and management difficulties [11]. It is estimated 34 million tons of plastic produced per year and only 7 percent is recycled with remaining 93 percent dumped into oceans and landfills [12]. Plastic wastes are considered as solid waste occupying space when discarded. Despite significant worldwide technical advances in management, treatment and recycling in the last three decades, a large part of plastic waste still ends up in dumpsites or landfills, or burnt openly letting out carbon monoxide and carbon dioxide into the air. Even in Europe and Japan, which have mechanically advanced and environmentally conscious waste and resource recovery systems deployed on the ground, approximately 50% of plastic waste is still directed to controlled landfill disposal [13].

Unfortunately, reasonable amount of plastic waste also end up as garbage in the marine and terrestrial ecosystems, creating various environmental, economic and social impacts [14]. Although the exact amount of plastics entering the marine environment is not yet known, the ocean is estimated to be in the range of 4.8 – 12.7 metric per year for land-based plastic waste entering, using global data on solid waste and the use of population densities [6].The marine environment and its organisms are particularly prone to plastic waste pollution. Different effects from macro, micro and nano plastic ingestion have been documented for various organisms including birds, turtles, fish larvae and other marine mammals. The effects include suffocation or blocking of digestive track causing death [15] on the other hand, leaching of additives may be more relevant for species with longer gut retention times, such as fish. While micro plastic intake may lead to increased bioavailability of plastic compounds, there is speculation that they may lead to decreased bio communication of unproven traditional persistent organic pollutants [16]. Nonbiodegradable polymers can be decomposed / fragmented by various mechanisms such as oxidation through heat and light, through ionizing radiations or through hydrolysis. Pollutants in air such as carbon monoxide, sulphur dioxide, nitrous oxide and ozone may also play a major role in the degradation of plastics [17]. However, a complete conversion of plastic material to its basic constituents such as CO_2, water and inorganic molecules via biodegradation or photodegradation is not possible. Under marine conditions any degradation that may occur will also be very slow owing to scant availability of solar radiation leading to slow thermal oxidation taking over a century [18]. Innumerable problems associated with solid waste management of conventional plastics have generated a heightened interest in the development of biodegradable plastics at a global level.

History of bioplastics

In the beginning of 1930s, before mastering the monomers derived from crude oil, many materials used in everyday life were made using biobased polymers. Soon the source shifted to refining oil based plastics with wide range of applications [19]. Early raw materials include natural rubber, cellulose, galactose and dairy components such as casein as starting materials for polymerisation. In 1947,Rilsan (or Polyamide 11, pa11 or Nylon11), made from castor oil was the first technical bioplastic introduced in the market, having exceptional mechanical integrity, thermal stability, chemical resistance[20]. In the 1950s-60s bioplastics such as Polyhydroxyalkanoate (PHA) and polyhydroxybutyrate (PHB) production on commercial scale from microbes and bacteria in particular was evaluated by W.R.Grace in the US, but was discontinued due to cost attached. Following global oil and energy crisis in the 70s, many researchers and manufacturers turned their attention towards bioplastics and late 1990s saw rapid production of polylactic acid (PLA), PHA, plasticized starch, made from rapid technological advances (https://bioplasticsnews.com/2018/07/05/history-of-bioplastics/). 40 different PHA synthase genes associated with PHA biosynthesis from more than 35 different bacteria were cloned by the end of the twentieth century. By beginning of 21st century, protein engineering advances led to betterment of PHA and PHB biosynthetic pathways through targeted manipulation, leading to cost effective and environmental friendly biopolymer synthesis. [21]. In addition to development of biodegradable plastics, focus is now on diversification of resources used to produce these products, with aim to recover by-products from wastes, thereby conserving resources.

Sources of bioplastics

In this section, we attempt to put together the various sources used for bioplastic production.
The figure below (Fig.1) illustrates the various resources used for generation of bioplastics.

Biomass resources

These include resources which are obtained from natural biomass, which include plant and animal resources, primarily obtained through agricultural activities and cattle rearing. They are further classified based on their biochemical properties.

Polysaccharides: These include predominantly the plant-based starch and cellulose. Pure starch is white in colour with no specific taste or odour. Also, pure starch cannot be dissolved in cold water or alcohol. It is non-toxic, biologically absorbable, and permeable to carbon dioxide. Linear and helical amylose and branched amylopectin are the two molecules present in starch. Amylose content may vary from 20 to 25%. Amylopectin is a much larger molecule than glucose and is made up of 2000 to 20,000 glucose units. When heated, the grains swell and long chains explode into smaller fragments, making them soluble [22]. Amylose provides crystallinity and starch complexity, highlighting the importance of amylose / amylopectin ratio during production and leading to improved strength and reduced strain. Due to low cost, renewability, and reasonable mechanical properties, it is used to produce decomposable films having the ability to replace partly or fully the plastic polymers [23].

Wheat: Wheat gluten is one of the best plant based source of bioplastics because of its user-friendliness, good biodegradability, low cost, unique viscoelastic properties and ability to crosslink on heating [24]. Wheat gluten can be processed into bioplastics through mold, extrusion and comparative modeling [25]. Various plasticizers have been used for wheat gluten based bioplastics such as water, glycerol, sorbitol, sugars, fatty acid, di-ethanolamine and triethanolamine to overcome their breakability through wide range interactions between the protein chains[26]. However, wheat gluten based bioplastics, have drawbacks, including long-term stability due to their sensitivity to water and their mechanical properties depending on their humidity and storage time. Mechanical properties and water resistance in gluten based bioplastics can be improved by combining with natural fibres such as hemp fibres, wood fibres, jute fibres and coconut fibres[25].

Potatoes: Potatoes are one of the world's main food sources, present as 100-180different species and thousands of varieties worldwide. Potatoes are an important source of nutrients for humans and animals, but their full potential for sustainable source of bioplastics has not yet been explored. The possible reasons could be their short shelf life, potential for use in food production and their reduced ability to adapt to different climatic conditions. Potato tubers contain starch, proteins, ascorbic acid, carbohydrates, minerals, vitamins and fiber, which is a low-fat nutrient. Optimal potato utilization can be achieved by placing fully grown potatoes in a suitable environment (average storage temperature, dark room) and ensuring that they are kept away from stress and physical injury [27].

Corn: Corn starch contain around 25% amylose and 75% amylopectin molecules. The amylose molecules loosen with water, thereby increasing the biodegradability and enhancing the plasticizer properties such as rapid gel formation, good absorption capacity and good flexibility of amylopectin molecules [28].Corn starch works as natural plasticizer with distilled water. Corn based biomaterials can be used in areas where biodegradation parameters are of paramount importance in items requiring short time use and disposed thereafter, such as glasses, plates, cups, spoons, packaging materials etc. corn starch-based bioplastics are efficient because of their biodegradability, non-toxicity, environment-friendly processing, low product cost and easy availability of raw material, thus holding huge potential for future development[29].

Cellulose: Cellulose is a linear homo-polymer of glucose and is the most abundant natural polymer on Earth. Glucose is incorporated into the beta configuration as opposed to the alpha configuration in starch. This allows the

crystals to be crystallized in linear compatibility in the form of micro fibrils with high crystallinity, high aspect ratio and sub-micron diameters combined with cellulose fiber (also known as final or single fiber). Except in few plants such as cotton where it exists as pure cellulose, plant fibre largely exists as a natural biocomposite made of cellulose, lignin, hemicelluloses, pectin, and wax. The components and their percentages vary depending on the type of plant. The direct use of cellulose in packaging can range from microfibers, pulp (short fibers), textile fiber or engineering fiber (long end fiber or fiber bundles) to relatively unrefined lignocellulose, like wood flour, straw etc. [30].

Cellulose fibre-based materials: The degradation of non-cellulose content in natural fibres such as hemicellulose and lignin result in cellulose microfibers (CMFs), which have enhanced performance. They hold potential for application in packaging such as transparent films, coatings, reinforcement in foams [31]. Widespread applications are currently restricted due to high cost associated with extraction process.

Cellulose derivatives: Multiple cellulose derivatives can be formed by dissolving insoluble cellulose, hydroxyl groups by different radicals and recovering from packaging to filmforming solution. These include cellulose film, cellulose ester, cellulose acetate (CA), butyrate (CAB) or propionate (CAP), cellulose ethers (such as hydroxyl ethyl/propyl and methyl cellulose), carboxy methyl cellulose, fatty acid ethers of cellulose etc. [32]. In the past, cellulosic derivative products have been criticized from an environmental point of view because of harmful emissions released when burnt. However, advances in technology and the drive to use renewable materials have revived the use of cellulose derivatives in recent years. They are transparent, stable to oil and grease resistance and have printable properties making them more desirable [30].

Lignocelluloses: Lignocellulosic fibres are made up of cellulose fibres reinforced by a matrix of hemicellulose and lignin or pectin in one or more layers, with the volume and orientation of cellulose fibres being variable in each layer. Lignin is closely related to hemi-cellulose to form subsystems in plants. The complex chemistry and polymer structure make it difficult to isolate and plasticize lignin by simple methods. For this reason, highly unrefined lignocellulose such as wood flour, cane baguette, palm fibers, grain straws and husks are used as low-cost fillers to yield compostable material. Lignin has the potential to function as a plasticizer, stabilizer, or bio-compatibilizer in bioplastics, which will produce different properties on bioplastics. Technical advancements involving simple modifications of lignocellulosic fibres, without changing the chemical content or composition of the fibres, can enhance the utility [33].

Natural fibre bio-composites: Natural plant fibre composites consist of a polymer matrix embedded with natural fibres. Biocomposites developed in this manner are renewable, energy efficient, lightweight and environmentally friendly as compared to other binder fabric composites. High performance natural fibres with flexibility such as jute, hemp and sisal, have found application in construction materials and automotive interior components as low-cost alternatives to synthetic fibers. The properties and performance of products made from natural fibre composites depend upon processing techniques, the properties of their individual components, as well as their compatibility and interfacial bonding between polymer and fibre. Natural fibres have drawbacks like higher water absorption, inferior fire resistance, and lower mechanical properties which limit their applications. Natural fibre bio-composites have many advantages such as low cost, good thermal and dimensional stability, low coefficient of friction, and low density and above all are environmentally friendly. Their properties can be further enhanced through the treatment, while their moisture absorption can be reduced through surface modification and addition of coupling agents thereby making better use of them [34].

Others: Gum, dextrin, pectin, chitin and chitosan are some of the naturally occurring polysaccharides, which have been extensively studied for bioplastics application. Chitin is the most abundant polysaccharides polymer after cellulose and synthesized by wide number of living organisms. Chitin occurs in nature as ordered crystalline microfiber forming structural components in shellfish or in the cell walls of many fungal species. Chemically, chitin

made up of 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose. Chitin is closely related to cellulose, except that some secondary hydroxyl is replaced by acetamide groups, so the features of cellulose technology are applicable to chitin. Chitosan is a linear polysaccharide made of distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan produced by complete or partial deacetylation of chitin [35]. Chitin on extraction can be used to produce chitosan and other bioplastic and nanostructured films. Biocomposite made of chitin and chitosan results ineasily degradable, thin, nanostructured films with good texture. These products have effective applications due to their biodegradability, biocompatibility and effective use as biomedical interface [36].

Proteins: Protein based bioplastics are an interesting alternative to synthetic polymers because of their excellent gas barrier and good mechanical properties [37, 38]. Protein are the functional polymers and are an important renewable source produced by all living organisms. Proteins are natural chains of alpha amino acids, whose amide bonds can be broken by enzymes such as proteases [39]. Since the 1930s, protein-based polymers have been used for bonding, coating, adhesives and surfactants. From their origin they can be divided into animal and plant proteins. The nutritional value of protein makes it especially attractive for making edible food packaging.

Animal based proteins

Casein: Milk contains many molecules of the protein casein. Casein extracted from milk when treated with vinegar and shaped in warm condition and cooled by plunging in coldwater is one of the simplest way of forming biopolymer. Waste valorisation is the process of reusing, recycling, or competing, from waste, valuable products or sources of energy [40]. In the 1930s, the Italian chemist Antonio Ferrety developed a successful method of converting casein plastics into fibres. Casein polymer was scooped and shaped from milk and was called as casein plastic, which was used to make several small items such as buttons, beads, hooks, knitting needles, etc. Casein plastic is prone to microbial attack and contact with acid, alkali and water can cause alteration in structure. Moreover, casein being a food protein, diverting a precious food resource such as milk towards bioplastic production needs to be ethically justified.

Whey: A product of the dairy and cheese industry, it is the liquid left after separating fat and casein from whole milk [41]. Whey has 7% dry matter of which 17% is protein which can be separated and used as protein coating in food based packaging. Whey protein coatings used as edible films on peanuts, salmon, fruits and cereals, showed good aroma, fat, humidity and oxygen barriers. These coatings also improved the shelf life by retarding rancidity caused by lipid oxidation. These coatings did not modify the sensory attributes of the food items and in fact provided some health benefits to the consumer [42]. They are also easily extruded and moulded, with good biodegradability after usage. Whey films as part of laminates have also been developed with great potential for use in various packaging application. Numerous studies have reported potential uses for whey protein in the packaging field, emphasizing in particular its good oxygen barrier properties, especially for its use as a coating on food items [43].

Collagen: Collagen is a complex macro protein which is found in 20%–30% of all proteins found in living organisms [44]. Collagen represent the main structural mechanisms of the extracellular matrix in all connective tissues such as skin, bones, ligament, tendons and cartilage [45]. When treated with strong alkali, it releases gelatine, which has been used for food dispensation and drug encapsulation. The physicochemical properties can be altered by plasticization, using glycerol or by blending with other polymers [30]. Collagen is widely used as a biomaterial in medical field due to its biocompatibility and degrading ability.

Plant based proteins

Plant based proteins as resource for bioplastics are getting attention due to their biodegradability and being a renewable resource. However, bioplastics derived from such proteins require improved mechanical and water absorption properties for wider applications [46].

Zein and Kafirin: Zein is the prolamin protein in corn, while Kafirin is a sorghum prolamin, both extracted through wet-milling process. Zein is 100% biodegradable, and is a sustainable bioresource. Zein protein has been processed into a bioplastic, developed into plant containers and evaluated for use in horticultural applications. [47]. There has been on-going interest in using prolamin proteins in zein and kafirin to make plastics. Prolamin bioplastics are hydrophobic, greaseproof, tough, resistant to microbial attack and have glossy appearance, but they all fall short of the superior properties of synthetic plastics and their high cost of production is also a drawback. They have huge potential in biomedical applications due to their relative hydrophilicity, bio- and cyto-compatibility, and biodegradability, but more studies relating to their immune-compatibility are needed for acceptance [48].

Soya: The soybean (US), or soya bean (UK), contains about 38% protein and 18% oil, both are used to produce soy-based polymers [30]. Soybean, one of the most abundant plants, is cultivated as a familiar crop around the world. A portion of degraded soybeans is used for human consumption and animal feed, although most of these are discarded as industrial waste worldwide. Therefore, we demonstrated a bioplastic product containing soy protein. Bioplastics have been tried with soy protein by crosslinking with formaldehyde and have shown functional and thermoplastic properties. Their relative abundance and low cost have created interest in developing as a biodegradable material in agricultural products, industrial parts and disposable products [49].

Gluten: Wheat gluten is a plant protein that is unique among plant proteins, because of its availability, good biodegradability, low price, unique viscoelastic properties and ability to crosslink on heating. Wheat gluten can be processed into a bio-plastic through forming, extrusion and compression molding [25]. The structure and final properties of wheat gluten based bio-plastics rest on both disulphide and hydrogen bonds [38]. Various plasticizers have been used for wheat gluten based bioplastics such as water, glycerol, sorbitol, sugars, fatty acids, di-ethanolamine and tri-ethanolamine in order to overcomes their brittleness due to the extensive interactions that occur between the protein chains. Gluten based bioplastics have some drawbacks including their sensitivity to water and poor long-term stability. These drawbacks have been improved by using different types of natural fibres, such as hemp, wood and jute fibres [50] and coconut fibres due to the good interactions between the wheat gluten matrix and the lignin in these natural fibres. Inorganic fillers such as attapulgite, natural montmorillonite, organically modified montmorillonite , silica and alumina have also been used to reinforce wheat gluten based bioplastics [26]. Bioplastics from natural or genitically modified organisms Poly(3-hydroxybutyrate) (PHB) was the first described bioplastic from the bacteria Bacillus megaterium by Lemoigne way back in 1926. Since then several polyhydroxyalkanoates (PHA) have been isolated from more than 250 microorganisms.

Polyhydroxyalkanoates (PHA)

PHA stands for polyhydroxyalkanoate, a carbon and energy storage polymer made by microorganisms under certain nutrient limiting growth conditions. These polyesters can be synthesised by growing in nutrient-deficient conditions, harvested by cell lysis and extracted using suitable solvents. PHAs are created from renewable sources, are biodegrade, and are biocompatible. Currently, PHA types such as Poly hydroxybutyrate (PHB), poly hydroxybutyrate-co-hydroxy valerate (PHBV), Poly Hydroxybutyrate-co-hydroxy hexanoate (PHBHHx) and poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) (PHBO) are explored for bioplastic production. However, PHAs are relatively expensive compared to petroleum-based polymers. By using PHAs in biocomposite materials along with bio-based agro-residues the cost can be reduced without compromising its performance. Organic fillers and fibres composed of cellulosic material can also improve the properties of these microbial polyesters, but their biodegradability in marine environments remains unexplored [51].

Polyhydroxybutyrate (PHB)

Polyhydroxybutyrate (PHB) is one of the first PHAs to be described in detail. It is produced by a wide range of microorganism including algae, bacteria, fungi, molds etc. These lipid inclusion bodies are accumulated in the cells as they enter the stationary phase of growth. Under limited nitrogen availability and in the presence of a carbon

source, some bacteria have the ability to accumulate around 60-80% of their weight as PHB. [52]. PHB finds wide range of application in industrial, agricultural, medical and several other fields due to its biodegradability and biocompatibility properties matching the petroleum based polymers [53].

Bioplastics synthesized from bio-derived monomers

Polylactic acid (PLA) is biobased and biodegradable under high temperature (58 °C) and is one of the most commercially exploited bioplastics in recent times due to its good mechanical properties, processability, renewability, and non-toxicity. It is widely used in single use application and disposable food packaging. PLA is synthesised from lactic acid by oligomerisation followed by cyclisation. PLA is a rigid thermoplastic polymer, mainly derived from renewable resources such as sugar and starch by fermentation using microorganisms. The PLA family includes poly (L-lactide) (PLLA), poly (D-lactide) (PDLA), poly (DL-lactide) (PDLLA), poly (meso-lactide) [54]. Recently, technology has been developed to obtain polyethylene from biological resources, by synthesising ethylene monomer by dehydration of bio-ethanol, obtained from glucose. Bio-PP as it is called however is not biodegradable [55]. Poly glycolic acid (PGA) has a similar chemical structure to PLA but without the methyl side group, which allows the polymer chains to pack together tightly and results in a high degree of crystallinity, thermal stability, exceptionally high gas barrier, high mechanical strength and stiffness. PGA is rapidly biodegradable and 100% compostable. Currently, PGA is mainly used in the form of copolymers, such as poly lactic-co-glycolic acid (PLGA), to provide additional mechanical strength. PLGA has high application in biomedical field to make components such as sutures, drug delivery vehicles, prosthetic devices, etc. [56].

Biodegradable polymers synthesized from petrochemicals

This group of materials consisting of aliphatic polyesters, aromatic co-polyesters and polyvinyl alcohols (PVOH) are specialty polymers synthesized from petrochemical monomers and possess weak linkages that are vulnerable to attack by enzymes like lipases and proteases, leading to biodegradation of the polymeric chains. As most of them are much more expensive than the common commodity polymers, they are rarely used alone for packaging applications and are often combined with starch, cellulose or as copolymers to produce compostable packaging materials.

Polycarprolatone (PCL)

It is a petroleum based, thermoplastic polymer. It is prepared by ring-opening polymerization of ε-caprolactone. It is a semi-crystalline polymer with a low Tm of about 50–64◦C. It is highly miscible, blends easily with other plastics, melts easily, non-toxic and is biodegradable. Its high ductility and low processing temperature have enabled it to be blended with other polymers such as PLA, PHA, starch, leading to better functional properties such as flexibility and toughness. Currently, many commercial blends such as Capa® are available as packaging materials. Polyester amide (PEA): Poly (ester amide)s (PEAs) are modified aliphatic polyesters obtained by polycondensation of butanediol with adipic acid and caprolactam, (www.biodeg.net/bioplastic). An example of this polymer is BAK®, developed by Bayer and widely used as a biodegradable consumable. PEAs have the combination of stiffness and excellent thermal and mechanical properties of polyamides and the biocompatibility and biodegradability of polyesters. These properties have made them popular in biomedical applications such as biocomposites, adhesives, drug carriers, and scaffolds in tissue engineering. The amenability in their amide bond/ester bond ratio provides this wide application [57]. Although it is very exciting that scientists are finding ways to harvest and utilize materials from sources like bacteria synthesis (PHA) and corn or sugarcane (other bioplastics like PLA), food crops being diverted for the production of bioplastics has raised a lot of criticism. Since the last decade, there has been focus on using waste materials such as banana peels, potato peelings, agricultural wastes, etc. to produce bioplastics. By utilizing waste products, optimal utilization of available resources is achieved.

Comparison of various bioplastics

The following table compares the various materials used for bioplastic production, on the basis of their biodegradability, melting temperature, sources, properties and application.(Table 1)

Waste management options for bioplastics

The sequential listing of waste management options based on their relative environmental benefits and order of sustainability, has been provided as "waste management hierarchy". It presents a framework to consider as to which option is preferable from an environmental perspective, as follows:

- Elimination/Minimize at source: This can have possible risk of hygiene issues and increased product damage.
- Reuse: Possibility to reuse the material thereby preserving energy and resources.
- Recycle or compost: reprocessing for reuse or composting in simple setup.
- Recovery of value in some other way, such as waste-to-energy through incineration.
- Disposal: the least favoured option of landfill.

Although landfill has been the most common practise in waste management, it has been placed lower down in hierarchy, as it is viewed as the least attractive option. The impact of bioplastics, when entering the waste stream and handled by currently available options (recycling, incineration and landfill) needs to be systematically analyzed. biodegradable plastics provide potential options for biological waste treatments such as composting and anaerobic digestion (AD), as ways of recovering the materials and to produce useful products, such as compost and gas for energy [58].

Biological waste treatments of bioplastics

Bioplastics, post their usage have the advantage of being treated biologically, through composting or by anaerobic digestion thereby generating compost for enriching soil or producing methane gas for energy [59]. However, biodegradation of these plastics depends on diverse factors such as physicochemical structure of the materials, environmental conditions such as humidity, temperature and the microbial populations involved in the biodegradation. Some of them take many years to decompose and in that time can end up choking marine animals, if released into water bodies. Composting as an option should also be viewed carefully as some may compost well in relatively higher temperatures under industrial composter set up, then in a simple home composter [58]. Due to numerous biotic and abiotic factors involved in the biodegradation process in natural environment, discrepancies among reported biodegradation level are observed and are difficult to standardize [60]. There is hence a need to categorise these biodegradable plastics, not only on their origin but also on their biodegradability and grade them accordingly.

Future Perspective

Major benefits of bio based plastics are that they reduce our dependence on fossil resources and, unlike fossil-based plastics, Some bioplastics are naturally compostable- in water, outside air, soil or in a combination of these. There are also certain bioplastics that are degradable in controlled conditions and not in nature. Bioplastics that are currently developed are either bio based and (naturally or industrially) biodegradable as those developed using PLA, PHA, PGLA and corn starch blends or bio based and non-biodegradable, developed from bio-PE and bio-PET. We need better clarity on classification of bioplastics. Simply being bio based need not be eco-friendly as bio-PET and bio-PE can have very slow degradation and may require bioengineering strategies like biocatalysts for enhanced degradation. Hence, a new guideline and standard for bioplastics should be develop for production, usage and waste management of bioplastics over the world. Thus, labelling legislation must be enhanced depend on products raw material usage, energy consumption, emissions from manufacture and use. While bioplastics serve as great alternative for the fossil-based plastics, their cost of production is high and their supply currently is insufficient to meet the demand. They make only 1% of the total plastic market at present. Although most bioplastics are made from renewable resources and are carbon neutral, they derive their raw materials from agricultural lands which could otherwise be used for raising food crops. Nonuniformity in their biodegradability is also another major challenge, as some take months to degrade posing risk to marine ecosystem and wildlife. Currently, bio-based and biodegradable plastics are mainly used for food packaging materials, disposable cups and cutlery, in agricultural and horticultural sectors and in making shopping bags. Application of bioplastics depend upon their characteristics. Different types of bioplastics need to be developed with more focus on utilizing the agricultural wastes as resources

and developing better technology that would bring the cost of production. Bioplastic materials developed henceforth must be not competing with traditional sources, especially feedstock but rather on agricultural wastes. Continued research in technology, innovation and global support is important to commercialize and demonstrate the importance of bioplastics. With sustained efforts, we can certainly reverse the dependence on fossil-based plastics with bioplastics in the near future.

CONCLUSION

Through this review, we have focused on the types of bioplastics available and have classified them based on the resources used in their production. There has been an increased focus on research on bioplastics as the world is moving away from the harmful fossil-based plastics owing to the awareness raised in the recent times. This has also led to everyone jumping into the bandwagon of making bioplastics, with many making them in combination with nonbiodegradable resources. The use of such plastics under the label of "bioplastics" can do equal harm to environment in the long run. This review has emphasized the need for proper classification of bioplastics, highlighting the various resources used in generation of bioplastics, in detail. If we are to replace single use plastics with bioplastics and avoid further plastic waste accumulation, it is also imperative that we understand what the waste management options are for these materials. The behaviours of different biodegradable plastics in a range of environments have to be hence assessed, and it must be determined under which conditions plastics show complete biodegradation. Proper technology involving use of sustainable resources and agricultural wastes with reduced production cost with complete awareness on impact on environment is the need of the hour.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Table 1: Comparison of properties of various bioplastics

Materials	Biodegradability	Melting temperature	Sources	Properties	Application
Sustainable plastics (BioPET, bioPE)	20 to 100% biobased, non- biodegradable and non-compostable	Approximately 230^{0}C	Sugarcane molasses, vegetable oils	Equivalent to traditional polymers, remain recyclable, non- biodegradable, ease of use	All type of packaging, technical parts, etc.
PLA	100% bio based and 100%biodegradable and compostable	130-150^0 C	Maize, Sugarcane, sugar beet, tapioca, etc.	Transparent, rigid, low thermal resistance, low barrier properties	Agro food Packaging (containers, films, cups,etc.), cosmetics injection moulded parts, as part of bio composites etc.
PHAs	100% biobased and 100% biodegradable and compostable	40-180^0 C	Microorganisms	Opaque to translucent, rigid to elastomeric, good thermal resistance and barrier properties	Biocomposite part, injection moulded parts, packaging film, etc.
Bio polyester	Partially biobased and 100% biodegradable and compostable	160-180^0 C	Sugarcane, starch, etc.	Opaque to translucent, rigid to flexible, good thermal resistance	Bag manufacturing, Mulching film, vials, injection moulded parts, etc.
Cellulosic derivatives	Mostly biobased, biodegradable and compostable	297^{0}C	Wood pulp	Transparent, rigid, good thermal, mechanical and barrier properties	Agro-food packaging as films, injection moulded parts, etc.
Bio- elastomers	Partially bio based and also 100% biodegradable and compostable	120-140^0 C	Different bio based polyols (vegetable oils, sugars, etc.)	Very flexible, good mechanical properties and easily transformable	Technical and injection moulded parts, primarily
Starch based compounds	Partially bio based and may be biodegradable and compostable	90-180^{0}C	Starch (corn, potato, zein etc.), flours	Flexible, sensitive to moister, controlled biodegradation	Bag manufacturing, mulching film, horticulture, etc.
Biocomposites	Partially biobased and may be biodegradable and compostable	297^{0}C	Wood fibers, hemp, flex, bamboo and bioplastics or conventional matrix	Rigid, good mechanical and thermal resistance, easily transformable	Technical and injection moulded parts, primarily

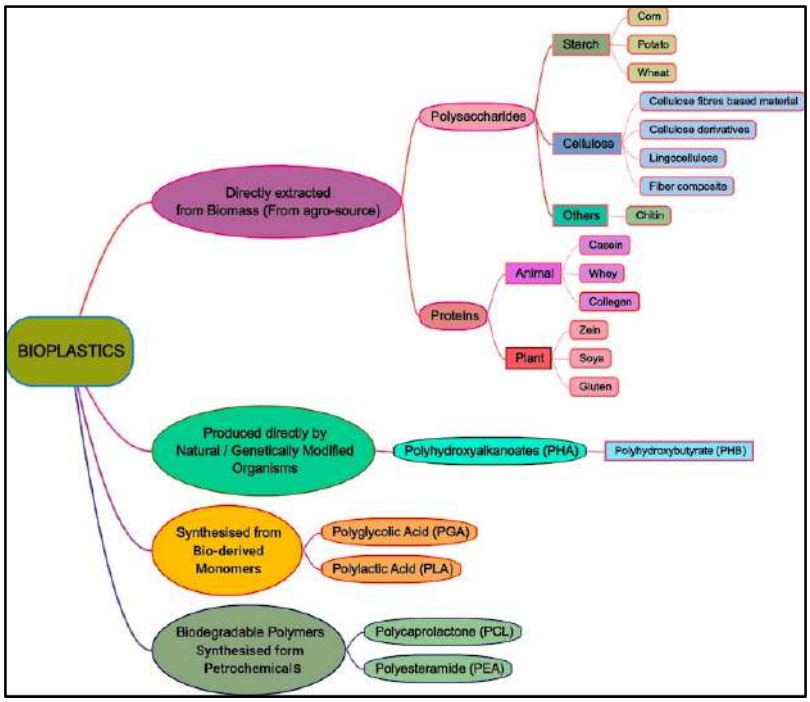

Fig. 1: Various resources used for generation of bioplastics

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RESEARCH ARTICLE

New Oscillation Criteria for Third Order Quasilinear Delay Difference Equation with Unbounded Neutral Co-Efficients

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ABSTRACT

The article deals oscillation criteria of third order quasilinear difference equations with unbounded neutral coefficients

$$\Delta(e_n(\Delta^2 z_n)^\alpha) + H_n x_{n-\lambda}^\gamma = 0, n \geq n_0$$

where $z_n=x_n+q_n x_{n-\sigma}$, α and γ are ratios of positive add integers, and obtain sufficient conditions for the oscillation of every solution of studied equation. This result is presented that extend and generalize those results given in the literature.

Keywords: Third order, Neutral difference equations, Oscillatory Solutions, Quasi-linear, non-Oscillatory.
2010 Mathematics Subject Classification: 39A10

INTRODUCTION

The article is concerned with new oscillatory behaviour of solutions of the third quasi-linear neutral difference equation

$$\Delta(e_n(\Delta^2 z_n)^\alpha) + H_n x_{n-\lambda}^\gamma = 0, n \geq n_0 \quad (1.1)$$

where $z_n=x_n+q_n x_{n-\sigma}$ and α, γ are ratios of positive add integers. Subject to the following assumed conditions without further notice.

(c_a) q and H are positive real sequences,

$pn \geq 1$, $pn \neq 1$ for large n.

(c_b) e_n is sequence of positive integers;

(c_c) σ and λ are positive integers.

By a solution of (1.1) mean a nontrivial and real sequence x, defined for every $n \geq n_0$ and satisfying (1.1). A nontrivial solution x of (1.1) is said to be non-oscillatory if it is either ultimately positive or ultimately negative and otherwise oscillatory the equation itself non oscillatory if everyone is non oscillatory solution.

Recently, it is easy to notice that multiplying the interest of studying the oscillatory behavior of third order quasilinear difference equations, see for examples [2 - 9], and the reference cited there in.

In [8], E. Thandapani et.al. deals with the oscillation theorem for second order quasilinear neutral type difference equation

$$\Delta(a_n(\Delta(x_n + p_n x_{\tau(n)})^{\alpha}) + q_n x_{\sigma(n)}^{\beta} = 0.$$

via Comparison theorem with the condition $\sum_{n=n_0}^{\infty} \frac{1}{a_n^{1/\alpha}} = \infty.$

In [9] authors discuss new oscillatorybehavior of third order half linear neutral type difference equation

$$\Delta(a_n(\Delta^2(x_n + b_n x_{n-\gamma})^{\alpha}) + q_n x_{n+1-\tau}^{\alpha} = 0.$$

and

$$\Delta(a_n(\Delta^2(x_n - b_n x_{n-\gamma})^{\alpha}) + q_n x_{n+1-\tau}^{\alpha} = 0,$$

where a_n,b_n andq_n are positive realssequence&satisfies $\sum_{n=n_0}^{\alpha} \frac{1}{a_n^{1/\alpha}} = \infty.$

Motivated by this literature. In this note, we scrutinize the oscillation and asymptotic properties of solutions of (1.1). Thisresult established in the paper content is extend and complement of known results.

1. MAIN RESULT

We stated with the following notations and lemmas which is important to proving main results. Define the following:

$$A = \sum_{s=n_1}^{n-1} \frac{1}{e_s^{1/\alpha}}, \; B = \sum_{s=n_1}^{n-1} As, \; C = e^x p[\sum_{s=n_1}^{n-1} \frac{As}{Bs}], \; n \geq n_1.$$

$$P_n^* = \frac{1}{P_{n+\sigma}}(1 - \frac{1}{q_{n+2\sigma}}) > 0;$$

$$P_n^{**} = \frac{1}{P_{n+\sigma}}(1 - \frac{C_{n+2\sigma}}{q_{n+2\sigma} C_{n+\sigma}}) > 0;$$

and,

$$E_n = \sum_{t=n+\sigma-\lambda}^{n+\sigma-\tau-1} \sum_{s=t}^{t+\sigma-\tau-1} \frac{1}{e_s^{1/\alpha}}, n \geq n_1.$$

Lemma:2.1 Let x_n be a non-negative solution of (1.1). Then, the sequence y_n satisfies either

I $yn > 0$, $\Delta yn > 0$, $\Delta 2yn > 0$ and $\Delta(an\ (\Delta 2yn)\ \alpha) \le 0$

(or)

II. $yn > 0$, $\Delta yn < 0$, $\Delta 2yn > 0$ and $\Delta(an\ (\Delta 2yn)\ \alpha) \le 0$

For sufficiently large n.

Lemma : 2.2 Assume Z satisfies case I of lemma 2.1 for every $n \ge n_1$. Then

$$\Delta z_n \ge A_n c_n^{1/\alpha} \Delta^2 z_n \text{ (2.1)}$$

$$z_n \ge B_n c_n^{1/\alpha} \Delta^2 z_n \text{ (2.2)}$$

$$z_n \ge \frac{B_n}{A_n} \Delta z_n \text{ (2.3)}$$

and $\frac{z_n}{c_n}$ is decreasing for every $n \ge n_1$(2.4)

Proof: By assumption Z_n satisfies first case of Lemma(2.1)

then $\Delta(e_n(\Delta^2 z_n)^\alpha \le 0,\ (e_n(\Delta^2 z_n)^\alpha$ is decreasing nature.

$$\Delta z_n = \Delta z_{n_1} + \sum_{s=n_1}^{n-1} \frac{(e_s(\Delta^2 z_s)^\alpha)^{1/\alpha}}{e_s^{1/\alpha}}$$

Hence,

$$\ge A_n e_n^{1/\alpha} \Delta^2 z_n.$$

Summing again from n_1 to n-1, wereach at

$$z_n \ge e_n^{1/\alpha} \Delta^2 z_n \sum_{s=n_1}^{n-1} A_s = B_n e_n^{1/\alpha} \Delta^2 z_n$$

In view point of (2.1),

$\frac{\Delta z_n}{A_n}$ was decreasing.

Since, $z_n = z_1 + \sum_{s=n_1}^{n-1} \frac{A_s \Delta z_s}{A_s}$

$$\ge \frac{B_n}{A_n} \Delta z_n.$$

From the above inequality, we get

$$\Delta\left(\frac{z_n}{c_n}\right) = \left(\frac{c_n \Delta z_n - \Delta c_n z_n}{c_{n+1} c_n}\right.$$

$$= \frac{\left(\frac{B_n}{A_n}\Delta z_n - z_n\right)\frac{A_n}{B_n}}{c_{n+1}} \leq 0.$$

Hence $\frac{z_n}{c_n}$ is decreasing. Lemma is complete.

Theorem :2.3 Assume conditions ((c$_a$)-(c$_c$)) hold, let $\tau > 0$ such that $n-\lambda \leq n-\tau \leq n-\sigma$ for every n≥n$_1$. If both the first order delay difference equations

$$\Delta X_n + H_n (P^*_{n-\lambda})^\gamma B^{*\gamma}_{n+\sigma-\lambda} \lambda^{\gamma/\alpha}_{n+\sigma-\lambda} = 0 \text{ (2.5)}$$

and $$\Delta w_n + H_n (P^*_{n-\lambda})^\gamma E^\gamma_n w^{\gamma/\alpha}_{n+\sigma-\tau} = 0 \text{ (2.6)}$$

oscillates then equation (1.1) is oscillated.

Proof : Let x be a non-oscillatory and non-negative solution of (1.1).we may choose $n_1 \geq n_0$ such that $x_n > 0$, $x_{n-\sigma} > 0$, and $x_{n-\lambda} > 0$. for every $n \geq n_1$. From Lemma 2.1, we say that z satisfies either one of the case of Lemma 2.1.

Assume the case (I) holds, then, from the definition of z, we have

$$x_n = z_n - q_n (x_{n-\sigma})$$

Denote $n = n+\sigma$, $x_n = \frac{1}{q_{n+\sigma}}(z_{n+\sigma} - x_{n+\sigma}),\ for\ n \geq n_1$

$$= \frac{z_{n+\sigma}}{q_{n+\sigma}} - \frac{x_{n+\sigma}}{q_{n+\sigma}}$$

$$= \frac{z_{n+\sigma}}{q_{n+\sigma}} - \frac{z_{n+2\sigma} - x_{n+2\sigma}}{q_{n+\sigma} q_{n+2\sigma}}$$

$$\geq \frac{z_{n+\sigma}}{q_{n+\sigma}} - \frac{z_{n+2\sigma}}{q_{n+\sigma} q_{n+2\sigma}} \qquad (2.7)$$

In view (2.4), we have $\frac{z_n}{c_n}$ is decreasing nature and $n+2\sigma \geq n+\sigma$ then from (2.7), we obtain

$$x_n \geq \frac{1}{q_{n+\sigma}}\left(1 - \frac{c_{n+2\sigma}}{q_{n+2\sigma} c_{n+\sigma}}\right) z_{n+\sigma}$$

$$x_n \geq p^{**}_n z_{n+\sigma}\ .$$

Also,

$$x_{n-\lambda} \geq p^{**}_{n-\lambda} z_{n+\sigma-\lambda} \quad (2.8)$$

Employing (1.1) with (2.8), we obtain

$$\Delta(e_n(\Delta^2 z_n)^\alpha) + H_n(p^{**}_{n-\lambda})^\gamma z^\gamma_{n+\sigma-\lambda} \leq 0, n \geq n_1. \quad (2.9)$$

It follows from (2.2), we have,

$$z_{n+\sigma} \geq B_{n+\sigma} e^{1/\alpha}_{n+\sigma} \Delta^2_{n+\sigma}, n \geq n_1.$$

Also, $z_{n+\sigma-\lambda} \geq B_{n+\sigma-\lambda} e^{1/\alpha}_{n+\sigma-\lambda} \Delta^2 z_{n+\sigma-\lambda}, n \geq n_1.$ (2.10)

Combining (2.9) & (2.10) which yields

$$\Delta(e_n(\Delta^2 z_n)^\gamma + H_n(p^{**}_{n-\lambda})^\gamma B^\gamma_{n+\sigma-\lambda}(e_{n+\sigma-\lambda}(\Delta^2_{n+\sigma-\lambda})^\alpha)^{\gamma/\alpha} \leq 0.$$

By letting $X_n = e_n(\Delta^2 z_n)^\alpha$ in last inequality, we have X is a non-negative solution of the delayfirst orderdifference inequality

$$\Delta X_n + H_n(p^{**}_{n-\lambda})^\gamma B^\gamma_{n+\sigma-\lambda} X^{\gamma/\alpha}_{n+\sigma-\lambda} \leq 0$$

By corollary [8], see that the equation (2.5), has non negative solution, a contradiction.

If case II holds, then z is decreasing & $n - \sigma \leq n$, we obtain

$$z(n+\sigma) \geq z(n+2\sigma).$$

By using the above in the inequality (2.7),

$$x_n \geq \frac{z_{n+\sigma}}{q_{n+\sigma}} - \frac{z_{(n+2\sigma)}}{q_{n+\sigma} q_{n+2\sigma}}$$

$$\geq \frac{1}{q_{n+\sigma}}\left(1 - \frac{1}{q_{n+2\sigma}}\right) z_{n+\sigma}$$

$$= P^*_n z_{n+\sigma}.$$

Then $x_{n-\lambda} \geq p^*_{n-\lambda} z_{n+\sigma-\lambda}$ (2.11.)

By utilizing the inequality (2.11) in (1.1) we obtain

$$\Delta(e_n(\Delta^2 z_n)^\alpha) + H_n(P^*_{n-\lambda}) z^\gamma_{n+\sigma-\lambda} \leq 0.\ n \geq n_1. \quad (2.12)$$

Let $n \geq s \geq n_1$, we have

$$\Delta z_n - \Delta z_s = \sum_{u=s}^{n-1} \frac{e^{1/\alpha}_u \Delta^2 z_u}{e^{1/\alpha}_u}$$

$$-\Delta z_s = (\sum_{u=s}^{n-1} \frac{1}{e^{1/\alpha}_u}) e^{1/\alpha}_n \Delta^2 z_n.$$

By summing from s to n-1, we get,

$$-z_n + z_s \geq \sum_{u=s}^{n-1}\left(\sum_{r=u}^{n-1}\frac{1}{e_r^{1/\alpha}}\right)e_n^{1/\alpha}\Delta^2 z_n$$

$$z_s \geq \sum_{u=s}^{n-1}\left(\sum_{r=u}^{n-1}\frac{1}{e_r^{1/\alpha}}\right)e_n^{1/\alpha}\Delta^2 z_n. \qquad (2.13)$$

We know that $n-\lambda \leq n-\tau$ and $\sigma > 0$, then

$n+\sigma-\lambda \leq n+\sigma-\tau$ and substitute $s = n+\sigma-\lambda$ and $n = n+\sigma-\tau$ in (2.13).

$$z_{n+\sigma-\lambda} \geq \sum_{u=n+\sigma-\lambda}^{n+\sigma-\tau-1}\left(\sum_{v=u}^{n+\sigma-z-1}\frac{1}{e_v^{1/\alpha}}\right)e_{n+\sigma-\tau}^{1/\alpha}\Delta^2 z_{n+\sigma-\tau},$$

Using the above in (2.12) we reach

$$\Delta(e_n(\Delta^2 z_n)^\alpha) + H_n(P^*_{n-\lambda})^\gamma E_n^\gamma (e_{n+\sigma-\tau}(\Delta^2 z_{n+\sigma-\tau})^\alpha)^{\gamma/\alpha} \leq 0.$$

By letting $w_n = e_n(\Delta^2 z_n)^\alpha$, we see that w is a non-negative solution of the first order delay difference inequality

$$\Delta W_n + H_n P^*_{n-\lambda} E_n^\gamma W_{n+\sigma-\tau}^{\gamma/\alpha} \leq 0.$$

The remaining proof is same as the case (I). Hence proof of theorem is compete.

Corollary: 2.4: Assume the conditions (c_a) - (c_c) hold and $\alpha = \gamma$. Let $\tau > 0$ such that $n-\lambda \leq n-\tau \leq n-\sigma$ for every $n \geq n_0$.

If $$\liminf \sum_{s=n+\sigma-\lambda}^{n-1} H_s(P^*_{S-\lambda})^\gamma B^\gamma_{s+\sigma-\lambda} > \left(\frac{1}{1+\gamma}\right)^{1+\gamma}. \qquad (2.14)$$

and $$\liminf \sum H_s\left(P^*_{s-\lambda}\right)^\gamma E_s^\gamma \geq \left(\frac{1}{1+\gamma}\right)^{1+\gamma}, \qquad (2.15)$$

then (1.1) is oscillates.

Proof: The proof follows theorem (2.3).

Corollary 2.5: Assume that the conditions (c_a) - (c_c) hold and $\alpha > \gamma$. let $L\tau > 0$ such that $n-\lambda \leq n-\tau \leq n-\sigma$ for every $n \geq n_0$. If

$$\sum_{s=N}^{\infty} H_s(P^{**}_{n-\lambda})^\gamma \beta^\gamma_{n+\sigma-\lambda} = \infty \qquad (2.16)$$

and

$$\sum H_s P^{*\gamma}_{n-\lambda} E_n^\gamma = \infty, \qquad (2.17)$$

for every $n \geq N \geq n_0$, then (1.1) is oscillates.

Proof: The proof follows from Theorem 2.3, shows that (2.5) and (2.6) oscillates. Then by Theorem 2.3 equation (1.1) is oscillates.

Theorem : 2.6 Let us assume the conditions (c_a) - (c_c) hold and $\alpha = \gamma$. let $\tau > 0$, such that $n-\lambda \leq n-\tau \leq n-\sigma$ for every $n \geq n_0$. If (2.15) holds and these exists a non-negative sequence η & non- decreasing such that

$$\limsup \sum_{S=n_1}^{n-1} [\eta_s H_s \left(P_{n-\lambda}^{**}\right)^{\alpha}] - \frac{(\Delta\eta_s)^{\alpha+1}}{(\alpha+1)^{\alpha+1} A_{s+1+\sigma-\lambda}^{\alpha}} < \infty. \quad (2.18)$$

then (1.1) is oscillates.

Proof: Let x be a solution of (1.1) and non-oscillatory and non -negative. we choose $n_1 \geq n_0$ such that $x_n > 0, x_{n-\lambda} > 0, and\ x_{n-\sigma} > 0,$ for every $n \geq n_1.$ Then the non-negative sequence Z satisfies either one of the case of Lemma 2.1.

Proof of theorem 2.3 case (I) for $\alpha = \gamma$, we reach at

$$\Delta\left(e_n(\Delta^2 z_n)^{\alpha}\right) + H_n(P_{n-\lambda}^{**})^{\alpha} Z_{n+\sigma-\lambda}^{\alpha} \leq 0,\ n \geq n_1. \quad (2.19)$$

Define

$$G_n = \frac{\eta_n e_n(\Delta^2 z_n)^{\alpha}}{Z_{n+\sigma-\lambda}^{\alpha}}\ n \geq n_1 .\ G_n > 0$$

$$\Delta G_n = \Delta(\frac{e_n(\Delta^2 z_n)^{\alpha}}{Z_{n+\sigma-\lambda}^{\alpha}})\eta_n + \frac{e_{n+1}(\Delta^2 z_{n+1})^{\alpha}}{Z_{n+1+\sigma-\lambda}^{\alpha}})\Delta\eta_n$$

$$= (\frac{e_{n+1}(\Delta^2 z_{n+1})^{\alpha}}{Z_{n+1+\sigma-\lambda}^{\alpha}})\Delta\eta_n + \eta_n[\Delta(e_n(\Delta^2 z_n)^{\alpha}\frac{1}{Z_{n+\sigma-\lambda}^{\alpha}} + c_{n+1}(\Delta^2 z_{n+1})^{\alpha}\Delta\frac{1}{Z_{n+\sigma-\lambda}^{\alpha}}]$$

$$\Delta G_n \leq \Delta\eta^n \frac{G_{n+1}}{\eta_{n+1}} - \eta_n H_n[P_{n-\lambda}^{**}]^{\alpha} - \alpha G_{n+1}\frac{\Delta(Z_{n+1+\sigma-\lambda})}{(Z_{n+1+\sigma-\lambda})}$$

In view of (2.1) and $e_n(\Delta^2 z_n)^{\alpha}$ is decreasing nature, we obtain

$$\Delta G_n \leq \Delta\eta_n \frac{G_{n+1}}{\eta_{n+1}} - \eta_n H_n[P_{n-\lambda}^{**}]^{\alpha} - \alpha\frac{G_{n+1}^{1+1/\alpha}}{\eta_{n+1}^{1/\alpha}}(\Delta_{n+1+\sigma-\lambda}) \quad (2.21)$$

By utilizing the inequality,

$$BK - AK^{1+1/\alpha} \leq \frac{\alpha^{\alpha}}{(\alpha+1)^{\alpha+1}}\frac{B^{\alpha+1}}{A^{\alpha}}\ for A > 0 and K > 0$$ in (2.21)

$$\Delta G_n \leq -\eta_{n+1} H_n(P_{n-\lambda}^{**})^{\alpha} + \frac{(\Delta\eta_n)^{\alpha+1}}{\eta_{n+1}^{\alpha}}\frac{1}{A_{n+1+\sigma-\lambda}}$$

Summing latter inequality from n to n-1,weget

$$\sum_{s=n_1}^{n-1}[\eta_{s+1} H_s(P_{s-\lambda}^{**})^{\alpha} - \frac{(\Delta\eta_s)^{\alpha+1}}{\eta_{s+1}^{\alpha}}\frac{1}{A_{s+1+\sigma-\lambda}^{\alpha}}] < \infty$$

By taking $n \to \infty$ and supremum of the above inequality we get contradicts to (2.18).

In the case (II), In view of (2.15). It is easy to see that equation (2.6) is oscillates. Hence by Theorem 2.3 equation (1.1) is oscillates. Now proof of theorem becomes to end.

Theorem: 2.7; Assume the conditions (c_a) - (c_c) hold and $\gamma \le \alpha$. let $\tau > 0$, such that $n-\tau \le n-\sigma$ for every $n \ge n_1$.

If $\lim_{n\to\infty} \sup A_{n+\sigma-\lambda}^{\gamma} \sum_{s=n}^{\infty} H_s (P_{s-\lambda}^{**})^{\alpha} \frac{B_{s+\sigma-\lambda}^{\gamma}}{A_{s+\sigma-\lambda}^{\gamma}} \begin{cases} = \infty if \gamma < \infty \\ > 1 if \gamma = \infty \end{cases}$ (2.22)

and

$$\limsup_{n\to\infty} \sum_{s=s+\sigma-\lambda}^{n+\sigma-\lambda} H_s (P_{s-\lambda}^{**})^{\gamma} E_1(n,s). \begin{cases} = \infty if \gamma < \infty \\ > 1 if \gamma = \infty \end{cases} \quad (2.23)$$

where $E_1(n,s) = \sum_{V=s+\sigma-\lambda}^{n+\sigma-\lambda} \sum_{s=u}^{v+\sigma-\lambda} \frac{1}{e_s^{1/\alpha}}$, then the equation (1.1) is oscillated.

Proof: Let x is non-oscillatory and non -negative solution of (1.1). we may choose that

Let $n \ge n_0$ such that $x_n > 0$, $X_{n-\sigma} > 0 and X_{n-\lambda} > 0$. for every $n \ge n_1$. By lemma 2.1, corresponding z satisfies case (I) or case (II).

In the case (I), by proof of Theorem 2.3, we see that

$$\Delta\left(e_n (\Delta^2 z_n)^{\alpha}\right) + H_n (P_{n-\lambda}^{**})^{\alpha} z_{n+\sigma-\lambda}^{\alpha} \le 0.$$

By utilizing (2.3) in the above inequality we have

$$\Delta\left(e_n (\Delta^2 z_n)^{\alpha}\right) + H_n (P_{n-\lambda}^{**})^{\gamma} \frac{B_{n+\sigma-\lambda}^{\gamma}}{A_{n+\sigma-\lambda}^{\gamma}} \Delta z_{n+\sigma-\lambda}^{\gamma} \le 0, \; n \ge n_1. \quad (2.24)$$

$$n+\sigma-\lambda \le n.$$

We conclude that

$$\left(e_n (\Delta y_n)^{\alpha}\right)^{1-\frac{\gamma}{\alpha}} \ge A_{n+\sigma-\lambda}^{\gamma} \sum_{s=n}^{\infty} H_s (P_{s-\lambda}^{**})^{\gamma} \frac{B_{n+\sigma-\lambda}^{\gamma}}{A_{n+\sigma-\lambda}^{\gamma}}$$

Letting n $\to \infty$ and lim sup in the last inequality. Which contradicts to (2.22). In the case(II), as the proof of Theorem 2.3 reach at (2.12) and (2.13).

Fact that $n+\sigma-\lambda > s+\sigma-\lambda$, for $n \ge s$. By substituting, $s = s+\sigma-\lambda$ and $n = n+\sigma-\lambda$ in (2.13)

We have,

$$z_{s+\sigma-\lambda} \ge \left[\sum_{u=s+\sigma-\lambda}^{n+\sigma-\lambda} \left[\sum_{s=u}^{n+\sigma-\lambda} \frac{1}{e_s^{1/\alpha}} \right] \right] e_{n+\sigma-\lambda}^{1/\alpha} \Delta^2 z_{n+\sigma-\lambda} \quad (2.26)$$

Summing (2.12) from $n+\sigma-\lambda$ to n-1 and update with (2.26).

$$e_{n+\sigma-\lambda} (\Delta^2 z_{n+\sigma-\lambda})^{\alpha} \ge \sum_{s=n+\sigma-\lambda}^{n-1} H_s (P_{s-\lambda}^{**})^{\gamma} E_1^{\gamma}(n,s) (e_{n+\sigma-\lambda} (\Delta^2 z_{n+\sigma-\lambda})^{\alpha})^{\gamma/\alpha}$$

We rewrite as

$$\left[[e_{n_0+\sigma-\lambda}(\Delta^2 z_{n_0+\sigma-\lambda})^{\alpha}]^{\gamma/\alpha} \geq \sum_{s=n+\sigma-\lambda}^{n-1}[H_s(P_{s-\lambda}^{**})^{\gamma} E_1^{\gamma}(n,s)]\right]$$

Assume that $y_n = \Delta z_n$, then $y_n>0$, $\Delta y_n > 0$, and $\Delta(c_n(\Delta y_n)^{\alpha}) \leq 0$, $forevery n \geq n_1$.

Hence, $e_n^{1/\alpha}(\Delta y_n)$ is decreasing nature and non- negative. We have, for $n \geq s \geq n_1$

$$\Delta y_s \geq \frac{e_n^{1/\alpha}\Delta y_n}{e_n^{1/\alpha}}.$$

Summinglatter inequalityfrom n_1 to n-1, we have

$$y_n \geq A_n c_n^{1/\alpha}\Delta y_n, n \geq n_1 \qquad (2.25)$$

By substituting (2.25) in (2.24) we obtain

$$\Delta\left(e_n(\Delta^2 z_n)^{\alpha}\right) \geq H_n(P_{n-\lambda}^{**})^{\gamma}\frac{B_{n+\sigma-\lambda}^{\gamma}}{A_{n+\sigma-\lambda}^{\gamma}} y_{n+\sigma-\lambda}^{\gamma}.$$

Again, summing from n to ∞, we obtain

$$\Delta\left(e_n(\Delta^2 z_n)^{\alpha}\right) \geq \sum_{s=n}^{\infty} H_s(P_{n-\lambda}^{**})^{\gamma}\frac{B_{n+\sigma-\lambda}^{\gamma}}{A_{n+\sigma-\lambda}^{\gamma}} A_{n+\sigma-\lambda}^{\gamma} e_{s+\sigma-\lambda}^{\gamma/\alpha}(\Delta y_s)^{\gamma}$$

$$= A_{n+\sigma-\lambda}^{\gamma}(e_{n+\sigma-\lambda}(\Delta y_n)^{\alpha})^{\gamma/\alpha}\sum_{s=n}^{\infty} H_s(P_{s-\lambda}^{**})^{\gamma}\frac{B_{s+\sigma-\lambda}^{\gamma}}{A_{s+\sigma-\lambda}^{\gamma}}.$$

We know that, $e_n^{1/\alpha}(\Delta y_n)$ is decreasing nature and letting n →∞ and lim sup of the above inequality, which contradicts with (2.23). Proof now complete.

2. Examples:

Example: 3.1 Let us consider third order neutral delay difference equation

$$\Delta\left(\frac{1}{8+4n}\left(\Delta^2(x_n + nx_{n-2})\right)\right) + 2x_{n-2} = 0,\ n \geq 2, \quad (3.1)$$

Here $\sigma = 2, \lambda = 2, \alpha = 1, \gamma = 1, \tau = 2, q_n = n, e_n = \frac{1}{8+4n} and H_n = 2.$

Easily to see that all important conditions in corollary 2.4 are satisfactory.The condition (2.14) becomes

And the condition (2.15) becomes,

$$\sum_{t=n_1}^{n-1} 2\left(\left(\frac{1}{t-4}\right)\left(1-\frac{1}{t+2}\right)\right)\sum_{s=t_1}^{n-1}\sum_{u=s_1}^{n-1}\frac{1}{8+4u} > \left(\frac{1}{2}\right)^2.$$

Hence every solution of equation (3.1) is oscillated, here $x_n = (-1)^{3n}$ is one such solution.

Example: 3.2 Let us consider the third order neutral delay difference equation

$$\Delta\left(\frac{1}{64n^2}\left(\Delta^2 x_n + nx_{n-1}\right)^3\right) + 2x_{n-3} = 0,\ n \geq 3 \quad (3.2)$$

Here $\sigma = 1, \lambda = 3, \alpha = 3, \gamma = 1, q_n = n, e_n = \frac{1}{64n^3}$ *and* $\mathrm{H}_n = 2.$

It is easily to see that all the important conditions in corollary (2.5) are satisfactory, and the condition (2.16) becomes

$$\sum_{t=n_1}^{n-1} 2\frac{1}{t+1}\left(1 - \frac{2(t+2)}{(t+2)2(t+1)}\right)\sum_{s=t_1}^{n-1}\sum_{u=s_1}^{n-1}\frac{1}{64}\left(u^3 - 2\right)^3 = \infty$$

And $\sum_{t=n_1}^{n-1}\frac{1}{t+1}\left(1 - \frac{2(t+2)}{(t+2)2(t+1)}\right)\sum_{s=t_1}^{n-2}\sum_{u=s_1}^{s-2}\frac{1}{64}u^3 = \infty.$

Hence every solution of equation (3.2) is oscillating. Here $x_n = (-1)^n$ is one suchsolution.

CONCLUSION

In this article, we establishing the Oscillation Criteria for third order Neutral delay difference equations comparing with first order equations. Which often were based Riccati substitution and involving limsup conditions for oscillation result. In last section two examples listed to supporting main results.

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REVIEW ARTICLE

A Review on Nanofiber Face Masks

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ABSTRACT

The fibers are basic building materials to human beings. The uniqueness and capability of nanofibers face masks are used to protect people from various airborne disease. The composition and membranes in nanofiber face mask are made of high quality and resistant to fluids. The working of nanofibers is based on physical adhesion barriers. The current invention has excellent blocking ability against foreign particles. The manufacturing of nanofibers involves two or more polymer resins with different melting points. The electro spinning and the centrifugal multispun method are the easiest techniques involved in manufacturing of nanofiber face mask. Inspite the N95 mask has the advantages it also has drawbacks. The nanofiber face masks are applied in various aspects.

Keywords: Fibers, fabrics, particulate matters, aerosols, electro spin, polymers, viruses

INTRODUCTION [1-3]

For now, the public health corporations of all countries have been methodically organized to make sure the safety in their citizens from virus threats. The use of the face masks is a popular running system for many people including those in healthcare. The WHO (global health enterprise, quote that wearing a medical mask can restriction the spread of breathing viral sicknesses, including COVID-19. The N95 is one of the most not unusual face mask filter requirements, posted by (NIOSH. the country wide institute for occupational safety and health. Filters composed of nano-sized fibers have a 99.8% efficiency in inhibiting viruses and are pretty at ease in use. Nanofibers have a completely high floor location per unit mass that improves capture efficiency which engineered into the fiber surfaces like ion trade or catalysis.

COMPOSITION OF NANOFIBER FACE MASK [4-5]

The N95 respirators is composed of many layers PP nonwoven material. The external protecting layer are produced using spunbond to cover both the inner and outer of the N95 respirator. there is a layer of prefilteration layer among the spunbond layer which can be as thick as 250g/m2 , make it stiffer and thicker, so it can be bendy enough to form the require shape. The final layer is a nonwoven soften-blown electric fabric of that controls filtration competence. The entire respirators are synthetic through converted equipment, welding the layers through ultrasonic and including belts and strips of metal to regulate the masks over the users face. Ultimately, respirators are sanitized earlier than shipment.

MEMBRANES IN NANOFIBER MASK

[6-8]

The face masks must have the precise standards and guidelines, primarily based on the nation or geographical area. The ASTM F2100 -1 requirements are certified with five overall performance metric for substances used to make the clinical face masks which includes resistance to fluid , breathability, bacterial filtration performance (BFE), particulate filtration performance (PFE), and flammability. ASTM attributes the substance barriers efficiency to a numerical rating.

a) Level -1, Barrier fluid contact at low risk
b) Level -2, Barrier fluid contact at moderate risk
c) Level -3, Barrier fluid contact at high risk

The N95 consists of an outer layer constructed of hydrophobic nonwoven PP (to prevent moisture), a filter out layer of melt-blown nonwoven PP (to seize oil and non-oil-primarily based debris), an assist layer, and an internal layer.

a) 1st layer (inhibits fluid carrier to enter)
b) 2nd layer (cut-off size filter retain viruses in both direction)
c) 3rd layer (adsorbs fluids from the wearer)

The respirators ought to offer protection at the very best concentration the individual will revel in. within the usa, the country wide Institute for Occupational safety and fitness (NIOSH) tests the filtration performance of particulate filtering, air-purifying respirators for certification purposes. NIOSH approves N-, R-, and P-series no powered air-purifying respirators, each at 95, 99, and 99.97% filtration efficiency tiers underneath forty two CFR

WORKING AND FILTRATION MECHANISMS OF PARTICLES [9-12]

Face masks are made of fine microscopic sieves that are the active layers using the mask's working mechanism. The first-rate microscopic sieve is made from entangled mats of very quality fibres capable of creating convoluted pathways that the air along with any particle or viruses and bacteria. Face mask and respirators have typically been used as protective devices for filtering airborne contaminants. Fibrous filters are used in contemporary masks and respirators, made from several flat, excellent, fibre layers of nonwoven mats, capable of capturing PM particles through physical adhesion boundaries.

Filtering of particles is basicallyachieved through five collections of mechanisms.

1) Interception
2) Inertial impaction
3) Diffusion
4) Gravitational settling and
5) Electrostatic attraction

There are four main types of applicable filtration mechanism using nanofiber filter media. These are,

1) Surface straining
2) Depth straining
3) Depth filtration and
4) Cake filtration

Although nanofiber lead to high performance, shorter lifestyles and a surface filtration technique. The beaded nanofiber is recommended for use for deep, high efficiency and short time filtration method.

CURRENT INVENTION OF NANOFIBER FACE MASK [13, 14]

In line with the configuration of the existing invention can gain all of the objects of the unique results, because the frame part of the face masks consists of a nanofiber layer having first-rate pores, the face mask in step with the existing invention has exceptional blocking capability in opposition to fine foreign substances which include bacteria and exceptional dust in addition, since the nanofiber layer is bonded by means of the recent soften sheet, the nanofibers may be well connected. And, because the nanofiber layer is sewn to the inner or outer pores and skin layer, the face masks according to the present invention is easy to manufacture and has a firmly bonded nanofiber layer.

The Inner Skin Layer [15]

It directly contacts the skin while the person wears the masks. As the inner skin layer, diverse substances including herbal fibers, synthetic fibers, and nonwoven fabrics may be used. Ideally, the inner skin layer is made from a cotton fabric having low irritation to the pores and skin and great breathability including gauze.

The Outer Skin Layer [16, 17]

It's far exposed to the out of doors without touching the pores and skin while the person wears the masks. The outer layer may be manufactured from the identical fabric as the internal layer. In a few cases, the outer skin layer can be fashioned of a fabric including nylon having high mechanical energy, thereby in addition increasing the damage preventing impact of the nanofiber layer.

The Nanofiber Layer[18-25]

It's far positioned among the inner pores and skin layer and the outer skin layer. Nanofiber oil layer includes nanofibers formed from fibers having a mean fiber diameter of fifty to 1,000 nm. Fine pores are shaped within the nanofiber layer to prevent the passage of microorganism or great dust. The size of the pores formed inside the nanofiber layer is preferably 05㎛ to 0.1㎛ diameter. If the size of the pores fashioned in the nanofiber layer is 0.1㎛ or extra in diameter, thinking about that the dimensions of most bacteria or best dirt is 0.1㎛ to 1.0㎛ it is hard to obtain a sufficient blocking impact against bacteria and excellent dust.

In addition, if the scale of the pores shaped inside the nanofiber layer is zero.05㎛ or less in diameter, the wearer may experience uncomfortable breathing. The nanofiber layer is shaped of polyvinylidene fluoride (PVDF) or nylon to which an antimicrobial substance, chlorhexidine gluconate (CHG) or polyhexamethylene biguanide (PHMB), is brought. Since the nanofiber layer consists of an antimicrobial material, the antimicrobial effect is in addition stepped forward. But, the prevailing invention does not restriction the material of the nanofiber layer to this. The nanofiber layer is formed through cutting the nanofiber adhesive sheet manufactured within the way defined under to the proper size after which thermally fusion to the internal skin layer or the outer pores and skin layer. Thereafter, the nanofiber layer may be sewn together to be bonded to one of the endothelial layer and the outer skin layer, or can be sewn collectively to be bonded to the endothelial layer and the outer pores and skin layer. Hereinafter, for example of a technique of manufacturing a nanofiber adhesive sheet that may be used as the nanofiber layer, a technique for generating a nanofiber adhesive sheet the usage of electro spinning method.

MANUFACTURING OF NANOFIBER FACE MASK [26-30]

In order to manufacture nanofibers, two or more polymer resins having unique melting factors are dissolved in a solvent to form a spinning solution. On this embodiment, polyvinylidene fluoride (PVDF) or nylon brought with chlorhexidine gluconate (CHG) or polyhexamethylene biguanide (PHMB) in which the spinning answer is an antibacterial substance it will be described as including. Next, the spinning solution is electro spun into the spinning space to form nanofibers on the recent soften sheet. Nanofibers are targeting the recent soften sheet to form a nanofiber web, and the recent melt sheet and the nanofiber internet are mixed to shape a nanofiber adhesive sheet.

The nanofiber adhesive sheet is conveyed by means of the conveyance belt pushed through the force rollers and wound via the winding device. Inside the manufacturing system, the nanofiber can be adjusted in size of pores formed inside the nanofiber via warmth treatment. Right now, the heat treatment temperature selects a temperature higher than the temperature at which as a minimum one type of the polymer resin is absolutely melted. The new melt sheet is a sheet which famous adhesiveness through heat and solidifies upon cooling to maintain adhesive energy, and is widely used for bonding.

For example [31-33]

A polyester hot melt cloth, a polyurethane hot melt nonwoven fabric, or a polyamide hot melt nonwoven cloth can be used. The new melt sheet need to then be fashioned so that the pores shaped in the nanofiber all through heat fusion can be properly maintained. The Nano melt is firmly bonded to the inner pores and skin layer or the outer skin layer of the masks through the new soften sheet. The fixing consists of a first catching strap and a 2nd catching strap. The primary and 2d locking straps are supplied at both sides of the safety unit with a purpose to be hooked to each ears, respectively. Two straps is shaped in the perfect length so that the protection unit may be in close contact with the face within the nation stuck on the ear.

TECHNIQUES IN NANOFIBER FACE MASK

Electrospinning Method [34-43]

Electro spinning is a unique technique to fabricate nanofiber, because it affords a quick method, low cost, and specific control of the nanofiber compositions and geometric membrane functions. In electro spinning, excessive voltages practice to melts or polymer solution droplets to get rid of the anxiety of liquid surface and ultrafine fibres with diameters between 40 and 2000 nm to be created. Selecting an appropriate solution concentration, suitable voltage, and the space between the assisting collector and the syringe tip is of huge importance for synthesizing uniform nanofiber. As a critical part of this generation, nanofiber-based filter media are the main additives for boosting filtration overall performance.

Electro spun nanofiber-based clear out media possess a high ratio of floor/extent, low-strain drop, right interconnectivity of voids, and controllable connectivity and morphology, rendering them proper to achieve outstanding filtering. Due to its fragility, electro spun nanofiber do no longer be used personally at filter out media, it ought to be deposited onto a substrate, usually fabric as nonwoven. Glass, polyester, nylon, and cellulose are the not unusual materials used to assist the electro spun nanofiber. The substrate need to have excellent mechanical houses to allow pleating, fabrication of filter out, and durability in usage. For the filtration recommend, substrates are selected for pleating, filter fabrication, sturdiness in use, and filter out cleaning.

Numerous research and patents on nanofiber had been diagnosed in unique face masks and respirator packages. The barrier development fabrics primarily based on nanofiber through electro spinning to be laminated onto face masks. This barrier protects from the permeation of microorganisms, dirt debris, and allergens. Prototype nanofiber-primarily based filter out media equipped face mask as compared to the N95 respirator are also produced. They discovered that the prototype significantly decreased airflow resistance, resulting in more face mask compliance and increased filtration efficiency, much like that received while the use of an N95 respirator. It became discovered that

electro spun nanofiber masks might be efficient at filtering out PM2.five particles and, at the same time, maintain appropriate breathability. Further, a nanofiber layer of cellulose acetate (CA) and polyvinylidene fluoride (PVDF) with one hundred% mechanical filtration for face masks and respirators capable of assembly the specifications of N95 respirators. The effect of nanofiber mat thickness, nanofiber diameters, and pore length on filtration performance turned into in comparison. The mean diameter of PVDF nanofiber (236.50 nm) turned into smaller than the diameter CA (319.02 nm) nanofiber. Consequently, CA nanofiber confirmed better filtration efficiency. The use of solution blow spinning (SBS) nanofiber is a massive step in developing a composite mask.

Preparation Procedure for Nanofibers [44, 45]

Polysulfone solution was organized at a concentration of 18 wt. % through dissolving in DMAc/acetone (9: 1) with vigorous stirring. The organized solution turned into stored overnight without stirring under room temperature to get rid of air bubbles. For the electro spinning, the 18 wt. % Polysulfone solutions were filled into a syringe with a metallic needle connected with a high-voltage energy supply. The voltage is 13 KV and the distance among the needle and the aluminium foil is 13 cm. The polymer answer become fed at a regular price of zero.4 mL/h by using a syringe pump. The nanofibers were collected on the floor of a non-woven PP on the grounded aluminium foil. The collective time of nanofibers turned into 15, 30, and 60 min

Centrifugal Multispun Method [46-48]

Nanofiber make right face masks filters because their mechanical interactions with aerosols particles deliver them a more ability to capture extra than 90percent of harmful particles including exceptional dirt and virus containing droplets. In response to this shortcoming, centrifugal spinning that utilizes centrifugal force instead of high voltage to provide polymer nanofiber has been advised as a more secure and extra cost effective alternative to the electro spinning. Smooth scalability is some other benefit, as this technology simplest calls for a rotating spinneret and a collector.

however, due to the fact the existing centrifugal pressure-based spinning generation employs most effective a single rotating spinneret, productivity is constrained and not tons higher than that of some superior electro spinning technology such as multi-nozzle electro spinning and nozzle less electrospinng. This hassle persists even if the scale when the size of the spinneret is elevated.

Inspired by way of those limitations, a research group led through professor do hymn Kim from the department of chemical and biomolecular engineering at KAIST developer a centrifugal multispinning spinneret with mass-reducibility, through sectioning a rotating spinneret into three sub-disks. This examine became published as front cowl article of ACS macro letters. While our device is scaled up from the size to a commercial scale, the massive-scale manufacturing of centrifugal multispun polymer nanofibers can be made feasible, and the cost of polymer nanofiber primarily based face masks filters can also be reduced dramatically.

APPLICATION

Thermal Management In Nanofiber Face Mask [49]

The concept of thermal management is brought into face masks for the first time to decorate the thermal consolation of the person. The device of nanofiber on nano porous polyethylene (fiber/nanoPE) is evolved in which the nanofibers with robust PM capture efficiency (99.6% for PM2.5) with low pressure drop and the nanoPE substrate with high infrared (IR) transparency (92.1%, weighted primarily based on human body radiation). Results in effective radiative cooling.

We further show that through coating nanoPE with a layer of Ag, the fiber/Ag/nanoPE masks indicates a higher IR reflectance (87.0%) and may be used for warming purposes. those multifunctional facemask designs may be explored

for both out of doors and indoor programs to shield people from PM pollution and concurrently reap personal thermal consolation.

DIY Nanofibers Face Mask [50]

An extensive range of portable, safe, battery-operated, and smooth to use electro spinners are available on the market which can be used to gain the electro spun nanofiber mats easily. Then, after acquiring the right electro spun filter out mats, they may be utilized while in a fully assembled operating face mask. A do-it-yourself (DIY) technique to gain a functional masks has been provided which may be followed at home. It's far critical to observe that face mask have to be made to comply with sure set standards.

Test For Nanofiber Face Mask [51]

Breathability test MIL-M 36954 C: ΔP

This measures the face mask's opposition to airflow, fluid resistance test ASTMF1862 which controls the resistance of the face mask to fluid diffusion.

Particulate filtration test ASTM F2299

This assesses the filterability of the face mask.

Bacterial filtration test ASTM F2101

This regulates the amount of bacteria larger than 3000 nm that can perhaps be filtered by the mask.

Flammability test 16 CFR Part 1610

Flame spread which events the flame resistance assets of the mask.

Other important test

Biocide efficiency, veridical efficiency, skin sensitivity, allergy, toxicity etc.

Nanofiber Mask In Sars-Cov-2 Airborne Disease [52]

The electro spun air filters showed incredible overall performance through shooting as much as 99.9% of coronavirus aerosols, which outperformed many industrial face masks," the look at states. "Further, we found that the identical electros pun air clear out or face mask eliminated NaCl aerosols equivalently or less correctly in comparison to the coronavirus aerosols whilst both aerosols have been generated from the same machine. Our work paves a new road for advancing air filtration through growing electros pun Nano fibrous air filters for controlling SARS-CoV-2 airborne transmission." Findings from examine showed that the cotton mask and neck gaiter removers around forty five% to 73% of the aerosols, at the same time as the surgical mask removed around 98%. But, the nanofiber clear out removed almost all the coronavirus aerosols, pretty much 99.nine%.

COST EFFECTIVE OF NANOFIBER FACE MASK [53-54]

The demand for face masks is growing exponentially due to the coronavirus pandemic and problems related to airborne particulate matter (PM). However, both traditional electrostatic- and nano sieve-primarily based mask filters are single-use and aren't degradable or recyclable, which creates extreme waste issues. This clear out is as efficient as the industrial N95 clear out and gets rid of 98.three% of 2.five µm PM. The nanofiber physically sieves fine PM and the microfiber offers a low pressure differential of 59 Pa that is comfortable for human respiration. In contrast to the dramatic overall performance decline of the industrial N95 filter out when exposed to moisture, this filter famous negligible performance loss and is therefore multi-usable due to the fact the everlasting dipoles of the chitosan adsorb ultrafine PM.

For example

1. Nitrogen
2. Sulfur dioxide.

Importantly, this filter completely decomposes inside four weeks in composting soil. An eco-friendly face mask filter with excessive-degree capability is advanced. Made most effective of biodegradable materials, it completely decomposes in the soil, thereby providing a essential waste hassle solution. moreover, the clear out is a sensible alternative to conventional disposable filters through integrating the physical sieving function of a polybutylene succinate fiber mat and the everlasting electrostatic adsorption roles of chitosan Nano whiskers.

ADVANTAGES

1. Emergingnovel masks and air filters by electrospinning is hopeful because of its high act in filtration, financial feasibility, and scalability, and it can meet on-site requirements of the masks and air filters[55]
2. Nanofibers are very lenient and delicate, especially with the aerosol flow driving through. But with sufficient care, perseverance, and luck, we ultimately got nice shots for our analysis[56]
3. The following liquid evaporation stage, meaningfullydipping the effective fiber length for capturing aerosols. They show hydrophobic and orthogonally woven fibers can lessen capillary forces and decrease the fiber combining rate [57]
4. Nanofiber filter has advanced heat release and carbon dioxide (CO2) emission concert and showssuperb breathability [58]
5. The filtration efficiency does not change even after the usage of 10 or more times.Nanofiber had lesser toxicity on human skin and vascular cells [59]

DISADVANTAGES

1. Nanofiber mask are presentlyhard to get, so there is an urgent necessity for a safe method of extending their usability through disinfection and reuse with leastcost of performance and integrity [60]
2. Ease of wearer and filter shape of mask changes after washing and disinfecting[61]
3. The biological care of nanofiber masks and maintenance of filtration efficiency after washing, which has newlydevelopa problematic[62]

CONCLUSION

Nowadays, people used to cover the face using N95 mask to safeguard them from foreign particles. The nanofiber face mask are increasing exponentially for the protection of people from various airborne disease and particulate matters. The hydrophobic layers and woven fibers in nanofiber face mask has good bacterial filtration efficiency. The N95 filters removes 98.3% of particulate matters hence it's considered to be finest fiber mask in this days. These mask are soft, flimsy and has its own comfort to wear. So in this days, wearing a nanofiber mask is must and considered to be significant role of every single person to provide themselves from the foreign particles. Though we wear a face mask, we also have to maintain social distance from people to prevent ourself.

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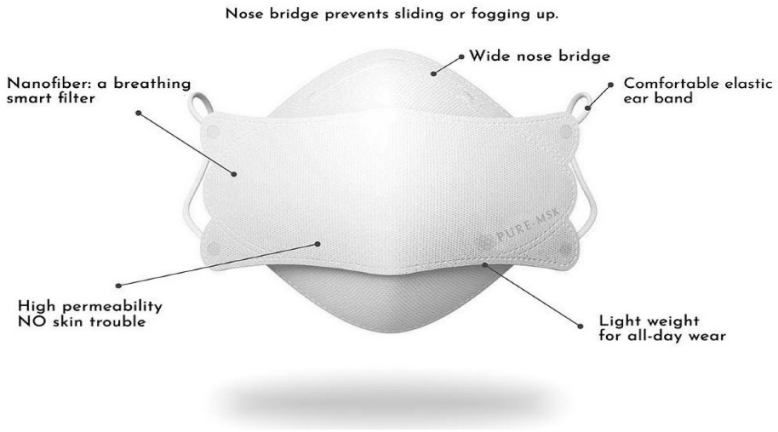

Fig.1.Nanofiber Face Mask

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REVIEW ARTICLE

A Review on Solid Propellant Rocket Engine Performance by its Design Optimization Techniques

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ABSTRACT

Over the past 80 years, the chemical rocket propulsion techniques namely solid propellant rocket engine, liquid propellant rocket engine and hybrid propellant rocket engine plays a important role in rocket propulsion. Even though liquid and hybrid propulsion technique have more advantages, the solid propellant technique was showed to reliable for wide range of applications. Solid propellant rocket engine is a type of rocket engine, where the propellant of both solid fuel and solid oxidizer used for oxidation or burning with air. The solid propellant rocket engine is mostly preferred by many aviation industries and organizations for its more advantages inclusion of simple manufacture, long time storage capacity, less time required to launch and etc.. this paper mainly focuses on review of design and investigation of solid propellant rocket engine performance in the aspect of optimization design techniques.

Keywords: chemical rocket propulsion, solid propellant rocket engine, oxidation, long time storage, design.

INTRODUCTION

The operation principle of solid propellant rocket engine is simple, where both the solid fuel and solid oxidizer get composed for combustion process in order to produce a thrust force to eject a rocket vehicle [3]. The solid propellant rocket engine is sometimes called as solid propellant rocket motor because of it does not having any moving parts. The design part of the solid propellant just begins with total impulse, which one determines the fuel and oxidizer mass. Solid Rocket Motor (SRM) is a type of rocket engine, which do the combustion process to breakdown the

chemical energy bond of the propellant [3,4]. The material which is used to manufacture the SRM motor plays a huge role because the performance of SRM is based on the motor case. The main application of the SRM is to launch vehicles, missiles and spacecraft. Due to the presence of aluminium powder, the main purpose of utilizing solid propellant is to increase specific impulse and combustion stability because of combustion instability causes severe problems inclusion of engine failure [1,2,3,6]. The performance of the solid rocket motor is based on many parameters including burning rate, specific impulse, cross sectional area or geometry, chamber pressure, mass of the propellant, volume of the propellant, exhaust velocity etc [2,6]… still research is going on solid propellant rocket motor technique in order to prevent the demerits of current version of SRM. Solid rocket motors are always preferable due to its simplicity when compare to other chemical propellant rockets like liquid propellant and hybrid propellant rockets [1,3,4,5].

SOLID PROPELLANTS

Solid propellants have been classified into two different types, such as homogeneous or double base propellants and heterogeneous or composite propellants [1,2]. Both the fuel and oxidizer are in the same molecule then the propellant is called "Homogeneous Propellant". combination of nitroglycerin-nitrocellulose is the best example to homogeneous propellant[2]. Both the fuel and oxidizer are in different molecule then the propellant is called "Heterogeneous Propellant". Some of the examples for heterogeneous propellant are Ammonium Perchlorate (AP), Ammonium Nitrate (AN), Nitronium Perchlorate (NP), Potassium Perchlorate (KP) and Potassium Nitrate (KN).

Propellant Characteristics

Selection of propellant for solid rocket motor deign is a critical process. The desirable propellant characteristics for smooth design of solid rocket motor includes high specific impulse or low molecular mass, low burning rate exponent and co-efficient of temperature, high density, low absorption of moisture, non-toxic exhaust gases and not prone to combustion instability[4,6,8,9]. Metal powders may be added with solid propellants for improving specific impulse and fuel density for successful burning process. aluminium, magnesium, boron and zirconium are the generally used metal powders with the particle size various from 10 to 40 μm[4,9]. Aluminium powder is the best suitable composition, which is having 12% to 22% of the total mass of the propellant for quite improvement of specific impulse as well as fuel density[3,9,12]. Powdered spherical aluminium is the most commonly used solid fuel, whose diameter is usually ranges 5 to 60 μm for both double-base as well as composite propellants. Boron, beryllium, aluminium hydride(AlH_3) and beryllium hydride(BeH_2) are preferred because of their high heat and gas volume[1,2,5,17].

Principle of operation of SRM

A solid fuel and oxidizer is mixed as a solid propellant and packed in the combustion chamber [1,2,3,4,5,18,22]. When the solid propellant is ignited it will burned and produce rapid rate of gases. This gases develop high pressure in the combustion chamber and the pressure forces the gases exit into nozzle section [2,5]. The function of the nozzle is, first reduce the area of the exit in order to increase the velocity of gases and finally supersonic gases reach at the nozzle throat due to the pressure energy of the gases converted into kinetic energy [4,7]. So nozzle exit gases are very high velocity. Due to high velocity gases from nozzle, a reaction force is produced and this force is propels the rocket in forward direction [2,3,4,18].

SRM design Problem

Generally the SRM design process consists of many subsystems inclusion of combustion chamber, nozzle, propellant grain and insulation, which are discussed in the above sections [6,7,8]. The propellant type selection is purely depends on many parameters including performance, availability of manufacturing technique and mainly requirement. In order to make a proper adjustment of designing parameter, a optimization techniques were used for the efficient of SRM design process. The basic problem is there is no any well definite universal procedure or method to SRM design[13,15,16]. Numerous approaches have been followed to SRM design problem and this approaching is depends on many parameters inclusion of type of the application, mission scenario etc[3,7,21,33].. through the

optimization techniques we can achieve optimum total impulse, optimum thrust-time profile, optimum nozzle configuration, optimum chamber pressure and solid propellant grain configuration[17,19,24]. In SRM design process, the independent design parameters(inclusion of propellant parameters and vehicle analysis parameter) should be evaluated and then dependent parameters need to be calculated[12,13]. The mission analysis parameters are weight, temperature, operating and ambient pressure. Propellant properties includes density, specific impulse, specific heat ratio, burning rate etc[12,14,15,17].. nozzle configuration and propellant grain configuration parameters are called dependent design parameters which includes length to diameter ratio, pressure, throat area, thrust coefficient, web fraction etc[11,15,19].

After evaluating the two types of design parameter, propellant grain shape need to be selected, analysed within the internal ballistic cycle[23,25]. This process produce the result in the form of pressure-time and thrust-time graphs. Based on these graphs, we can make any changes or modifications until the process is converged[3,11,19]. Physical model of the internal ballistic analysis can defect the performance or results. Few physical as well as chemical processes will occur during the motor operation, which are still have no well definite procedure. But physical models have been processed with assumptions[19,20,21]. Probably zero dimension(0-D) analytical modes were used for small to moderate SRM design. One dimension (1-D), two dimension (2-D) and three dimension (3-D) used for dynamic and erosive burning condition [17,23].

SRM design optimization

In the optimization process, the initial step is to formulate mathematical model, which one is going to be optimized [9,13,14]. Majority of SRM design optimization cases, 0-D internal ballistics preferred because of its fast analysis [6,8]. A first step in SRM design optimization technique is to set a design coal based on the requirements including maximum thrust, minimum weight etc[13,19].. then it is need to formulate optimization design function which gives a mathematical representation. Optimized 3-D grain design parameters for achieving maximum thrust. Optimized 2-D design for achieving maximum thrust and total impulse [1,2,3,4]. The main objective of the optimization technique is minimizing the gross lift-off mass because of total vehicle mass can cause a big impact in the aspect of cost as well as performance. It was examined that, maximum range and minimum weight would be multi objective for optimization techniques [6,7,9]. In order to make efficient optimization process, both the technical parameters as well as cost effect need to be considered during the optimization process. It was examined that, through decreasing the propellant mass, we can achieve a maximum specific impulse [10,12,15].

Optimization techniques for SRM design

The application of optimization technique is based on many interior as well as exterior properties inclusive of pressure, temperature, density, area, requirements, application scenario, affecting of internal factors etc[3,4,8,11,17]... the optimization techniques used in SRM design have been classified into four types namely (1) Metaheuristic optimization (2) Hybrid optimization (HO) (3) hyper-heuristic optimization (HHO) (4) MDO optimization.

Metaheuristic optimization Technique

Metaheuristic optimization algorithms evaluating methods, which will perform design space exploration[3,7]. This method is frequently utilized in SRM design along with simulated annealing, particle swarm optimization etc. These algorithms have been derived from natural physical process [3,7,8,9]. The main advantage of this method is, it does not require any gradient information to proceed the process like conventional method. This method used either propulsion based approach or single-solution based approach. Through the propulsion based approach, set of solutions randomly evaluated [1,7,8,10]. By using a single solution based approach, we can generate and replace the procedure into single solution. This approach provides a solution to variety of optimization problems, through proved having more tendency to determining a global optimum because it has capability of determining the global minimum region in large space[4,7]. It was studied that, it is act as a dual-objective and multi-objective optimizer to correct the SRM design optimization process. To analyzed and optimized a three and four stage solid propellant launch vehicle, in order to minimize the overall vehicle mass GA technique preferred [2,5,8,9]. By using slotted

propellant grain, it is possible to increase specific impulse and decrease propellant mass through GA technique. Individual Metaheuristic techniques have proved notable merits in spite of conventional method to solve the SRM design optimization process with the following advantages (1) This method have longer time and gathering more functions, compare to traditional method in order to converge the smooth solution. (2) by using this method, different optimal solutions may be achieved from same algorithm. (3) at least local optimum is confirmed upon convergence[3,5,6,8,9,12,13].

HO approach

The main function of the HO approach is combine two algorithms to perform the process in a single attempt to evaluate specific features of each unit of the composition in order to enhancing the overall optimization process[3,6,11]. For the composition process this method is always preferred because it has done either improves the quality of the solution or decrease the computation time or both. It is examined that, hybridization of GA and SQP to provides ground based interceptor missile conceptual design, which uses a three stage solid rocket propulsion system. from the simulation results, the GA/ SQP hybrid algorithm improved the solution by 10% then single GA approach[3,17]. Hybrid optimizer is more efficient in the aspect of convergence speed and solution quality[1,17]. By using different tapered grain geometries, an SRM design may be match to different burn profiles. Different method to formulate a hybrid optimizer is combining two Metaheuristic algorithms together(one for global search and another for local search). For example, formation of HO with GA (for global search) and SA (for local search) proved its performance in processing in high speed and high-quality solution and this hybridized efficiently provides multidisciplinary design as well as optimization[3,4,5,8]. It provides the high quality model, through modules the mass properties, characterization of flight dynamics, propulsion and aerodynamics. It was studied that, the hybridization approach applied to a dual thrust SRM propulsion system which basically consists of boost-phase thrust and sustain-phase thrust[2,3,5,11,17]. GA used for identifying the regions and SA used to local optimality within that region.

HHO Approach

Both the Metaheuristic and hybrid techniques showed their successful in solving of real-world optimization problems, but still they facing some difficulties in the aspect of universality and flexibility[11,14,16]. These methods facing difficulties on selection and manipulation of large set of parameters in a algorithm for a particular problem. HHO is alternative method for search and optimization, which raise the level of generality through automating the selection[16,19,35]. It can able to find a optimal solutions without modification on algorithm for different problem conditions[11,17]. For example, this method is applied to ASLV which uses three stage SRM as a propulsion system[11,13,15,17]. in this study nearly 19 variable have been considered in MDO technique reduce the mass of vehicle. For minimizing the gross motor mass under specified geometrical and ballistic constraints through optimizing eight variable including of combustion chamber, nozzle and propellant grain[18].

MDO Approach

MDO provides a structural methodology in order to find best suitable design in a multidisciplinary environment[21]. This is preferred to design and optimization of complex systems for its coordination in multidisciplinary analyses in order to realize effective solutions. Generally the MDO technique proceeds with the following steps (1) system is decomposed into multiple subsystems (2) improving mathematical models which describes overall as well as subsystems (3) choosing of MDO formulation and algorithm (4) solving on MDO problem for generating solutions. MDO technique is naturally deals with different model analysis, which is a really critical task for proper implementation of the process. After preloaded a specific properties of solid propellant to propulsion model, the fundamental SRM characteristics including of burn time, combustion pressure, thrust and grain mass and through this process each stage propulsion characteristics were calculated[9,25]. For example, analyzing of each subsystem of nozzle, combustion chamber, structure and grain will be considered as a single discipline for MDO problem[23,27]. Through this we can explained the action between subsystems and design variables in structure matrix[18,32]. MDO techniques generally allows achieving optimum solutions over than to traditional methods. The complexity of the

same are indicated as (1) formation of problem exclusively heterogeneous models need to be composed properly and run as a single problem during process. (2) mathematical model limits the algorithm selection. (3) from the results on design variables design volume can be increased exponentially[3,11,29].

RESULTS AND DISCUSSION

The optimum design of a solid rocket motor is a critical process, which requires very high integration of many subsystems[1,2,3,4,5,6,7,9]. The tools or techniques of optimization process becomes necessary in order to effectively complete the process. It is found that, GA is one of the most widely used optimization technique either in individual algorithm or global search algorithm along with HO approach[3,7]. GA technique have been proved its capability in the aspect of exploring more design space, increasing level for solution of global optimum. in order to overcome limitations of individual Metaheuristic algorithms hybrid optimization approach is a best solution[3,7,13]. HHO is preferred for universal design process especially complex multidisciplinary design problems. For a complicated rocket based system design MDO optimization technique is highly recommended[1,3,4,5]. A complicated step in the procedure of SRM optimization process is selection of design parameters and material selection. it was studied that, cost and operations are proportional to the size and weight of the vehicle[2,5,6]. It appears that, still there is no any globalized procedure or method in order to compensate the demerits, which are arising during the SRM design process. The above discussed optimization techniques may improve the effectiveness of parameters usage, but still it is a critical for complicated or multidisciplinary design. Because of solid rocket motor design involves many parameters such as grain geometry characteristics, fuel/oxidizer weight and total impulse required etc[1,2,5].. The optimization techniques may provides possibility to determine solutions to progressive/regressive problems which will cause combustion instability later[28]. In the aspect of materials for SRM casing, carbon fibre composites are best choice for fabrication of casing for solid rocket motor[1,5,19]. Carbon epoxy composite materials with their high strength can reduce the weight of the structure. Fabricated SRM can be inspected through many Non-Destructive Techniques[3,23]. When selecting the composite materials for SRM process, it must be able to tolerance high pressure and light weight[2,29]. The grain materials of the solid propellant rocket motor is incompressible probably they have bulk modulus in compression at least 1400 MPa[31,32,34].

The motor physical properties are directly changing with time rate of load application. Fast pressurization binding materials such as hydroxyl-terminated polybutadiene (HTPB) have a good elongation and stronger strength compared to other polymers[1,2,3]. Ammonium Perchlorate (NH_4ClO_4) is mostly preferred crystalline oxidizer in SMR for its compatibility with other materials, performance, quality, good physical properties and availability. Occasionally ammonium nitrate and potassium Perchlorate were used[4,8]. Ammonium nitrate capable of changing its crystal structure at various phase temperatures. Comparison of some frequently used crystalline oxidizers were listed in table for better understanding.

For a stable combustion of solid rocket motor, a burning rate exponent must be less than 1[1]. Large pressure oscillations within chamber can lead to engine failure and this status is called combustion instability which will occur in SRM process[3,6]. This combustion instability is a dangerous one especially in large rockets. High frequency oscillation often screaming is not familiar to understood till now because of it is associated with more vibration modes of the chamber like longitudinal, radial and tangential[1,4,5]. Solid rocket motors are also depends on energy release Non-uniform pattern. The performance plots of HTPB based composite propellants were indicated below for showing specific impulse improvement[1].

CONCLUSION

The various design affecting parameters was discussed in this paper inclusive of solid rocket motor design, important geometrical parameters, optimization techniques, different type of propellants with

ingredients for achieving improved performance on SRM design process[34,35]. Base of solid rocket motor propellants have been taken main consideration for this work. Based on the internal parameters changing, the affected factors, variants along with methods have been illustrated in this paper[6,7,9]. From the reviewed work, it is concluded that still some research is essentially required in this field for further processes and achievements in order to complete the efficient design of SRM by improving some parameters in its optimization techniques[1,2,3,5,11,17,35].

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Table.1 crystalline oxidizers with their properties [1]

Oxidizer	Chemical Symbol	Molecular Mass (kg/kg-mol)	Density (kg/m^3)	Oxygen content (wt %)	Justification for selection
Ammonium Perchlorate	NH_4ClO_4	117.49	1949	54.5	Low cost, easy availability
Potassium Perchlorate	$KClO_4$	138.55	2519	46.2	Low burning rate, medium performance
Sodium Perchlorate	$NACIO_4$	122.44	2018	52.3	High performance
Ammonium Nitrate	NH_4NO_3	80.0	1730	60.0	Smokeless, medium performance

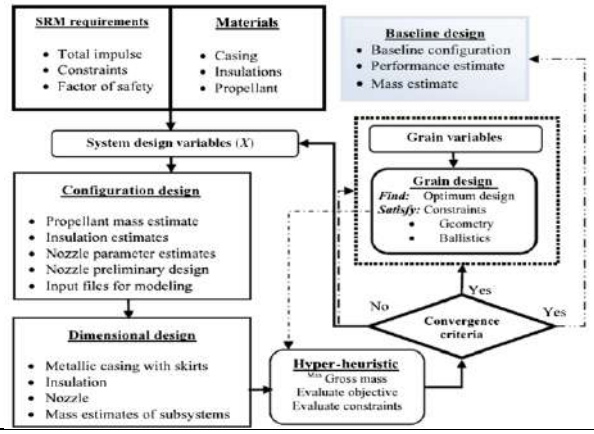

Figure. 1 SRM system design analysis[3]

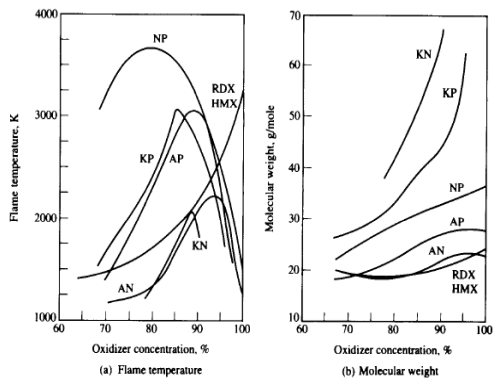

Figure.2 HTPB based composite propellants for flame temperature and molecular weight [1].

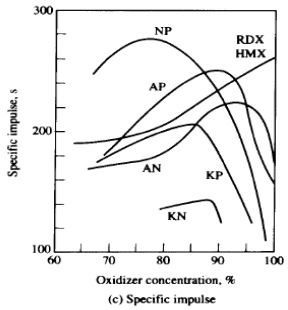

Figure.3 HTPB based composite propellants for specific impulse[1]

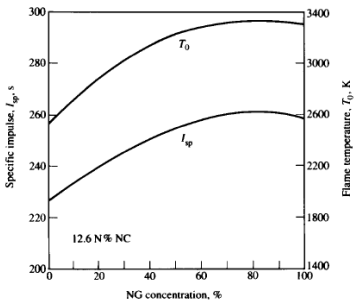

Figure. 4 Specific impulse and flame temperature versus nitroglycerin concentration of double base propellants [1]

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REVIEW ARTICLE

Systematic Review on Fresh Freeze Technology

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ABSTRACT

Freezing technique is one of the perseveration process and it is the old method of preservation compared with recent preservation technique, and most generally used methods for drug preservation, which preserve the taste, and nutritional value in pharmaceutical product which is best than the other methods. The process of freeze can be carried out under completely sterile conditions, thereby prevention microbial contamination. The fresh freeze technology is formed by Japan; it is one of the Japan's top inventions in freezing technology. The fresh freeze that focused to assist freeze drug without destroying its cell structure a "fresh freezing" system. In this freezing process may be a combination of the beneficial effects of low temperatures at which microorganisms cannot grow cellular metabolic reactions. Freezing could be a very well-established drug preservation process that produces long storage life to the drug and will be maintains the standard of the pharmaceutical product.

Keywords: Freezing, Preservation, Microorganism, Freshness, Magnetic field.

INTRODUCTION [1-5]

Freezing is technique is one of the perseveration process and it is the old method of preservation compared with recent preservation technique, and most generally used methods for drug preservation, which preserve the taste, texture, and nutritional value in pharmaceutical product which is best than the other methods. The freezing process could even be a combination of the beneficial effects of low temperatures at which microorganisms cannot grow, chemical reactions are reduced, and cellular metabolic reactions are delayed.

Freezing well is an extremely well-established drug preservation process that produces long storage self-life to the drug and may be maintains the standard of the pharmaceutical product. However, freezing is not suitable for all drugs, and freezing can cause physical and chemical changes in some drugs that are consider as reducing the quality and standard of either the defrosted material or the end product. The physical state of drug material is modified when energy is removed by cooling below freezing temperature at 18°C. Freezing methods are employed like as cryogenic freezing, air blast freezing, indirect contact freezing, direct contact freezing, and immersion freezing. These traditional methods, it is very difficult to preserve the perishables because there occurs discoloration. Several emerging technologies are recently proposed for ice nucleation control during freezing.

HISTORY OF FREEZE TECHNOLOGY [6-8]

Freezing technique used as a way of preservation, and history reveals it had been mostly shaped by the technological developments within the process low quantity of ice produced without employing a "natural cold" in 1755 was considered the primary discovery within the freezing process. The process of freeze-drying was invented in 1906 by Arsened Arsonval and his assistant Frederic Bordas at the laboratory of biophysics of school de France in Paris. In 1911 Downey Harris and Shackle established the lyophilization process of preserving live rabies virus which eventually led to development of the first antirabies vaccine. Freeze drying was first actively established during World War-II for transport of serum. The fluidized bed freezer, can be a recent modified reasonably air-blast freezer for particular product types, consists of a bed with a perforated bottom through which cold air is blown vertically upwards Rahman in 1992. The fresh freeze technology is formed by Japan; it's one amongst the Japan's Top inventions in freezing technology. This time, an invention that uses attraction to assist freeze drug without destroying its cell structure: a "fresh freezing" system. It had been developed by a Japanese venture firm in 1995 as a technology which will help freeze drug without altering its therapeutic effect.

GOAL OF FREEZING [9-10]

To prevent growth of microorganisms by

- ✓ Killing some bacteria
- ✓ Reduced water activity
- ✓ Mechanical formation of ice crystals and Osmotic changes in cell fluids
- ✓ Typing up some free water (reduced the amount of free water)

Physical, biochemical and microbiologic degradation of drugs controlled by heat removing process.

NEED FOR FREEZING [11]

1. The process of freezing involves lower temperature which acts as a productive agent.
2. The process of freeze can be carried out under completely sterile conditions, thereby prevention microbial contamination.
3. Dehydration of the material takes place in a rapid manner.
4. The process of freeze for drugs and food materials is completely approved by food and drug safety regulations and is safe for consumption for users.

FACTORS AFFECTING DURING FREEZING

Freezer Burn [12]

Freezer burn is caused by the sublimation of ice on the surface region of the merchandise when the water pressure of ice is on top of the pressure within the environment. Freezer burn produces changes within the looks and texture on the merchandise surface and may be the reason for off-odors and flavors. Moisture migration causes weight losses during freezing and frozen storage, unless the merchandise is packed employing a cloth with tide vapor permeability.

Freezing Burn And Dehydration [13]

If the freezing burn and dehydration, the air when and where it has access to product, starts withdrawing water from the product and the sport or area from where water is withdrawing gets dehydrated and appears as white spot called freezer burns. Unpacked foods and drugs are more susceptible to freezer burns. Longer freezing time will result in larger freezing losses IQF type of freezing result in 2- 4%; slower methods may contribute to even 10% or more weight loss.

Biological and chemical changes [14]

The biological changes include reduction of micro flora on the surface and interior of drugs, freezing has inhibiting effect on the metabolism and reproduction of microbes. As water gets converted into ice and it is not freely available to microbes for their metabolic and physiological activity. Due to this effect reduction in the bacterial load during freezing is witnessed however freezing is not a sterilization process.

FRESH FREEZE TECHNOLOGY [16-21]

Fresh freeze technology is also known as Magnetic resonance assisted freezing in this system patented by the Japanese company of ABI Corporation Ltd, Chiba, Japan. It's one of the Japan's Top inventions in freezing technology. It had been developed by a Japanese venture firm in 1995 as a technology which will help freeze drug without altering its therapeutic effect. The concept of fresh freeze technology is provide a process where a product can be frozen 'in such a manner attraction to assist freeze drug without destroying its cell structure: a "fresh freezing" system. The thought came from the experiments conducted on freezing the water droplets with magnetic fields under 0–0.5 T, which resulted in significant degree of super cooling of water molecules and increased the steadiness in hydrogen bonding. Later, this study was granted patent when same results were found that magnetic resonance is assisted to helped in maintaining stability of the drugs. They proposed that application of magnetic assisted freeze in unidirectional motion created an imbalance within the electronic spin of drug molecules and thus ultimately prevented the freezing by providing force field. It also resulted in removing heat energy of freezing from the water molecules without undergoing into the frozen form.

PRINCIPLE INVOLVED IN FRESH FREEZE [22-26]

Fresh freezing with induced magnetic field has the potential to enhance the freezing rate and to improve the quality of frozen drugs. The process of magnetic resonance assisted freezing or fresh freezing includes the conversion of the material directly from the solid phase to gaseous phase, without going through the liquid phase. For perishables, freeze is the most appropriate method for preservation.

- ✓ Refrigeration not necessary
- ✓ Material can be stored at the room temperature
- ✓ Reconstituted with water within a short period of time
- ✓ Long-term stability(for about two years)

It is also a universal truth that magnetic field greatly affects the properties of water which is associated with the movement of tides in the sea called process of geomagnetism. It has been proposed by the various scientists that magnetic field act on water molecules by aligning the electronic and nuclear spins of the atoms in the direction of the magnetic field. The latter is more acceptable as it is possible to vary the strength of filed applied as per the need and freezing requirements of a particular food product. The electro-magnetic field can be further divided into two categories, i.e. (Static magnetic field, Oscillating magnetic field).

WORKING OF FRESH FREEZE TECHNIQUE [27-32]

Fresh freeze technology is also known as Magnetic resonance assisted freezing. Freezing with induced magnetic field has the potential to enhance the freezing rate and to improve the quality of frozen drugs. An electrical current flowing during a wire creates a magnetic flux round the wire, consistent with the Ampere's law.

Consistent with **Ampere's law**, magnetic fields are associated with the electrical current produced in them. The law specifies the magnetic flux that's related to a given current or vice-versa, as long as the electrical field doesn't change with time

B = μo x N/L x I

Where;
B = Magnetic field, **μ0** = Permeability of free space ($4\pi \times 10{-}7$), **N** = Number of loops , **L** = Number of turns per unit length (m) , **I** = Current flowing through the wire (A) So, as to generate the magnetic field in an electro-magnet, a coil of wire is wound around the magnetic core with many turns. So, when the electric current is passed through the wire, the magnetic field of all the turns of wire passes and penetrates the iron coil, causing the domains to turn and tiny magnetic fields of core are added to the magnetic field of wire, thus creating a large magnetic field.

The intensity of magnetic field generated is influenced by the number of turns in the winding N and the current flowing through the wire I. The changes brought by the magnetic field depend on the strength of magnetic field, exposition time, and the temperature. Magnetic field could be applied in the form of permanent magnets or by using electromagnetic coils, The latter is more acceptable as it is possible to vary the strength of filed applied as per the need and freezing requirements of a particular food product. The electro-magnetic field can be further divided into two categories, i.e. (static magnetic field and oscillating magnetic field). The magnetic field assisted freezing system with metallic coil generating magnetic field around the drugs product is prevented which would cause the cold air to transmit easily to the inner portion of the object and enhance the cooling rate and brings free water in a super cooled state. It also results in the cold air to be transmitted quickly inside the core of the product, thus resulting in even and quick cooling of product. This phase change leads to temperature shift which is proportional to face of the applied magnetic flux.. The decreasing cluster size of free water makes it possible to increase the amount of non-freezable bound water and finishes up in better freshness of products. .

QUALITY LOSSES IN FROZEN PRODUCT

Frozen product has expanded worldwide over the last 50 years to include a variety of pharmaceutical products. Even if a drug product is adequately frozen, physico-chemical and biochemical changes during storage can lead to degradation in its quality. Quality of frozen drugs is extremely captivated with storage temperature and there's a requirement for a continuing and systematic control on maintaining the specified temperature throughout the frozen product distribution within the cold chain, from production to final consumption.

Physical Changes During Frozen Storage [33-37]

The main physical changes during storage of frozen drugs are moisture migration and ice recrystallisation.

Moisture migration

A slow freezing process can allow sufficient time for water migration because of osmotic forces from the inner region of a product to the freeze-concentrated intercellular region. These phenomena affect not only the texture of the frozen product, but also a major drip loss during thawing and resulting in a loss of therapeutic effect. During frozen storage the existence of temperature gradients within a product creates water vapors pressure profiles leading to moisture migration and relocation of the water, both within and from the merchandise.

Recrystallisation of Ice

Slow freezing leads to a coffee rate of nucleation and therefore the production of a little number of huge ice crystals, whereas fast freezing causes a high rate of nucleation leading to the formation of an outsized number of small ice crystals.

Recrystallisation reduces the advantages of fast freezing and includes any change within the number, size, shape, orientation, or perfection of crystals following completion of initial solidification. In frozen aqueous solutions recrystallisation is that the method by which the standard ice crystal size increases with time.

Chemical Changes During Frozen Storage [38-42]

During the freezing of product, water is transferred into ice crystals and solutes concentrate in the unfrozen matrix. Slow freezing results in a maximum ice crystal purity and maximum concentration of solutes in the unfrozen phase, leading to equilibrium conditions.

Oxidation

Oxidation is a reaction that severely limits the shelf-life of a frozen product, leading to loss of quality flavor, appearance, nutritional value, and therapeutic effect. Oxidation is a complex process that proceeds upon a free radical process.

Flavor Deterioration

Flavor deterioration is produced in both plant and animal products. It's identified more with frozen muscle than with frozen vegetable products, because blanching is usually applied to vegetables before freezing.

PHARMACEUTICAL APPLICATIONS [43-46]

The fresh freeze process is one of the important applications in the pharmaceutical and biotechnology industries, and pharmaceutical fresh freeze is latest process used to stabilize, store or increase the shelf life of drug products and other biologicals. Pharmaceutical companies often use freeze to increase the shelf life of products, such as vaccines and other injectable. Pharmaceutical companies use freeze as tool to extend the shelf-life of medication and vaccines. If a liquid drug is converted to its powdered from and stored in an exceedingly vial, it are often easily reconstituted as necessary. Pharmaceuticals is newly subjected to the fresh freeze process include vaccines, hormones, proteins, plasma, antibiotics, ect. In chemical synthesis, products are often lyophilized to form them more stable, or easier to dissolve in water for subsequent use. However, the freezing process is employed more commonly within the pharmaceutical industry.

ADVANTAGES 0F FRESH FREEZE TECHNIQUE

The most important advantages of fresh freeze are listed below:

- ✓ Minimum damage to the heat labile material [47].
- ✓ Speed and completeness of rehydration [48].
- ✓ The ability to sterile filter liquids just before dispensing [49].
- ✓ The substance may be stored at room temperature without refrigeration and be protected against spoilage for many years [50].

DISADVANTAGES 0F FRESH FREEZE TECHNIQUE:

The most important advantages of fresh freeze are listed below:

- ✓ Maintaining frozen storage is costly and takes up a lot of space [51].
- ✓ High capital cost equipment, and high energy cost [52].
- ✓ Possible damage to products due to change in pH and tonicity [53].
- ✓ Transportation of frozen materials can be difficult and expensive [54].
- ✓ Failure of freezing equipment would risk the total loss of the product [55].
- ✓

PHARMACEUTICALS AND BIOLOGICALS SUITABLE FOR FRESH FREEZE [56, 57]:

- ✓ Vaccines and antibodies
- ✓ Blood plasma
- ✓ Proteins

- ✓ Enzymes and hormones
- ✓ Viruses and bacteria.

CONCLUSION

Pharmaceutical companies use freeze as tool to extend the shelf-life of medication, vaccines and other injectable. In fresh freeze is latest process used to stabilize, and maintain the freshness of drugs. By removing the water from the product and waterproofing the material in vial, the drugs can be easily stored. Fresh freeze technology is also known as Magnetic resonance assisted freezing system. In this process increase the shelf-life of drugs and other biologicals compared with other methods of freezing technique. Fresh freeze technique is newly invented method it is very useful in know a days for storing the vaccines and other injectable. The researchers to focus on this freezing technique to improved their features and extend the technique in all over world.

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RESEARCH ARTICLE

Design, Development and Evaluation of pH Sensitive Delayed Release Multiparticulate Formulations of Mesalamine

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ABSTRACT

Pellets were attracted as multiparticulate formulation with the objective of improving bioavailability, avoiding first pass effect and extending the controlled release of drug in pH sensitive manner for a prolonged period. In the present study extended released mesalamine pellets were prepared using drug layering on sugar sphere followed by various delayed release polymer coating on it, which were further processed into capsules. Amount of Eudragit RSPO & RLPO were taken as the formulation variables for percentage release of drug. The pellets were evaluated for physical parameter, Assay, aspect ratio, density, particle size analysis, in vitro drug release and stability studies. The formulation with 796% drug loading, 10% Eudragit RSPO & RLPO and 15% Eudragit L100 coating was consider as a best pellet with respect to perfect size, shape and in-vitro drug release study. The release profile of mesalamine pellet was studied for 8 hours in pH 6.8 phosphate buffer with 84% release with no lag time for drug release. The Multiunit particulate drug delivery system gives unique release pattern with no significant change with respect to shape, colour, surface and in vitro drug release pattern and demonstrated its potential for colonic delivery.

Keywords: Delayed release pellets, Mesalamine, in vitro drug release, particle size analysis,stability studies.

INTRODUCTION

Delayed release drug delivery systems are typically modified release dosage form either enteric-coated or targeted to the colon aimed to protect the drug from an unfavorable environment in the gastrointestinal tract, or to target a specific region of absorption or action[1]. There are numerous ways of achieving prolonged drug release, including

the use of ion exchange resins, pH-independent formulations, prodrugs, barrier-coating, embedment in hydrophilic, plastic or slowly eroding matrices, repeat action, polymer resin beads, drug complex formation, bioadhesives and local targeted systems[2,3]. Out of these, a polymer membrane surrounding the pellet controls the extended and delayed drug release, and embedment in different types of matrices, resulting in homogeneous dosage systems through which the drug diffuses at a controlled rate.

The modified release dosage form "Pellet" has been used to describe a variety of systematically produced, geometrically defined spherical or semi spherical units of agglomerates ideally in the size range between 0.5 – 1.5 mm, mainly aimed fororal multidrug release dosage forms having gastro resistant or sustained-release properties or the capability of site-specific drug delivery. Pellets possess great flexibility as to target multiple drugs which may be chemically compatible or incompatible, at the same sites or different sites in the gastrointestinal tract add. Due to spherical shape and low surface area- to- volume ratio, the polymer coating can be successfully applied on pellets.The coated pellets are encapsulated in capsules or compressed as disintegrating tablets enhances the release of contents in GI area[4]. The dosage form design of pellets adaptable for different dosages are given in figure no.1

Advantages of pellets[5,6]

- Pellets can be provided as desired dosage strength without changing the formulation variables.
- As pellets are multiple unit dosage regimen, they offer different drug release at predetermined time intervals in the gastrointestinal tract making them superior over single unit dosage forms**.**
- They are suitable for the safe delivery of incompatible drugs.
- Pellets offer sustained release of drugs with great flexibility in the design and development of dosage form.
- Pellets protect the drug from GI environment and so the dosage form is suitable for drug targeting.

Disadvantages of pellets[7]

- Pellets doses are calculated based on the volume and not with number basis.
- Formulation of pellets as disintegrated tablets or filling into capsules are tedious process and highly costly.
- The different sized pellets are available depends on the formulation.

THEORY OF PELLETIZATION

Different pelletization theories have been postulated based on either experimental results or visual observations in order to optimize the process related to granule formation and growth. The pelletization process, involves three different mechanisms namely; nucleation, transition and ball growth. In nucleation the powder is wetted with liquid and it is the first step in all pelletization/granulation processes. The tiny particles are agglomerated together and are bridged together by liquid molecules to form three-phase air-water-liquid nuclei. The enhanced bond strength is observed with reducing size of particle. The nuclear formation rate, extent and size depends on the size, viscosity, moisture content of primary binding particles, the processing conditions, such as tumbling and drying rates. The next growth mechanism is transition phase and it is the combination of two processes namely, coalescence and layering. Coalescence is the formation of large-sized particles by random collision of well-formed nuclei, and layering involves the successive addition of fragments and fines. In coalescence stage the number of nuclei is progressively reduced, the total mass of the system remains unchanged whereas in layering step, the number of particles remains the same but the total mass got increased due to increasing particle size. The fragments or fines produced by size reduction are picked up by large pellets. Fragment formation, coalescence and layering steps continues until the number of favourable collisions declines rapidly, thereby leading to a reduction in the rate of growth of the pellets. Balling is the third growth phase, which involves the transfer of materials from one granule formed to another in a directionless manner and it is a slow growth agglomeration stage. This situation does not result in a change in the total number or mass of the particles but the particles, however, undergo a continuous change in size. In balling the finely divided particles are converted, upon the addition of appropriate quantities of liquid prior or during agitation stage, to spherical particles by a continuous rolling or tumbling motion. In compression process, the pellets with perfect shape and size are produced by compacting mixtures or blends of active ingredients and excipients are compacted under pressure. Globulation or droplet formations describe the two

related processes of spray drying and spray congealing. In spray drying Drug entities in solution or in suspension form are spray dried, with or without excipients, in to a hot air stream to generate dry and highly spherical particles. Spray congealing generates spherical pellets in which a drug is allowed to melt, disperse, or dissolve in hot melts of guns, waxes, fatty acids, etc., and is sprayed in to an air chamber where the temperature is below the melting points of the formulation components, to provide, under appropriate processing conditions, spherical congealed pellets. Depending on the physicochemical properties of the ingredients and other formulation variables, pellets with immediate or controlled release behavior can be produced[8]. Extrusion-spheronisation is a multiple-step compaction process comprising dry mixing of the ingredients with excipients, wet granulation of the mass, extrusion of the wetted mass, charging the extrudates into the spheroniser to produce a spherical shape, drying the wet pellets in a dryer and, finally, screening to achieve the required size distribution[9,10,11]. The granulation step can be performed both in batch-type processors, including a conventional planetary mixer, and in vertical or horizontal high-shear and sigma-blade mixers, and high-shear twin-screw mixer-extruders[12,13].

MATERIALS AND METHODS

Trial & error method was used for the development of formulation of Mesalamine Extended-release pellets. So, different steps adopted are:

- Sugar Sphere as a core material
- Step: 1 Drug layering on Sugar Sphere
- Step: 2 Extended-release polymer coating on drug layered pellets.
- Step: 3 Delay release polymer coating on ER polymer coated pellets.

Step: 1 Drug layering on sugar sphere

Different percentage of mesalamine coating was made on sugar spheres to optimize the formula and was shown in the Table No. 1

Coating suspension preparation

Dissolve HPMC 50cps in slightly warm water and stir well until the clear solution was obtained. Then talc, PEG-400 and mesalamine were added to above solution under stirring condition. The suspension was homogenized in colloidal mill for 20 mins. Sugar Sphere was loaded in fluid bed coater by maintaining the parameters inlet temperature: 35-40°C, bed temperature: 30-35°C, fluidisation rate: 1.6-2 Kg/cm3, atomisation rate: 1.6-2 Kg/cm3, spray rate: 2-10 rpm and drug suspension was coated on sugar sphere by using wurster technology[14,15].

Step: 2 Polymer coating on Drug loaded Pellets

Different concentration of different polymers used for controlling the release of mesalamine from the drug loaded pellets[16,17,18]. The composition of formulation of trial series for obtaining extended drug release from pellets was shown in the Table No.2

Step: 3 Delay release coating on extended-release polymer coated Pellets

The delay release coating was done with Eudragit L100. The film of Eudragit L100 is not degrade in stomach and protect the formulation from the acidic environment and also soluble in basic pH[19,20]. The formulation were shown in table no.3.%[21,22].

Preparation of coating solution

First the talc was homogenized with half quantity of water and Isopropyl alcohol for at least for 30 min. Now the Eudragit L_{100} is dissolved in remaining quantity of water and Isopropyl alcohol. Triethyl citrate was added in Eudragit L_{100} solution under stirring condition. Now Homogenized talc suspension was added in above solution under stirring condition. Coating solution was sprayed on the SR coated pellets with continuous stirring.

Step 4 Capsule filling of delayed release polymer coated pellets:

Capsule filling was done by laboratory scale capsule filling machine or by manually method. Capsule was filled with two different kinds of pellets in different percentages[23,24]. The capsule filled by following formula: Batch F02G pellets were the drug loaded pellets followed by the 15 % enteric polymer coated (Eudragit L 100) pellets which was withstand the acidic environment and release the drug only in the intestine while batch F02G pellets were extended-release polymer (Eudragit RSPO & RLPO) coated pellets followed by the enteric polymer coating which release the drug after long time and so desire profile was obtained by this formula. During the filling of the capsule the bulk and tapped density of the pellets is so much important. Different capsule size has different volume capacity. Filled weight in the capsule is directly affected by the density of the pellets which can easily understand by the following table data.

EVALUATION OF PELLETS

Description and Density

The density of the pellets was measured by USP-I auto tapped density apparatus[25,26]. On the basis of tapped density capsule size would be calculated for the capsule filling of the desired quantity.

Bulk density = weight of granules / Bulk volume of granules

Tapped density = weight of granules / Tapped volume of granules

Particle Size Analysis

Particle size analysis was done by the Sieve analysis by USP sieve shaker[27,28]. Pellets were passed from the 16# and retained from the 30# sieve.

Aspect Ratio

Hot stage microscope was used for the measurement of the height and width of the pellets[29,30,23]. Aspect ratio was calculated from following formula:

$$\text{Aspect ratio} = \frac{Length\ of\ pellets}{\text{Width of pellets}}$$

Assay

Assay of Mesalamine was carried out by the HPLC method using methanol and acetonitrile in the ratio of (50:30) as mobile phase by maintaining Hypersil BDS C8, (150 mm X 4.6 mm), 5µm, column temperature 25^{0}c, flow rate 1.0ml/minute and detected at 220nm[31].

Solution Preparation

Standard preparation

Transfer accurately weighed quantity of about 40 mg of drug in about 200 ml of volumetric flask. Add 5 ml of 1 N Hydrochloric acid and sonicate to dissolve. Make up volume up to the mark with diluent and mix.

Sample preparation

Transfer accurately weighed quantity equivalent to 100 mg of drug to a 200 ml of volumetric flask. Add about 150 ml of diluent and sonicate with occasional shaking for about 45 minutes. Make volume up to the mark with diluent and mix. Dilute 5.0 ml 0f this solution to 25 ml with diluent and mix. Filter the solution through 0.45 µm Millipore PVDF filter; collect the filtrate by discarding first few ml of the filtrate.

Procedure

Separately inject mobile phase, standard preparation and sample preparation into the chromatograph. Run the chromatogram and measure the responses for the analyte peak. Follow the injection sequences as mentioned below. Calculate quantity in mg of drug per net content of pellet by using following formula.

$$\text{Drug Content} = \frac{ATi}{\text{AS}} \times \frac{\text{WS}}{50} \times \frac{5}{50} \times \frac{200}{\text{WTi}} \times \frac{\text{P}}{100} \times \frac{153.13}{X} \times \text{Net content for respective strength}$$

Where,
ATi= Peak area of sample injection of the respective strength
AS= Peak are of standard injection
WS= Weight of working standard taken in mg
WTi= Weight of sample taken in mg (i = 1&2)
P= Percentage purity of working standard (on as is basis)
153.13 = Molecular weight of Drug
X= Molecular weight of drug with salt

$$\text{Assay} = \frac{Drug\ content\ (practically)}{\text{Drug content (theorically)}} \times 100$$

***In-vitro* Drug Dissolution Profile**

In-vitro drug dissolution study was performed for the % drug release of the API from the formulation at the regular time interval. Drug release parameters should be studied under acid stage (900ml 0.1N Hydrochloric acid as medium for 2 hrs), phosphate buffer at pH 6.4 for 6hrs at a temperature 37ºC ± 0.5ºC, at100 rpm, in USP TYPE II apparatus[32,33,34]. Following are the major parameters for the In-vitro dissolution study for the pellets. The absorbance of the solution was measured at 227 nm using water as a blank.

$$\%\ \text{drug release} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{1000} \times \frac{DT}{LC} \times \frac{P}{100} \times \frac{153.13}{X} \times 100$$

Where,
AT = Absorbance of sample
AS = Absorbance of standard
DT = Dilution of sample preparation
WS = Weight of working standard in mg
LC = Label claim
277.4 = Molecular weight of API
X = Molecular weight of API with salt
P = Percentage purity of working standard

RESULTS

Physical description of pellets wasup to the standard. The density of prepared pellets was given in table no.6.

Particle Size Analysis

By the particle size analysis uniform pellets can obtained which are useful for the capsule filling.

Aspect Ratio

Aspect ratio should be very near to 1 for the best spherical shape.
From the above result aspect ratio of the pellets is 1.060

Assay

For Uncoated Pellets

Pellets were tested as above method for the drug content in pellets, which were shown ***99.80%*** in the final formulation.

For Coated Pellets

Pellets were tested as above method for the drug content in the coated pellets, which were shown ***99.83%*** in the final formulation.

***In-vitro* Drug Dissolution Profile**

The percentage of drug release 84.7% at the end of 8th hour in phosphate buffer.

DISCUSSION

ER pellets was formulated by doing drug loading (796%) on sugar sphere. The aqueous drug loading was done by fluidized bed coating. The prepared pellet was coated with sustain release polymer and then coated with the delay release polymer. The prepared pellets were evaluated for physical parameter, Assay, aspect ratio, density and in vitro drug release. Stability study was carried out for the optimized formulation according to ICH guide lines 40° C / 75% and 60° C / 85% RH for 1 month. The result shows that there is not any significant change in physical and chemical parameter of the pellets; hence the formulation was found to be stable.

CONFLICT OF INTEREST

The authors report no declarations of interest.

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Table No 1:Formulation Of Aqueous Drug Layering On Sugar Sphere

Ingredients	F01 (530%)	F02 (796%)
Sugar sphere	600	400
Mesalamine	3000	3000
HPMC 50 cps	144(4% w/w)	144(4% w/w)
Talc	24	24
PEG - 400	16	16
Purified water	9552	9552
Total	**3784**	**3584**

Table No. 2: Formulation Of Polymer Coating On Drug Loaded Pellets.

Ingredients	F02A (10%) (7:3)	F02B (12%) (6:4)	F02C (10%)	F02D (14%)	F02E (7%) (7:3)	F02F (10%) (7:3)	F02G (15%)
Drug loaded pellets (F01 & 02)	250	250	250	250	250	250	275
Ethyl Cellulose 7 cps	17.5	18.0	--	--	--	--	--
Hypromellose 3 cps	7.5	12.0	--	--	--	--	--
Eudragit NE 30 D*	--	--	83.30	--	--	--	--
Eudragit RLPO	--	--	--	35.0	5.25	7.5	--
Eudragit RSPO	--	--	--	--	12.25	17.5	--
Eudragit L_{100}	--	--	--	--	--	--	41.25
Acetyl mono Glycerate	1.0	1.2	--	--	--	--	--
Tri ethyl Citrate (20%)	--	--	--	7.0	3.50	5.0	8.25
Talc (30%)	--	--	7.50	10.5	5.25	7.5	12.38
Isopropyl Alcohol	222.3	266.76	--	301.87	151.0	215.6	569.2
Acetone	--	--	--	301.87	150.0	215.6	--
Methylene Chloride	222.3	266.76	--	--	--	--	--
Purified water	49.4	59.28	83.4	--	--	--	142.30
Total	**286**	**281.2**	**340.8**	**302.5**	**276.25**	**287.5**	**336.9**
All quantity are in gm							

* Solid of 25 g of Eudragit NE 30 D ≈ 83.30 g of Eudragit NE 30 D Solution

Table No. 3: Formulations For Dr Pellets

Ingredients	F02F_a (5% w/w)	F02F_b (10% w/w)	F02F_c (15% w/w)
SR coated pellets (F08)	300	300	300
Eudragit L_{100}	15	30	45.0
Talc (30%)	4.5	9.0	13.5
Tri ethyl citrate (20%)	3.0	6.0	9.00
Isopropyl Alcohol	207.0	414.0	621.0
Water	51.5	103.0	155.0
Total	**322.5**	**345.0**	**367.5**
All quantities are in gm			

Table No.4: Filled Composition In Capsule

Ingredients	Quantity	Quantity per capsule
Pellets of F02Fc	80%	485.2 mg
Pellets of F02G	20%	110.0 mg
Total	100%	595.2 mg

Table No.5: Empty Gelatin Capsule Specification

Capsule Size	Target Weight	Volume	Fill weight (mg) if Tapped Density is					
			0.4 g/ml	0.5 g/ml	0.6 g/ml	0.7 g/ml	0.8 g/ml	0.9 g/ml
00	119	0.95	380	475	570	665	760	855
0 elongated	104	0.78	312	390	468	546	624	702
0	96	0.68	272	340	408	476	544	612
1	76	0.48	192	240	288	336	384	432
2	63	0.37	148	185	222	259	296	333
3	50	0.27	108	135	162	189	216	243
4	39	0.20	80	100	120	140	160	180
5	28	0.13	52	65	78	91	104	117

Table No.6: Physical Description

Sr. No.	Parameters	Observation
1	Colour	Light pink
2	Appearance	Rough surface pellets
2	Flow	Free flowing
3	Shape	Round

Table No.7: HPLC Parameter in Assay of Pellets

Sr. No.	Sample	No. Of Injections
1	Mobile phase	1
2	Standard preparation	5
3	Sample preparation	1

Table No.8: Density Of Pellets

Sr. No.	Parameters	Result
1	Bulk Density	0.77gm/ml
2	Tapped Density	0.82gm/ml

Table No.9: Particle Size Analysis of Pellets

Sr. No	Sieve No.	Empty Weight of Sieve (g)	Filled weight of Sieve (g)	Weight of Pellets (g	% Retains
1	#16	432.5	432.5	0.0	0.0
2	#20	485.5	488.0	2.5	2.5
3	#30	452.0	548.0	96.0	96.0
4	#40	421.0	422.0	1.0	1.0
5	#50	420.0	420.0	0.0	0.0
6	Bottom	374.5	374.5	0.0	0.0

Table No.10: Observation of Aspect Ratio

Sr.No.	Length (µm)	Width (µm)	Aspect Ratio
1	1420.0	1370.0	1.078
2	1255.3	1210.6	1.036
3	1191.0	1089.2	1.093
4	1099.1	1070.8	1.026
5	1245.7	1163.2	1.070

Table No.11: Result of *In vitro* Drug Release from Pellets

Medium	TIME (Hrs.)	% Drug Release		
		F02G	F02Fc	F02G (20%) and F02Fc (80%)
0.1 N HCl	0	0	0	0
	2	0	0	0
pH 6.8 Phospahte Buffer	3	17.3	36.1	36.2
	4	31.8	46.9	50.7
	5	45.7	57.7	63.5
	6	58.8	67.3	71.2
	7	70.7	77.6	79.9
	8	78	80.1	84.7

Above results are averages of the 6 samples

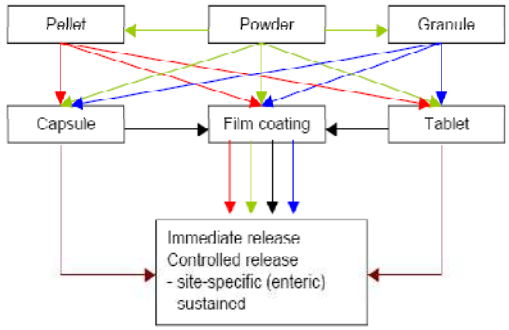

Figure1: Dosage form design of pellets.

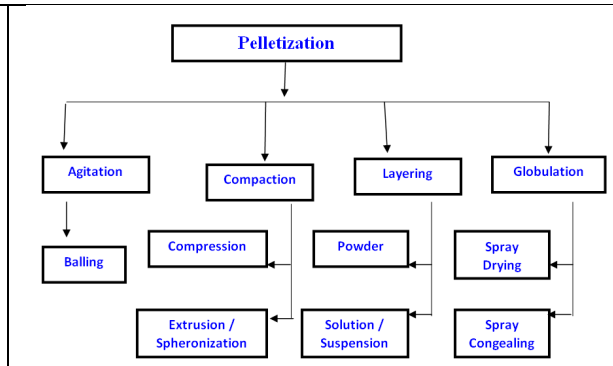

Figure 2 Techniques of Pelletization

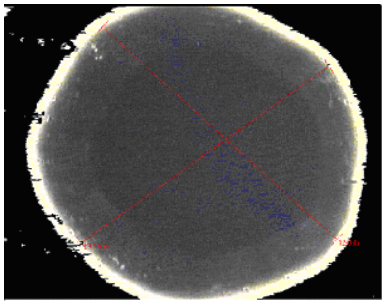

Figure No:3 Diagram of pellet in hot stage microscope

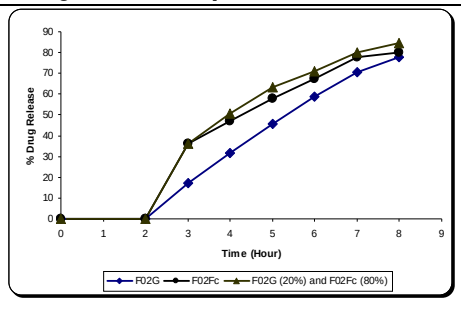

Figure No:4 Graphical presentation of %Drug dissolution versus Time (hrs.)

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RESEARCH ARTICLE

Novel Perceptions on Anticancer Activity of Bromelain

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ABSTRACT

Bromelain, a proteolysis enzyme, nowadays a substantial area of exploration as an anticancer agent. Numerous *in vitro* and *in vivo* studies are conducted to subsume its full potential, due to the absence of undesired effects. Bromelain based nanoparticles found increased bioavailability, and is a desired therapy since there is accumulation via EPR effect. Synergistic activity of Bromelain along with various chemotherapeutic agents exhibits notable activity in reducing cancer. Novel studies also manifest the benefit from functionalization of many drug carriers, results in better anti-proliferative effect than standard Bromelain. This review integrates its effect in varied type of cancers such as breast cancer, skin cancer, Leukemia, Non-Hodgkin lymphoma, colorectal cancer and Lung cancer. Apoptosis, cell survival regulators inhibition and immune modulation are recognized to be the major mechanisms by which it is effective in cancer.

Keywords: Bromelain; Cancer; Tumors; Apoptosis; Cell line

INTRODUCTION

Bromelain is regarded as an effective anticancer agent, blend of sulfhydryl proteolytic, that is protein digesting enzymes [1] found in pineapple (Ananascomosus), isolated from peels, core, leaves etc. belongs to the family of Bromeliaceae. They are mainly grown in tropical and subtropical countries such as China, Indonesia, Thailand, India, Philippines, etc [2]. and obtained as crude aqueous extract from stem and fruit. Also they have numerous medicinal uses, such as it helps in preventing and minimizing cardiovascular disease, relieves osteoarthritis, prevents diarrhea, treatment of chronic inflammatory, malignant and autoimmune diseases, [3] potentiation of antibiotic effects, etc. Marcano in 1891 first established the existence of Bromelain and it was Chittenden, who first extracted from pineapple juice and detailed its action [4]. Breakthrough in the history of Bromelain has outstretched as a potential

candidate in inhibiting or preventing the symptoms of novel disease COVID-19, by utilizing its anti-inflammatory and anti-coagulation property, as such it found to decelerate the progression of cancer.[5] The ideology of adopting Bromelain as a potential anticancer compound can be regarded as a result of several other studies previously conducted which demonstrates the high susceptibility of extracellular matrix to proteolytic enzymes such as Papain, Collagenase and Bromelain [6]. Cell death doesn't occur in cancerous cells, whereas it occurs naturally in healthy cells. And the cancerous cells can metastasize to nearby tissues via circulation.[3] The effect of Bromelain on cancer patients was first observed by Gerard in 1972 and Nieper in 1976 established the beneficial effects following oral administration of Bromelain in cancer patients.[7] Bromelain has antimetastatic property and exhibits reduced damage to healthy cells,[3] such that the activity is due to its direct impact on cancer cells.[8] They have the capacity to modify key pathways that support malignancy [3]. Bromelain can be used as a combination with other chemotherapeutic agents, improves the efficiency of later in curing cancer. It is found that Bromelain can reduce volume of CD44 protein in leukemia and melanoma cells [9]. Different studies have shown in vivo antitumor activity of Bromelain in cell lines such as Ehrlich ascetic carcinoma, P-388 leukemia, adenocarcinoma etc [3]. Bromelain inhibits the metastasis through inhibition of cell surface proteins, which are essential for cell adhesion. Numerous nanoformulations have been developed that utilizes the higher diffusion ability of Bromelain to the tumor parenchyma. Wang X et.al. (2018) formulated Doxorubicin nanocarriers in which Bromelain is immobilized on the surface of Lactobionic acid modified chitosan. Also the results have proved these nanoparticles show higher diffusion ability and better growth inhibition than free Doxorubicin and those without Bromelain.[6] The treatment of human epidermoid carcinoma and melanoma cells *in vitro* resulted in cell cycle arrest at G(2)/M phase and subsequent induction of apoptosis [10].

Framework of Bromelain

This phytotherapeutical drug is mainly composed of amino acid sequences, and is a weave of thiolendopeptidases, protease inhibitors, cellulases, glucosidases, carbohydrates and glycoproteins [11]. The potential therapeutic value of Bromelain is due to the presence of glycoprotein together with insoluble materials such as minerals, protease inhibitors, organic solvents etc.[12]It always manifests an optimum enzyme activity over a pH range of 5 to 8. It is copious in the amount of Alanine, Glycine, Serine, while little amount of Histidine is observed. Both stem and fruit bromelain are different in terms of immunity and specificity. This enzyme favorably cleaves glycyl, alanyl and leucyl peptide bonds [11].

Bromelain in Breast Cancer

Breast cancer is one among the leading causes of deaths in women worldwide. There are several risk factors that contribute to this condition, especially the amount of endogenous oestradiol, age, menstrual cycle, childbearing, oral contraceptives, exercise, ionizing radiation etc.[13]Early in vitro studies proved that it has potential anticancer activity in breast cancer cell lines such as MCF-7 and MDA-MB 231.[14] It is considered as a safe surrogate as the current therapies have numerous side effects and most effective drugs are highly toxic. [15] Dhandayuthapani S et.al. 2012, in their study investigated Bromelain's effect on GI-101A breast cancer cell lines, and the extent of apoptosis was determined by assessing activities of caspase-9 and caspase-3 [14]. Caspase-3 belongs to cysteine proteinase family and have potent role in regulating apoptosis by the lysis of certain key cellular proteins and gets activated in the presence of chemotherapeutic agents [16]. And those treated with Bromelain showed significant decrease in proliferation by inducing apoptosis, such that a maximum 67% of cell death observed at a concentration of 20µg/ml with 24 hour treatment interval [14]. Increased dosage of Bromelain shows enhanced apoptotic activity in mammary cancer cells [9]. Mohamad et. al in his study suggests that bromelain treatment enhance the effect of Cisplatin on 4T1 breast cancer cells through moderating tumor environmental inflammation. Previous findings have shown the antitumor activity was active both on slow growing and aggressive tumors *in vivo* [17]. Similarly combination of Cisplatin and Bromelain on MDA-MB-231, exhibited a synergistic effect in the induction of apoptosis while single agent treatment with either Cisplatin or Bromelain results in dose and time- dependent decrease in viability of cancer cells [18]. Early 80's remarked the potential benefit of orally applied Bromelain either as a single treatment or in combination with chemotherapeutic agents in breast carcinomas [19]. Oliveira et.al (2017) put forward a hypothesis

on antitumor activity of Bromelain surface functionalized nanoparticles and conducted in vitro studies on MCF-7 human breast cancer cells. The result manifests that functionalization shows much more anti-proliferative effect than standard Bromelain solution [20].

Bromelain in Skin Cancer

In Australia, 300 over 100000 people are affected with skin cancer according to the reports in 2018 [21]. Kalra N et al. in their study exhibits that application of Bromelain hold up the onset of tumorigenesis and an exhaustion in cumulative number of tumors, tumor volume and average number of tumors in DMBA (7,12-Dimethylbenzanthracene) initiated and TPA (12-O-Tetradecanoyl Phorbol-13-Acetate) promoted mouse skin model [22]. Both agents are capable of producing skin papillomas by topical application [23]. Nano-chemoprevention is regarded as the best concept to increase the potency of Bromelain against solid tumors. Bromelain exert its anticancer activity in skin papillomas by inducing apoptosis by increasing the expression of activators of apoptosis, p53 and Bax [24]. p53, a tumor protein is a gene that codes for a protein helps in regulation of cell cycle [25]. Increase in p53 expression indicates increased tumor suppression [26].

Bromelain in Leukemia

Bromelain was found to downregulate the level of COX-2 and PGE-2 in human monocyticleukemia cell lines. [12,27]COX-2 belongs to Cycloxygenase family, which is upregulated during the incidents of inflammation and cancers, and can moderate apoptosis and regulate cell proliferation. COX-2 inhibition is a potential target in cancer therapy [28]. Numerous studies have shown that intraperitoneal administration of Bromelain after 24 hours of tumor cell inoculation resulted in tumor regression,[27b]and the application on P-388 leukemia cell line shows an effect of increased apoptosis, reduced expression of NF-κβ (Nuclear Factor- κβ), at the same time reduced growth and metastasis [29]. NF-κβ has a significant role in tumor development and evolution, as activated signaling boosts cancer cell survival. Hence it always took attentiveness for a product that obstruct or reduce its activity [30]. Debnath et.al. conducted a study to identify the candidate with higher potential in inducing apoptosis on different cell lines, whether it is Bromelain alone or the combination of Bromelain and peroxidase. Both Bromelain and Peroxidase are sourced from the fruit juice. And it has been found that the mixture induces apoptosis better than Bromelain alone in acute myeloid leukemia cells via mitochondria dependent pathway. Previous studies have indicated that peroxidase is the reason that increases the efficiency of Bromelain against proliferation of cancer cells [31]. Also Bromelain efficiently reduces the volume of CD44 protein in leukemia cells [32].

Bromelain in Non-Hodgkin Lymphoma

Non-Hodgkin lymphomas are heterogenous cancers involved from B-lymphocytes [28]. Debnath et. al. conducted the study of using combination of Bromelain and Peroxidase against ascitic Dalton's Lymphoma Cells, shows a reduction in Non-Hodgkin Lymphoma via upregulation of antioxidant enzymes and modulating apoptotic protein expression. The rapid upsurge of ascitic fluid, which is an important nutrition source for lymphoma cells found to be decreased after treatment with the combination [33].

Bromelain in Colorectal Cancer

Bromelain treated Kras mutant colorectal carcinoma cell lines exhibited reduced cell growth and proliferation. The mechanism is by ferroptotic cell death, which is regulated by differential action of Bromelain set up by the differential expression of ACSL-4 (Long-chain-acyl-CoA synthetase 4). It has been found that there is a recovery of an intestinal inflammation, and survival rate has increased with Bromelain treatment [34]. Another mechanism that hinders colorectal cancer cells proliferation is via activation of ROS (Reactive Oxygen Species) production and autophagy. In gastric cancer, the effect of Bromelain can be visualized with reduced cell growth accompanied by significant DNA perturbation. It induces production of oxidative stress and superoxide, also increases levels of cleaved caspase-3, caspase-8, caspase-9 and PARP [35]. Romano et.al. 2014 during his study on chemopreventive action of stem bromelain on colon carcinogenesis found that Bromelain reduced cell proliferation and promoted apoptosis in Caco-2 cells. In concentration-dependent manner, it reduces the ^{3}H-thymidine incorporation in

proliferating Caco-2 cells. Also exhibited an increased activity of caspase 3/7, indicating a proapoptotic effect in the corresponding cells [36]. Brom Ac, which is a combination of Bromelain and Acetylcysteine, is used to treat a rare mucinous tumor, pseudomyxomaperitonei. This can affect tumor's biological functions as well as oncogenes which results in cytotoxicity [37].

Bromelain in Lung Cancer

Majumder et. al. studied the combinatorial treatment Oleaeuropaea, which are olive leaves and Bromelain in curing lung cancer under the consideration as an anti-inflammatory agent which could reduce the pulmonary inflammation regulated by Nrf2 (nuclear factor erythroid 2–related factor 2) and NF-κβ. And it has been found that there was an increased translocation of Nrf2 from cytoplasm to nucleus [38]. Nrf2, is a regulator of cellular resistance to oxidants [39]. Usually this is inactivated in the nucleus and reactivated on return to the cytoplasm. Cytoprotective effects are mediated by cytoplasmic return of Nrf2 with increased reactivation or refresh-rate under stress condition [11]. The combination treatment has shown least number of mast cell accumulation in lung tissue [40].

Future Directions

Bromelain is a hopeful delegate in future anticancer therapy [12]. Its effect on apoptosis and cell survival regulators directs it into researches in the field of oncology. Numerous in vitro and in vivo studies have been conducted and the proved mechanisms includes the inhibition of cell growth, alterations in tumor microenvironment, hemostatic system regulation, immune-modulation etc [29]. Recent studies on the formulation of Bromelain as an anticancer drug, reached upto the development of nanoformulations and utilizing its full potential in producing maximum effectiveness. Parodi et. al. conducted studies on surface modification of silica nanoparticles by Bromelain to increase diffusion into tumor extracellular matrix. And this approach is found to be essential in imparting proteolytic effect on tumor microenvironment, thus increasing the diffusion of chemotherapeutic agents [41].

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RESEARCH ARTICLE

Study of Structural, Thermal and Electrical Properties of PVP Polymer with $ZnSO_4.7H_2O$

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ABSTRACT

In recent years, electrochemical devices are more popular for different purposes so solid sate polymer electrolytes have become very important for rechargeable batteries and super capacitors. Poly-vinylpyrrolidone (PVP) is an important amorphous polymer material for polymer electrolytes. In this paper, we are reporting PVP blended films with Zinc Sulphate (ZnS). The prepared film characterized various experimental techniques using like SEM for morphology, XRD for structural study, Differential scanning calorimeter (DSC), AC Impendency spectroscopy. Experimental studies have performed for PVP and ZnSO4.7H2O with 70:30 molecular weight percentages.

Keywords: Polymer electrolyte, Ionic conductivity, . Poly-vinylpyrrolidone, thin films.

INTRODUCTION

Fental had been introduced polymer electrolyte In 1973 [1] and its importance for technical application was recognized in early 1980 [2]. The salts dissolved in the polymer matrix for electrolyte [3]. Host polymer materials have great potential for fabrication of energy storage devices. Those polymer chains which belong to polar groups are important for salt complexes. In the recent years we have found that polymer materials are more useful for electrochemical devices, energy storage devices as a rechargeable batteries, solar cells, super capacitors, sensors. [4-8]

Polymer electrolytes have been developed as ionic conductors. Metallic salts are dissolving in the polymer matrix [5]. In this paper, we are studying Zinc ion and PVP based electrolyte. Zinc-ion batteries are regarded as a promising candidate for next generation energy storage systems due to their high safety, resource availability and environmental friendly. Zn-ion have been more popular for rechargeable batteries in the recent past due to its non toxicity, low cost material, excellent mechanical strength [9]

EXPERIMENTAL

Polymer polyvinyl pyrrolidone PVP (Sigma Aldrich) of average molecular weight 40,000 and salt $ZnSO_4.7H_2O$ Zinc sulphate heptahydrate has been used for sample preparation materials. Triple distilled water was used as a solvent. 70% weight percentage of host polymer material PVP and 25% salt $ZnSO_4.7H_2O$ were dissolved in the triple distilled water and continuously stirred with a magnetic stirrer for 20 hours to obtain homogeneous mixture. The solution was casted in the Petridis and kept in oven under 50^0C to dry thick film.

RESULT AND DISCUSSION

XRD Analysis

The amorphous nature of PVP has observed in the XRD pattern (a) with broad peak of 2θ at 19^0-24^0 degree and almost similar amorphous nature is remain in fig1(b) of our sample study with 70% PVP and 30% $ZnSO_4.7H_2O$.

SEM Analysis

The figure (2a) and figure(2b) are the SEM images of PVP blend with $ZnSO_4.7H_2O$ (70:30) at 100 µm and 50 µm resolution respectively. The blending of the material is showing with homogeneous morphology in the entire range of concentration. The surface morphology of the film (70:30) shows amorphous in the nature and there is no phase separation observed in the blend polymer matrix.

EDX Data

The energy dispersive X-ray data is important to find the presence of different element in the PVP blended with $ZnSO_4.7H_2O$ (70:30). Different elements Zn, S, C, O and N have found in the sample with certain percentages.

FTIR Analysis

The FTIR spectrum of pure PVP has been shown in Fig(4a) and PVP blended with $ZnSO_4.7H_2O$ (70:30) has been shown in Fig(4b). The characteristic vibrational bands 2061 cm^{-1} for C-N stretching, and other stretching peaks are appearing like 1612 cm^{-1}, 1240 cm^{-1} and 746 cm^{-1}. [10,12, 13]

Impedance Analysis

The impedance of blend PVP polymer was measured with using AC impedance analyzer. The graph is showing a semicircle. The semicircle in the figure (5) is important to find the bulk resistance of parallel combination.
The bulk resistance of the PVP blend electrolyte (R_b) calculated by using the intercept of the straight line of Z'.
The value of conductivity calculated by using the formula of [11]

$$\sigma = l/R_b AS$$

Where l and A are thickness and area of the PVP blend electrolyte.

DSC Analysis

DSC technique is applicable to understand the response of polymers to heating. It is useful to find the glass temperature or melting point of crystalline polymer material. The T_g value our PVP electrolyte has been found 82 ^{o}C.

CONCLUSION

XRD broad peak showing the amorphous nature of our PVP blend electrolyte and significant morphology has been found in SEM analysis. In FTIR study molecular bonding has found with small shifting of wave number. DSC analysis confirmed amorphous nature of electrolyte till 82 ^{0}C . After the calculation of ionic conductivity by using bulk impedance, the ionic conductivity of pure PVP Is found 2.3 $\times 10^{-6}$ Scm^{-1} which has improved in PVP blend elctrolyte with ionic conductivity value 1.8 $\times 10^{-5}$ Scm^{-1}.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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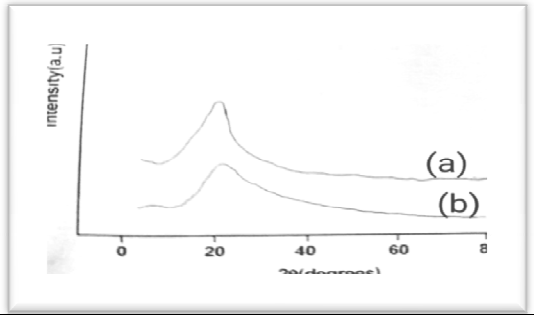

Fig. 1. X-Ray diffraction pattern (a) PVP (b) PVP with $ZnSO_4.7H_2O$

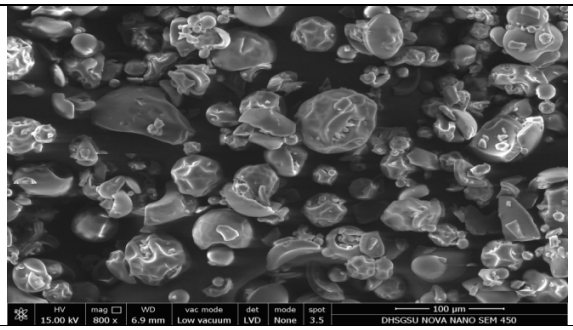

Fig. 2a. SEM image of PVP blended with $ZnSO_4.7H_2O$

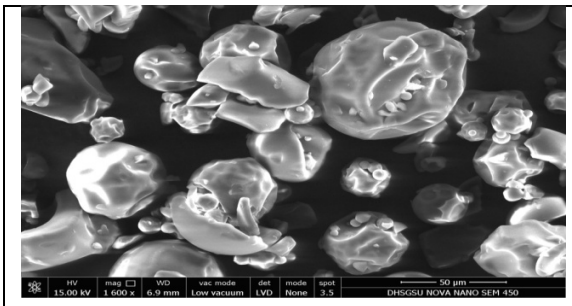

Fig. 2b. SEM image of PVP blended with $ZnSO_4.7H_2O$

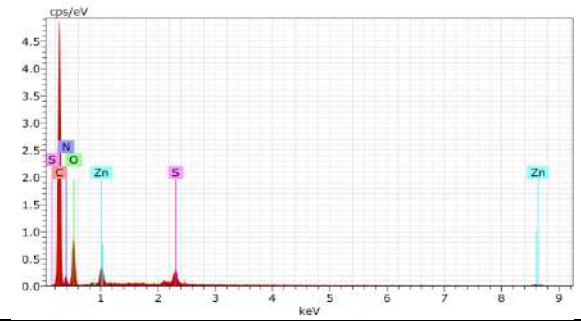

Fig. 3. EDX data of PVP blended with $ZnSO_4.7H_2O$ (70:30).

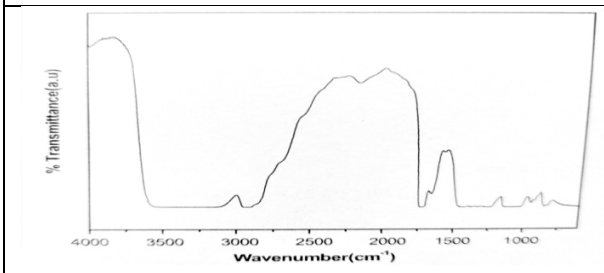

Fig. 4a. FTIR for pure PVP

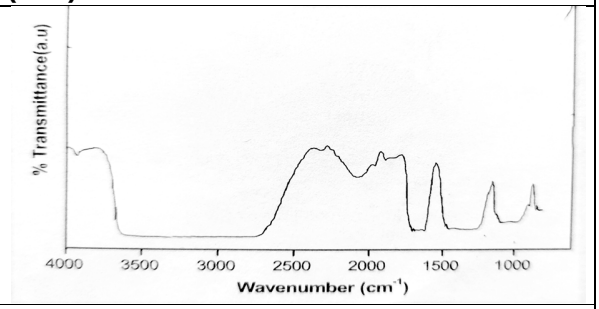

Fig. 4b. FTIR for PVP blended with $ZnSO_4.7H_2O$ (70:30).

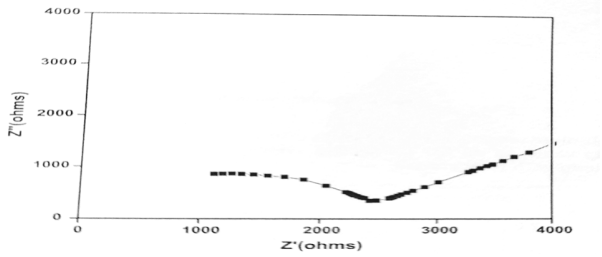

Fig. 5. Z' and Z'' plot for PVP blended with $ZnSO_4.7H_2O$ (70:30).

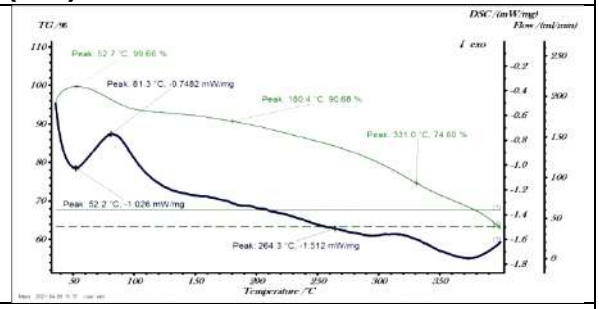

Fig. 6. DSC for PVP blended with $ZnSO_4.7H_2O$ (70:30).

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RESEARCH ARTICLE

Isolation and Characterization of Flavanoids from *Rhynchosia minima* (Linn.) DC

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ABSTRACT

Plants have been the basis of different traditional medicinal systems throughout the world and continue to provide mankind with new remedies. The plant *Rhynchosia minima* locally known as Nela Alumu (Telugu) is an indigenous medicinal palnt used traditionally as abortifacient, antihelminthic, used in the treatment of wounds, asthma and piles. Extraction of the flavanoids can be performed with solvents that are chosen according to their polarity. The selective crude extract is subjected for isolation by column chromatography by using different solvents. The separation of flavanoids from each fraction by column chromatography was monitored by thin layer chromatography. The pure compounds obtained after separation by the column chromatography were characterized by IR, NMR and Mass Spectroscopy for the structural elucidation.

Keywords: *Rhynchosia minima*, Extraction, Coloumn chromatography, Isolation, Thin layer chromatography, Structural elucidation.

INTRODUCTION

Herbal medicines serve as major remedy in traditional system of medicine, even in 21st century these are the primary source of health care system in rural areas and poor countries. According to WHO, about 80% of the world populations still depend on herbal medicines for primary health care. Herbal medicine practices continue still today because of their biomedical superiority over modern medicine [1,2]. Among the plants of Fabaceae family which are used in traditional medicine, *Rhynchosia* species have occupied a prominent role. *Rhynchosia* genus consists of approximately 300 species circulated throughout the tropical and subtropical areas around the world, out of which twenty-two species occur in India [3,4]. The plant *Rhynchosia minima* Synonym(s): *Dolicholus minimus*, *Dolichos minimus*, *Rhynchosia minima* var. *diminifolia* Family: Fabaceae, locally known as Nela Alumu (Telugu) is an indigenous medicinal palnt used traditionally as abortifacient, antihelminthic, used in the treatment of wounds, asthma and piles. The seeds are bitter and poisonous and seed extract shows specific agglutinating action on human RBC [5].

The species belonging to the genus *Rhynchosia* (Fabaceae) are herbs, twining or erect shrubs. Previous phytochemical investigations on several species of *Rhynchosia* showed that the genus is exclusive to profuse production of *C*-glycosyl flavonoids [6]. Some of the isolated compounds of *Rhynchosia* genus and their plant extracts exhibit interesting biological activities, including antioxidant, anti-inflammatory, antimycobacterial and antiproliferative [7,8].

Traditional uses

Rangaswamy *et al*., (1974) studied the phytochemistry of seed coat and pericarp and found to contain gallic acid, Hydroquinone diacetate and other phenolics. Elisabeth *et al*., (1977) studied phenolics and flavonoids in the leaves and reported that all flavonoids of the leaf extract were present in the form of C-glycosyflavones [9]. The hydroquinone present in the seeds of *R. minima* is supposed to be involved in seed germination. Flavonoid profiles of seven species of *Rhynchosia* including *R. minima* were reported by Adinarayana *et al*., (1985) [10,11]. New flavonoids were identified in the leaf extract of *R. cyanosperma* (Adinarayana *et al*., 1980; 1981) [12,13]. In all these studies the medicinal uses of the phytochemical principles were not discussed. However, Gundidza *et al*., (2009) [14] demonstrated range of 8 essential oils which showed high antibacterial activity against several bacterial and fungal species. N. Yellasubbaiah et al., (2015) studied the anti-oxidant and anthelminthic activity of ethanolic extract of *Rhynchosia minima* [15]. Aqueous, ethanol and ethyl acetate extracts of *R. minima (Linn) DC* were screened against pylorus ligation induced ulcers in rats reported by N. Yellasubbaiah et al., (2017) [16].

MATERIALS AND METHODS

General

IR spectra were recorded (KBr discs) on an FT-IR spectrometer (mmax in cm^{-1}). 1H NMR spectra were recorded on a Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in d, ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (kmax in nm). Mass spectra were recorded on a Varian 3800 mass spectrometer. TLC was performed with silica gel GF_{254}. All solvents were analytical reagent grade.

Plant Material

Rhynchosia minima (Linn) DC plant was procured in the spring season, from Medicinal garden of CES College of Pharmacy, a Chinntekur locality in Kurnool. The Leaves were identified and authenticated by botanist Dr. M. Palanisamy, Scientist 'C' Botanical survey of India, Southern regional center, Coimbatore. A specimen voucher of the plant has been deposited in the Department of Pharmacognosy, CES College of Pharmacy, Chinntekur, Kurnool.

Preparation of extracts

Whole plant of ***Rhynchosia minima (Linn) DC*** was shade dried under room temperature for one week and before extraction the leaves were grounded into coarsely powdered mechanically. About 100g of the finely powdered plant material is subjected for the extraction of the flavonoid compounds by the maceration. In this fine slurry is made by mixing the coarsely powdered plant material with solvent aqueous-alcoholic (ethanol) 700ml/liter, leave the mixer for 24hrs, with frequent shaking of the contents of the flask. The extract was filtered under vacuum and residue was further extracted with aqueous-alcoholic (ethanol) 500ml/liter for another 12hrs and the filtered. Repeat the same procedure until to obtain a specified amount of the crude extract[17].

Isolation by column chromatography

The aqueous-alcoholic extract (50 g) was suspended on water and extracted successively with n-hexane, chloroform, ethyl acetate, methanol and acetone to yield n-hexane (5.4 g), chloroform (12.8 g), ethyl acetate (9.6 g), methanol (8.2 g) and acetone (8.5g) fractions, respectively. Chloroform soluble fraction (10 g) was subjected to chromatography on silica gel (60–120 mesh, Merck) eluted with methanol-acetone (3:7) solvent system. By repeated chromatography to give six major fractions (Fraction-1, 0.198 g; Fraction-2, 0.257 g; Fraction-3, 1.085 g; Fraction-4, 0.126 g; Fraction-5, 0.219 g).

Fraction-1

Fraction-1 obtained from column chromatography was further purified by preparative TLC over silica gel GF_{254} using hexane-ethyl acetate (3:4) as the developing solvent. It was a pale yellow crystal, m.p. 183–185º C; MS: (M+, 287); UV: 342, 262, 208 nm; IR (KBr): 3465, 3156, 2920, 1625, 1578, 1398, 1372, 1125cm^{-1}; 1H NMR (δ values, DMSO-d_6): 5.35 (m, 3H, 4- OH), 5.35 (s, 1H 4'-OH) 6.08 (s, 1H, H-8), 6.65 (d, 2H, H-3',5'), 6.71 (s, 1H, H-3), 7.59 (dd, 1H, H-1',6'). It was characterized as 5,6,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one[18] (Isovexitin).

Fraction-2

Isolated fraction was further purified by preparative TLC over silica gel GF_{254} using hexane-ethyl acetate (3:4) as the developing solvent. It was crystallized from methanol as a white powder (2, 6 mg); m.p. 158–161º C; MS: (M+ 304); UV: 342, 262, 208 nm; IR (KBr):3475, 2985, 1715, 1596, 1572, 1464, 1342, 1195, 1158, cm^{-1}; 1H NMR (δ values, DMSO-d_6): 5.42 (m, 3H, 4- OH), 5.38 (s, 1H 4'-OH) 6.12 (s, 1H, H-6), 6.69 (d, 2H, H-3,1'), 6.98 (s, 1H, H-5'), 7.34 (s, 1H, H-6'). It was characterized as 2-(3,4-dihydroxyphenyl)-5,7,8-trihydroxy-4H-chromen-4-one[18] (Orientin).

Fraction-3

The isolated mixture was worked-up in the usual way and purified by preparative TLC over silica gel GF_{254} using hexane – acetone - ethyl acetate (4:6:3) as the developing solvent. It was crystallized from methanol as brown colour needles (13 mg); m.p. 167 - 169ºC; MS: (M+, 305); UV: 238, 268, 375 nm; IR: 3470, 1654, 1587, 1579, 1369, 1358 cm^{-1}; 1H NMR (δ values, DMSO-d_6): 6.21 (m, 4H, 4- OH), 5.98 (s, 1H 4'-OH) 6.78 (s, 1H, H-4), 6.97 (d, 2H, H-3',5'), 7.28 (d, 2H, H-1',6'). It was characterized as 5,6,7,8-tetrahydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one[18] (Vicenin).

Fraction-4

Isolated fraction from the column was further purified by preparative TLC over silica gel GF_{254} using n-hexane-acetone-ethyl acetate (6:4:5) as the developing solvent. It was crystallized from ethanol as orange colour needles (8 mg); m.p. 177º C; MS: (M+, 316); UV: 218, 248, 364 nm; IR: 3465, 1652, 1615, 1595, 1425, 1385 cm^{-1}; 1H NMR: (δ values, DMSO-d_6): 3.87and 3.92 (2s, 6H, OCH_3×2),5.95 (d, 2H, 5-OH, 4'-OH), 6.25 (s, 1H, H-8), 6.72 (d, 2H, H-3',5'), 6.82 (s, 1H, H-3), 7.64 (dd, 1H, H-1',6'). It was characterized as 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxy-4H-chromen-4-one (Schaftoside).

Fraction-5

It was purified by preparative TLC over silica gel GF_{254} using n-hexane-acetone-ethyl acetate (7:3.5) as the developing solvent. It was crystallized from ethanol as pale brown coloured needles (6 mg); m.p. 185º C; MS: (M+, 316); UV: 218,

248, 364 nm; IR: 3465, 2987, 2865, 1652, 1615, 1595, 1425, 1385 cm^{-1}; 1H NMR: (δ values, DMSO-d_6): 3.87and 3.92 (2s, 6H, OCH3×2), 7.95 (s, 1H, , 4'-OH), 6.25 (s, 1H, H-8), 6.72 (d, 2H, H-3',5'), 6.82 (s, 1H, H-3), 7.64 (dd, 1H, H-1',6').It was characterized as 6-hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-chromen-4-one (compound 5).

RESULTS AND DISCUSSION

The aqueous-alcoholic extract of the leaves was extracted with n-hexane, chloroform, ethyl acetate, methanol and acetone. The chloroform fraction was purified and five compounds were obtained (Scheme I). By means of spectroscopic analysis, they were characterized as 5,6,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one[18] (Isovexitin), 2-(3,4-dihydroxyphenyl)-5,7,8-trihydroxy-4H-chromen-4-one[18] (Orientin), 5,6,7,8-tetrahydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one [18] (Vicenin), 5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxy-4H-chromen-4-one[19] (Schaftoside), 6-hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-chromen-4-one (compound 5). The compound 5 was isolated from this plant first time. 6-hydroxy-2-(4-hydroxyphenyl)-5,7-dimethoxy-4H-chromen-4-one (compound 5) was obtained pale brown coloured needles crystal. High resolution mass spectrum exhibited molecular ion at m/z 316, which is consistent with the molecular formula $C_{17}H_{14}O_6$. UV spectra displayed characteristic absorption bands for conjugated double bond at 248 nm. IR spectra of compound (5) showed frequencies at 3465 cm^{-1} and at 2987-2865 cm^{-1} indicating the presence of hydroxyl group and C-H in conjugation, respectively and the absorption peaks at 1652, 1615 and1595 cm^{-1} indicated the presence of C=O (Carbonyl group) and unsymmetric ethylenic double bond and aromatic rings. The ^{1}H NMR spectrum of the compound (5) indicated the presence of di methoxy groups by a sharp singlets at 3.87and 3.92 revealed the presence two methoxy groups at conjugation. A singlet at 6.82 indicated the presence of H-3 proton for the flavone nucleus. Also, a singlet at 7.95 indicated the presence of a hydroxyl group. Compound (5) was also obtained as pale brown coloured needle showing a pink colour on TLC silica gel plate when heated with concentrated sulphuric acid at Rf (0.74) in n-hexane-acetone-ethyl acetate (7:3.5). All the isolated known triterpenes were identified on the basis of UV, IR and ^{1}H NMR data and compared with the literature.

CONCLUSION

Flavonoids are one of the most important classes of secondary metabolites from natural products due to their several applications in medicine, foods, diet industries, and so on. Even though a huge number has been reported from natural and synthetic sources, scientists are still interested in flavonoids and derivatives. In present study after purifying the chloroform fraction five different compounds i.e Isovexitin, Orientin, Vicenin, Schaftoside and compound 5 were obtained. The compound 5 was isolated from this plant first time and characterized by established spectroscopic techniques. Further chemical pharmacological investigation on this newly isolated compound is needed to explore its biological properties.

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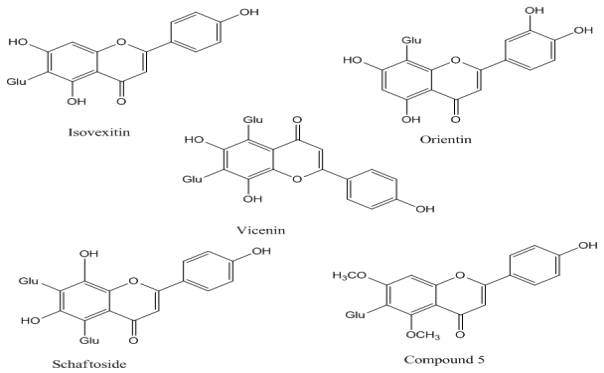

Scheme I

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REVIEW ARTICLE

An Overview on Microneedles in Transdermal Drug Delivery Systems

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ABSTRACT

Drug delivery systems are targeted delivery or controlled unleash of therapeutic agents. Medications have long been wont to improve health and extend lives. The microneedle is also known as collagen induction therapy (CIT) that involves repeatedly puncturing the skin with small, sterilized needles. Drug delivery systems control the rate at which a medication is delivered and the area in the body where it is delivered. Microneedles area unit wide utilized in stratum drug delivery system as a result of they are economical, safe, convenient and painless. Microneedle are avoided the First-pass metabolism and increasing patient compliance and frequent drug administration are avoided. The microneedle drug delivery technology may include either of the five design sorts of microneedles like; Solid microneedles, Coated microneedles, Dissolving microneedles, Hollow microneedles, Hydrogel forming microneedles. A microneedle ought to be sharp and thin enough so it will basically enter into the skin and even be durable enough all together that it doesn't break once inside the skin.

Keywords: Microneedles, Polymers, Absorption, Permeation, Nanoparticles

INTRODUCTION [1-3]

Drug delivery systems are targeted delivery or controlled unleash of therapeutic agents. Medications have long been wont to improve health and extend lives. Drug delivery systems control the rate at which a medication is delivered and the area in the body where it is delivered. The stratum drug delivery system may be a technique that has drug absorption via the skin. Stratum drug delivery involves drug diffusion through distinct layers of the skin into general or blood circulation to electrify therapeutic result.

Transdermal patches deliver medication locally, wherever they're absorbed by the skin and into the blood. They supply a uniform delivery of tiny amounts of a drug into the blood stream over an extended amount of your time. The length of damage time and therefore the quantity of drug delivered is completely different from patch to patch.

TYPES OF TRANSDERMAL DRUG DELIVERY SYSTEM

1. Single-layer drug in adhesive.
2. Multi-layer drug in adhesive.
3. Reservoir system.
4. Matrix system.

SINGLE-LAYER DRUG IN ADHESIVE [4]

The adhesive layer of this technique additionally contains the drug. During this kind of patch the adhesive layer not solely serves to stick the varied layers along, in conjunction with the complete system to the skin, however is additionally chargeable for the cathartic of the drug. To the outer facet of adhesive layer there's lining of temporary liner and a backing.

MULTI-LAYER DRUG IN ADHESIVE [5]

It is like the only layer systems within the respect that each adhesive layers are also responsible for the cathartic of the drug. The multilayer system is totally different but that it adds another layer of drug-in- adhesive, typically separated by a membrane (but not altogether cases). This patch additionally enclosed by a brief liner-layer and a permanent backing.

RESERVOIR SYSTEM [6]

In this system, the drug reservoir is embedded between associate fast backing layer and a rate dominant membrane. The speed dominant membrane may be microporous or nonporous solely which may unharnessed the drug. Within the drug reservoir compartment, the drug could also be within the type of a solution, suspension, gel or dispersed during a solid polymer matrix.

MATRIX SYSTEM [7,8]

Drug in adhesive system

In this sort, the drug reservoir is made by dispersing the drug in associate adhesive chemical compound then spreading the medicated adhesive compound by solvent casting or softening (in the case of hot melt adhesive) on associate fast backing layer. On high of the reservoir, immediate adhesive chemical compound layers square measure applied for defense purpose.

Matrix dispersion system

This type, the drug is distributed homogeneously during a deliquescent or oleophilic compound matrix. This drug containing compound disk is fastened on to associate occlusive base plate during a compartment fictional from a drug impermeable backing layer. instead of applying the adhesive on the face of the drug reservoir, it's unfolded at the side of the circumference to form a strip of adhesive layer. Rather than applying the adhesive on the face of the drug reservoir, it's unfolded at the side of the circumference to make a strip of adhesive rim.

MICRONEEDLES [9-11]

A microneedle could also be a micron-sized needle with a height 10-2000 µm and a width of 10-50 µm which can penetrate through the cuticle layer to dermal tissue directly while not pain. Microneedles area unit wide utilized in stratum drug delivery system as a result of they are economical, safe, convenient and painless.

Microneedle device is made by arrangement many microneedles in arrays on a touch patch (the same as that of a typical transdermal patch accessible within the market) so on deliver decent quantity of drug to supply a needed

therapeutic response. It pierces the stratum so bypassing the barrier layer. Microneedles square measure made of a spread of fabric starting from chemical element, titanium, chrome steel, and polymers. Some microneedles square measure manufactured from a drug to be delivered to the body however area unit formed into a needle so as that they will penetrate the skin.

ADVANTAGES OF MICRONEEDLES

1. Large molecules are are often administered and painless administration of the active pharmaceutical ingredient [12].
2. Specific skin areas are often targeted for desired drug delivery [13].
3. Decreased microbial penetration as compared with a needle, the microneedle punctures only the epidermis [14].
4. Rapid drug deliveries are often achieved by coupling the microneedles with an electrically controlled micro pump [15].
5. Microneedle are avoided the First-pass metabolism [16].
6. Self-medication as described in the previous point is uncomplicated and reduces the risk of self-medication errors [17,18].
7. Reproducibility available in the microneedle and avoid the needle stick injury [19].
8. Increasing patient compliance and frequent drug administration are avoided [20].

DISADVANTAGES OF MICRONEEDLES

1. Dosage accuracy may be less than with hypodermic needle [21].
2. Careful use of the device could also be needed to avoid particles bouncing off the skin surface [22, 23].
3. The external environment, like hydration of the skin affects the drug delivery [24].
4. Small amounts of drug (less than 1 mg) are often given by bolus iven by bolus [25].
5. Repetitive injection may collapse the veins. Use of microneedle produces the local inflammation of the skin [26, 27].
6. This method is more expensive. This method used to produce skin irritation due to allergy [28, 29].

TYPES OF MICRONEEDLES [30]

Classification of microneedles depends on their mode of drug delivery. The microneedle drug delivery technology may include either of the five design sorts of microneedles like

1. Solid microneedles
2. Coated microneedles
3. Dissolving microneedles
4. Hollow microneedles
5. Hydrogel forming microneedles

SOLID MICRONEEDLES [31-35]

Solid microneedles area unit usually within the vary of 150-300 μm long tapered at a tip angle 15-20°. Their wear time ranges between thirty seconds to 10 minutes. These microneedles are often created from number 14, glass, and metal. Metal and glass area unit comparatively non-biodegradable. Metals employed in the fabrication of microneedles square measure stainless-steel, iron, and nickel. These microneedles are often ready either by coating with the drug then inserted into the skin or inserting the uncoated needle into the skin and making micro channels and later applying drug formulations like cream, gel or spray (two step processes). Solid microneedles square measure associate degree array of 1 uniform material with micron- scale protrusions, and do not contain any drug or Excipient related to the array. The formulation is applied over the pores; they facilitate the permeation of drugs into the skin either for native or general result.

COATED MICRONEEDLES [36-38]

Coated microneedles square measure solid microneedles coated with appropriate coating answer. Safety aspects of that square measure almost like solid microneedles. To boot, the aggravation capability of the covering answer and its fixings has to be inspected to stay away from any undesirable response of skin at the appliance website. One methodology of coating microneedles is through the work of electro hydraulics atomization (EHDA). During this procedure, treated steel microneedles with a height differ "between"600-900 μm tall were in addition to a ground conductor with a changed arrangement of ethanol: methyl liquor extent connection of 50:50. By and large, this technique was allegedly utilized in making of nano and micrometer-scaled coatings of drug item. Benefit of this approach is that the traditional dance use of its utilization contrasted with the uncoated strong microneedles that are partner dance strategy. This may restrict the work of covered microneedle to convey intense particles.

DISSOLVING MICRONEEDLES [39-44]

Since dissoluble microneedles square measure factory-made from polymers or sugars, they don't leave behind sharp biohazardous squanders so are more secure contrasted with strong or empty needles, in any case, the biocompatibility of the polymer/sugar utilized must be evaluated for patient consistence. The manufacture strategy for dissolving microneedles is regularly dispersed by embeddings compound answer into female molds and filling the micro cavities of the mold. The primary issue with these sorts of microneedles is addressing similarity of sugars or polymers with dynamic fixings and furthermore the cycle conditions like outrageous pH, warm temperature, and solvents will impact the relentlessness of joined proteins, antibodies and elective drug. The subsequent concern is that the extent connection of medication to compound or sugar since it significantly impacts the mechanical strength of microneedles.

HOLLOW MICRONEEDLES [45-47]

Hollow microneedles obliges a medication supply (normally allowing up to 200 milliliter of medication plan or medication alone) with an empty bore inside the focal point of the needle and predominantly indented to direct an outsized portion of medication answer to keep away from the constraint of coated microneedles. These needles are of 300 μm, with a130 μm external width and 110 μm inward distances across at the tip followed by 80 μm internal measurement and a 160 μm external breadth at the base were created abuse this procedure. These microneedles may also be invented mistreatment another system like micro-electro-mechanical system technologies like optical device micromachining, deep reactive etching, and associate degree integrated planographic printing molding technique, X-ray lithography.

HYDROGEL FORMING MICRONEEDLES [48-51]

Microneedles are regularly made out of dissolvable or transitory materials like polymers or sugars that typify the medication inside the MN network. These MNs when application totally breaks down or corrupts inside the skin, accordingly passionate the typified drug payload, and leave behind no hazardous leftovers. On account of hydrogel-shaping MNs, the needle tips of compound swell by grasping humor to deliver drug release. They also produce channels simultaneously, thus grant the medication liberated from repository to enter the microcirculation. They leave none or insignificants compound buildup when expulsion from skin. These are the most part concocted from double compound mixes of poly (methyl vinyl ether/maleic corrosive) and poly (ethylene glycol) through a little trim strategy abuse siloxane molds or by abuse optical gadget designing innovation.

This are generally suitable for the conveyance of minimal hydrophilic drug like caffein, thiazine and high mass mixtures like (ox-like egg whites and insulin).

PREPARATION OF MICRONEEDLES

Laser Cutting [52-55]

Metal microneedles are often factory-made by 3D laser cutting, laser ablation, and electroplating or electro less plating of metal onto positive or negative microneedle molds. Varieties of strong microneedles are created by cutting treated steel or titanium sheets looking like microneedles with an infrared laser.

The required form, geometry, and dimensions of microneedles area unit created mistreatment a number of the computer-aided design (CAD) computer code. These producing methodologies are often accustomed manufacture one row of MNs of different geometries, further as two-dimensional lines of bronze microneedles. Small machines 2020, 11, x 9 of 30 the microneedles region unit adequately hardened to infiltrate creature item models while not making harm. They based that this method are often utilized for programmable medication conveyance or liquid testing. Optical maser Cutting Metal MNs are frequently production line made by 3D optical maser cutting, optical maser removal, and electroplating or electro less plating of metal on positive or negative MN molds. The required form, calculation, and measurements of MNs are made utilizing a portion of the PC supported plan (CAD) programming.

The laser pillar follows the foreordained state of the needle, and afterward MNs are cleaned in major trouble and bowed at 90 degrees, in an upward direction from the plane of the base. These producing methodologies are often accustomed manufacture one row of MNs of various geometries, further as two-dimensional rows of bronze microneedles.

Laser Ablation [56- 58]

This approach could be a hierarchical procedure for measure materials, just as metals. Light-weight beats give the lump of the necessary structure on a metal plate, so framing strong metal clusters. However, because of the high-intensity optical maser pulses, the arrangement of plasma of particles and electrons isn't suitable for the manufacture of organized materials. The authors wrote about the manufacture of a tantalum MNs with an upward stature of more than ten µm and impressively little tip radii. This buildup was broadly used inside the infusion forming strategy for the get together of the MNs.

MICROMOLDING METHOD (SOLVENT CASTING) [59, 60]

Dissolving MNs region unit regularly made by filling an aforesaid prepared MN mold with the fluid definition. For the most part, the mold is shaped from a nuclear number 14 wafer as a starting material. Thereafter, the wafer is oxidated at a thousand a thousand. Needle unadulterated science is mottled abuse lithography ways, trailed by RIE, while CVD is utilized for covering a wafer. A fluid polymeric arrangement is filled arranged molds, and afterward, air voids are taken out with vacuum or rotator. Accordingly, the molds are dried in the broiler, and MNs are eliminated subsequent to cooling. Likewise, the creation of biodegradable polymer MNs, comprising of both regular and engineered materials, with a proper calculation and adequate solidarity to enter the skin.

ATOMIZED SPRAYING METHOD [61, 62]

This strategy conquers the issues related with the restricted limit with regards to large scale manufacturing of dissolving MNs with the ideal calculation and actual attributes. Likewise, the issues connected with the impacts of fluid surface pressure and thickness when filling the MN molds can be limited. Dissolving MN can be delivered from the sugars (trehalose, fructose, and raffinose) or polymers (PVA, PVP, CMC, HPMC, and sodium alginate). Momentarily, a spout associated with an air source and fluid detailing produces an atomized shower.

MICROELECTROMECHANICAL SYSTEMS (MEMS) [63-70]

Strong and empty MNs, just as molds for dissolving MNs, have been produced straightforwardly from a reasonable material substrate utilizing MEMS techniques. The creation includes a definitively controlled three step process: affidavit, designing, and scratching of materials. Complex three dimensional (3D) structures are, subsequently, shaped because of contrasts in the selectivity to the etchant between various materials. In the initial step, a film with a thickness between a couple of nanometers and 100 µm is framed on a substrate by a compound (CVD) or actual fume testimony (PVD). In the PVD cycle, the movie is framed by iota's moved straightforwardly from the source to the substrate through the gas stage. In the CVD cycle, the compound response on the substrate surface outcomes in film arrangement.

The most widely recognized kind of lithography is photolithography, a cycle dependent on the way that a few materials, for example, metals are not straightforward when presented to UV light (λ = 193–236 nm), while others, for example, glass are straightforward. In this interaction, an optic veil, a murky layout for producing. The silicon substrate is first presented to steam or humidified oxygen at around 900 ◦C to deliver an oxide layer, and afterward, pivoted and covered with a natural polymer touchy to UV light, the purported photo resist material. The warmth of 75–100 ◦C followed by UV radiation eliminates the dissolvable and structures the ideal photograph safe example. In the positive oppose, the chains of the photograph safe polymer separate after openness to UV light, making them more solvent in the synthetic arrangement the engineer, in contrast with the negative oppose, where the compound securities are reinforced.

The carving is accomplished by applying a solid corrosive or acidic specialist to scratch out the revealed portions of the substrate to shape a plan on the outside of the material. The scratching can be performed at something similar (isotropic drawing) or various rates (anisotropic carving). Then again, the dry carving measure is accomplished by utilizing a fume stage or plasma etcher. The quantity of particles that impact the level of isotropy can be changed by controlling the gas pressure. This strategy is utilized to deliver empty MNs with a lumen of a few hundred micrometers. To start with, the chromium veiling material was applied to a silicon wafer and framed into spots of a measurement equivalent to the foundation of the ideal MNs. Therefore, the wafers are presented. The MNs were warmed to 60 ◦C to expand exemplification in the miniature channels by diminishing its thickness. The MNs were created by anisotropic dry carving, isotropic dry drawing, and the channel topping off measure. They established that this framework can be utilized for programmable medication conveyance or liquid inspecting.

MATERIALS USED IN MICRCONEEDLES PREPARATION

Silicon [71, 72]

The first microneedle was made of silicon inside the 1990s. Silicon is anisotropic in nature and silicon crystalline structure. Its versatile nature permits manufacturing needles of various sizes and shapes. Chemical element substrates are definitively made and are fit for cluster creation. The cost of silicon and its since quite a while ago confounded creation technique restricts its utilization in microneedle. The most restrictions of silicon are its intricate manufacture, long creation times, high worth and muddled multi-step measure.

Metal [73- 75]

He principle metals utilized are tempered steel and titanium. Palladium, nickel, palladium-cobalt compounds additionally are utilized. They need shrewd mechanical properties and brilliant biocompatibility. Titanium could be a smart various to stainless-steel. Hollow microneedles also are created of metals. Ceramic alumina (Al_2O_3) is especially used owing to its chemical resistance. various assortments of earthenware production utilized square measure salt dry out ($CaSO_4$ $0.2H_2O$) and phosphate get dried out ($CaHPO_4.2H_2O$). Ceramics square measure solid materials composed of inorganic compounds of metals, non- metals or metalloids. Ceramics microneedles are usually ready mistreatment micromoulding of ceramic suspension followed by sintering at high temperatures.

Silica glass [76]

Silica glass is physiologically dormant anyway fragile in nature. Salt glass that is made of silicon dioxide and boron silica is a lot of elastic. They're largely fictitious manually, so are less time economical. Glass MNs aren't used currently commercially, however just for experimental functions.

Polymers [77, 78]

Polymers attract wide attention of microneedle fabrication due to their biocompatibility, biodegradability and low value. Varieties of present polymers are used for casting of microneedles. These embody present proteins, polysaccharides, semi synthetic and synthetic polymers. Polymers are used to prepare solid soluble or swell able microneedles or used as a coating on structures manufactured from different materials. Miniature needles created with these polymers have less strength than various materials anyway are tougher than glass and pottery. These

microneedles are wont to convey varying sorts of medication, from small particles to macromolecules or nanoparticles across the skin.

EVALUATION OF MICRONEEDLES

Characterization methods [79, 80]

The drug can be stacked onto or into the microneedles either in suspension/scattering structure or exemplified structure (liposome's, nanoparticles, nanoliposomes).The medication can be covered with the polymer arrangement or can be applied as a fix. Different physicochemical portrayals including molecule size, polydispersity file, consistency, and zeta potential can be assessed for stacked medication relying upon the sort of detailing utilized in the microneedles. The size, inner design and crystallinity of the liposome's or nanocarriers can be performed utilizing a powerful light dissipating, X-beam dispersing, and transmission electron microscopy procedure. Different tests like solvency examines, drug content, in-vitro discharge tests, and biocompatibility studies are likewise performed on planned microneedle.

DIMENSIONAL EVALUATION [81, 82]

Evaluation different techniques are utilized to survey the needle calculation and to gauge the tip range, length, stature of the microneedle. Commonest procedures are optical or electrical microscopy. Produces a picture of an example by making utilization of a focused light emission that demonstration with the particles inside the example though filtering and produce varied signals that provide data concerning test surface geography and structure. Confocal laser magnifying lens creates high-goal pictures.

MECHANICAL PROPERTIES [83]

A microneedle ought to be sharp and thin enough so it will basically enter into the skin and even be durable enough all together that it doesn't break once inside the skin. Two fundamental components for a safe and affordable plan of microneedles are the power at that the microneedle loses its underlying honesty and in this manner the inclusion power. The ratio is most popular to be as high as attainable.

***IN-VITRO* SKIN PERMEATION STUDIES [84, 85]**

Diffusion cell equipment is employed to search out the permeation of the drug through the skin. Pig ear skin is usually utilized in the experiment that is mounted between the receptor and donor compartment. The cumulative permeation profiles of microneedle treated and untreated skin are compared. These tests additionally can be wont to analyze the profundity of entrance of the atom. They reported the concentration of the dye to be very weak below 80 mm depth. They also evaluated the penetration of model drug using Franz diffusion cell across the microneedle-treated and untreated skin and reported enhancement in penetration by 104 to 105 times with use of microneedles.
In vitro and ex vivo by infusing Rhodamine B color. For in-vitro testing 1 Chronicles agarose gel was utilized and for ex-vivo testing, chicken bosom tissue, research facility mouse and an anesthetized bunny were utilized

***IN-VIVO* TESTING OF MICRONEEDLES [86]**

To lead the in vivo preclinical investigation, for the most part mice, hares, guinea pigs, mouse and monkey and so on are utilized. The primary intention of the in vivo testing is the assurance of wellbeing too poisonousness of the tried compound. The vital goals behind in vivo testing of the microneedles incorporates to perform skin poisonousness test, assurance of entrance power in various skin, mechanical dependability, twisting breakage power, to perform different non-clinical wellbeing study and pharmacological investigation, assurance of different boundaries like immunogenicity, genotoxicity, skin sharpening and allerginisation, study, formative harmfulness, intense and constant dermal harmfulness, cancer-causing nature.

BIOLOGICAL SAFETY TEST [87]

It is not really settled extractable synthetic substances from microneedles as per ISO 10993-12:2002 norm: 'Test Preparation and Reference Materials'. Extraction of synthetic substances from microneedles was finished by

drenching microneedles in physiological saline at 37°C for 72 h. The concentrate was then straightforwardly applied on shaved unblemished human skin for genuinely taking a look at dermal bothering. Adverse consequence of the test uncovered the natural wellbeing of the microneedles.

APPLICATIONS

VACCINE THERAPY [88]

A vaccine is a natural arrangement. It gives dynamic gained insusceptibility to a specific infection. Vaccine establishes a killed or debilitated type of infection causing miniature life form, its poisons or one of its surface proteins. Vaccine treatment invigorates the invulnerable arrangement of the body and gives assurance against the future miniature creature experience. Microneedle approach was observed to be successful in antibody treatment.

A less portion is required when the medication is directed utilizing empty microneedles when contrasted with intramuscular infusion. After the inoculation, the immune response titers were essentially higher with intradermal immunization with microneedles when contrasted with subcutaneous infusion on fifteenth day. Dissolving microneedles were additionally researched for intradermal inoculation.

CANCER THERAPY [89]

Cancer affects many people every year in the world and cancer treatment faces lots of challenges. Microneedles have been researched for different anticancer medications conveyance. Self-degradable microneedles were researched for melanoma treatment by conveying anti-PD-1 (aPD1) in a supported way. Anti-PD-1 and glucose oxidase stacked pH-touchy dextran nanoparticles were conveyed through microneedle skin cream containing 5-fluorouracil is utilized to treat basal cell carcinoma. The porousness of 5-fluorouracil was upgraded up to 4.5 occasions when the cream was applied on the skin treated with strong microneedles. Examined the conveyance of chemotherapeutic specialists tamoxifen and gemcitabine through microneedles for the therapy of malignant growth.

OCULAR DELIVERY [90]

Numerous back portion signs ready to be treated by focusing on drug conveyance. Iontophoresis was utilized to convey nanoparticles through the suprachoroidal space. Without iontophoresis, the particles were found to confine at the infusion site. When joined with microneedles over 30% of nanoparticles were conveyed to the back section of the eye.

HORMONE DELIVERY [91]

Insulin is a peptide chemical. The medicine is utilized to bring down the high glucose levels. Conveying insulin utilizing microneedle was found to bring down blood glucose levels all the more effectively. Created strong microneedles and examined the impact on blood glucose levels in diabetic mice on conveyance of insulin. The outcomes showed the diminished blood glucose level to 29% of the underlying level at 5 h which affirmed the further developed penetrability of insulin to the skin utilizing microneedle. In any case, the fix was not found to work adequately. These enhancers showed the emission of insulin from the β-cells cases. The aftereffects of clinical examination led for parathyroid chemical (I-34) covered microneedles showed the multiple times more limited Tmax and multiple times more limited obvious $T_{1/2}$ contrasted with regular infusion treatment. Furthermore, iontophoresis in mix with microneedles can likewise be investigated for conveyance of different chemicals.

LIDOCAINE DELIVERY [92]

Lidocaine is utilized for neighborhood sedation. Managing Lidocaine through microneedle causes less agony when contrasted with hypodermic infusion and accordingly shows better understanding consistence. These microneedles showed predictable in vitro skin entrance and upgraded conveyance of the medication in 2 min. Thus, microneedles can be utilized for torment free and fast neighborhood sedation. In one examination, microneedles covered with PEG-Lidocaine scatterings showed further developed medication conveyance inside 3 min contrasted with the skin detailing.

GENE DELIVERY [93]

The use of microneedles for gene delivery is another area that is being explored and preliminary research has shown some promising results. Employed dissolving protrusion array device (PAD) loaded with small interfering RNA (siRNA) agents for delivery through skin. Effective silencing of the reporter gene expression was reported by the authors in transgenic mouse model also demonstrated delivery of plasmid DNA (pDNA hydrogel formulations) to the epidermal cells of the human explants using silicon microneedles for genetic vaccination. Freshly excised human skin (viable epidermis) was used for diffusion and gene expression experiments. Light and fluorescent microscopy was used by the authors in this study to demonstrate the delivery of beta-galactosidase and non-viral gene vector through the micro channels into the viable epidermis.

CONCLUSION

The Transdermal drug delivery system is a technique that provides drug absorption through the skin. In this drug delivery system is involves drug diffused to distribute through different layers of the skin into systemic or blood circulation to provoke therapeutic effect. The drugs delivery system is mainly topically, where the drugs are absorbed by the skin and it will be penetrate through the bloodstream by using microneedles. It will provide a consistent delivery of drug in a small amount into the blood stream over a long period of time. The microneedle is also known as collagen induction therapy (CIT) that involves repeatedly puncturing the skin with small, sterilized needles. Currently, the system is not limited to topical application, and is tested for systemic delivery of drugs and biologics. Most of the studies have provided results in the favor of the system. The technology has the potential to provide therapeutic benefit in a variety of branches from dermatology to oncology. There has been encouraging research conducted on MNs, which has focused on profiling their safety concerns for elucidating potential medical/pharmaceutical use Attention has to be given in standardizing scalable methods of manufacturing of MNs. This will help in exploiting the system and associating technology to a greater extent.

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RESEARCH ARTICLE

Machine Learning based Sentiment Analysis for Twitter Social Networking Service

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ABSTRACT

Sentiment analysis context refers to the analytical and computational assessment of public user group opinion, school of thoughts, attitude, emotion, and viewpoint towards a particular content. Opinion mining is the proceeding in which opinions are categorized by positive, neutral, and negative used for interpreting the text or the words used to determine the author's attitude towards the context produced. Basically, it is the elucidation and classification of emotions. Bag of words is a method to extract features from text documents. In this method the collection of all the unique words that are turning up in the training set document are created as a vocabulary. The goal is to approximate the mapping function. So, from the splitting of the dataset and the features are used from the Bag-of-Words. Data analytics techniques are used to find the polarity and subjectivity of the gathered data. Further the sentiment is visualized as a word cloud and sentiment graph obtained in a web application. Mining the opinions of people is necessary in recent impact of social media sites because most of the conflicts arise from these social media platforms which can produce revolutionary changes that create societal impact. As a result, automating emotion analysis and summarization systems is required to overcome subjective biases and mental constraints to achieve the goal of sentiment analysis.

Keywords: Opinion mining, Sentiment analysis, Bag-of-words, Data analytics, social media

INTRODUCTION

Sentiment analysis is the ability to look through the subject matter and decide its polarity using the techniques of Data Science and AI. Human race evolution has seen many astonishing stages, one of the exciting fields of

astonishment in the current century is AI with rightful tools and packages and with python sentiment analysis can be delivered with better understanding of the sentiment of the text. Sentiment analysis is performed with python and various packages of it. Authentication APIs and python modules are used to connect with the respective social media site. Sentiment analysis of a selected social media account can be performed using APIs and Natural Language. Algorithm generates an overall sentiment score from the input data in terms of positive, negative, and neutral. The task is to analyze the posts of a social media account in terms of Subjectivity and Polarity. Positive, negative, neutral, and visualized posts are assigned to each other. Sentiment analysis is particularly useful for monitoring social media posts from afar, in addition to the number of likes and comments, and for providing qualitative opinions. The key feature in the sentiment analysis system is deploying it as an application which is user friendly. Using the streamlit which is an open-source library in python it is possible to create and share custom web pages for machine learning and data science. An add on feature is that the application is mobile friendly and completely responsive which means that the application works fine with all devices.

Literature Survey

Sentiment analysis and opinion mining used for evaluation of emotions and opinions from social media. Nowadays, social media have huge impact on the societal factors, so people tend to share their opinions on social networks like Facebook, Twitter, and LinkedIn, regarding many topics. The data can be analyzed with the following techniques,

Machine Learning methods

- ➢ Support Vector Machine (SVM)
- ➢ Naive Bayes (NB)
- ➢ Logistic Regression
- ➢ Multilayer perceptron (MLP)

These approaches build a model from a training dataset and a testing dataset to ensure that the model is optimized.

Lexicon-based methods: The lexicon-based technique infers the semantic orientation of words or phrases in the training set. Because sentiment values from the dictionary are allocated to the phrases in the paper, the technique is straightforward and effective. Text is assigned a polarity value using lexical features or lexicon. Senti Word Net is the most widely used lexicon in the domain of sentiment analysis. However, because of the various word senses, this approach may not produce the best results in particular fields [1]. To solve this issue, domain-dependent lexicons are created for the proposed system. To improve sentiment analysis performance, a technique based on several filters was developed. This system already has a specialized task such as misspelt detections and special character recognition. Furthermore, numerous alternative techniques have been presented that can manually filter text to retrieve useful information [3]. Finding irrelevant content by evaluating each word through numerous filters or manually verifying each sentence, on the other hand, is a time-consuming operation [6]. As a result, SVM-based data filtering, which divides data into relevant and irrelevant information classes, is an effective way to deal with the challenges.

Sentiment Analysis

Ontology Based Modelling: Opinion mining is an intriguing study issue, particularly in the field of microblogging. Even though many systems have been built to far, there is still a lot of potential for future research in this field. Prior sentiment analysis work on the material for customer reviews and sentiment analysis at the phrase level has been completed. To express the author's opinion on Twitter, the user is forced to condense the post within a word limit of 140 characters. Proper and formal comparisons between these outcomes acquired by numerous features and classification strategies are essential to identify the best features and most efficient classification procedures for applications. APIs are used to access information. In the realm of sentiment analysis, academics have used ontology and LDA in recent years. Subjects are extracted from online travel assessments using topic-based sentiment analysis. The main purpose of the current research system is to determine the most essential issues and emotion phrases that tourists have major concern. This topic analysis is used to capture human language to infer

patterns and unlock semantic forms from the training set to extract features that aid in data-driven decision-making. The scope of the topic-based analysis is divided into three levels: document, sentence, and sub-sentence. Topic-based sentiment analysis provides a qualitative insight into the total subjective matter, with topic tagging being the most important duty in efficiently analyzing the massive volume of data. An LDA-based ontology learning method was used to update the domain of a civil aviation system. An ontology and LDA-based topic modelling technique for transportation data classification was provided. Classic ontology, which is built on dealing with crisp and flawless logic and avoids dealing with ambiguous information, does not fit the transportation domain well enough. Non-crisp data, on the other hand, is difficult to represent. As a result, the ontology is supplied with LDA-based extracted data to create a fuzzy transportation ontology.

Classification

The suggested system's goal is to improve topic modelling, sentiment categorization, and representation performance. Various techniques, such as LDA, are used to represent words along with the most relevant subjects for opinion classification are Ontology and deep learning. Using the LDA technique, statistical correlations between topic terms and in massive sets of documents can be discovered. Traditional LDA, on the other hand, creates subjects that are incompatible with human thought. The LDA approach starts with a topic of high probability words and then moves on to low probability words. Transportation mining can also be used to effectively separate low likelihood terms. Furthermore, the LDA approach does not allow for the discovery of semantic links between words. As a result, ontology-based semantic knowledge is used to improve the LDA model and extract the subjects more precisely related to transportation.

Implementation

The following are the various stages involved in sentiment analysis of the social media opinions. The first step is the Gathering of training data. The second is the cleaning of the gathered data. Next is the Analyzing the data. The final stage is the visualizing of the optimized learning model.

Training dataset: Data is obtained in the form of raw posts using the Python package "tweepy," which is a twitter streaming API for twitter, "Facebook graph API" for Facebook, and additional dataset from Instagram and LinkedIn. Any filtering criteria can be specified by a programmer. The goal is to improve the generality of data, so it is collected in pieces at different times rather than all at once. If the alternative strategy is adopted, the posts' generality may have been reduced because a significant number of the posts would be referring to a certain trending issue and hence share the same general attitude or sentiment. This method yields a large amount of raw data, which may or may not be helpful for the application. It takes the form of a python "dictionary" data type with a variety of key-value pairs. A list of some key-value pairs is given below:

Whether a post from the social media has been favorited
User ID
Screen name of the user original text of the post
Geo-tag location of the post date and time when the post was created

Because there is such a large amount of data involved, only the information that is required is filtered, while the rest is deleted. Only the postings in the sample test are iterated and saved in a data frame using pandas in this investigation.

Data preprocessing

Data preprocessing consists of the following methods:
Tokenization
Stemming
Bag of words

Tokenization: It is the process of breaking down a stream of text into meaningful parts, words, and symbols known as "tokens." Tokens can be separated by whitespace and/or punctuation characters, and they are treated as separate parts that make up a whole post. Emoticons and abbreviations are identified as part of the tokenization process and treated as individual tokens.

Stemming: It is the process of reducing inflection words to their corresponding root, base, or stem, from which irreverent letters are usually eliminated as a suffix. To generate the word stem, disrespectful suffixes such as "ing," "ly," "es," "s," and others are removed.

Bag of words: The method of extracting features from text documents is known as feature extraction. This function can also be used to train machine learning algorithms. A vocabulary of all the unique words found in all the papers in the training set is constructed at the same time.
Each word in the lexicon has specific scores in terms of three different parameters

1) Polarity: negative vs. Positive (-1.0 => +1.0) 2)
2) Subjectivity: objective vs. Subjective (+0.0 => +1.0)
3) Intensity: modifies next word? (x0.5 => x2.0)

Modules and packages

Tweepy: Twitter API was used to get information from Twitter. A Twitter developer account is required for this. An access token and an access token secret are generated after an API key and API secret key are received. Tweepy makes an authentication call before calling the function to get the most recent tweets from the specified Twitter account.
Pandas: A data frame is created with specific columns using pandas in which the data is fitted in.
Regex: A *cleantx*t function is created which removes mentions, hashtags, retweets URLs and other noises using regular expressions.
TextBlob: To calculate the subjectivity and polarity of the posts TextBlob is used, these functions are applied to the Data Frame and two new features Subjectivity and Polarity are created in the data frame. By using *get Analysis ()* function the score of the tweets in the data frame are calculated and categorized as positive, negative, neutral by adding another feature called Score in the data frame.
matplotlib. pyplot: Using matplotlib. pyplot, the data is visualized as Positive, Negative, and Neutral. It keeps track of the current figure and plotting area, preserving multiple states across function calls.
Seaborn: Seaborn is used to show the counts of observations in each categorical bin using bars. And pandas objects are used because the associated names will be used to annotate the axes.
Word cloud: A word cloud is generated to see the themes and most common words used in the posts we are analyzing. The purpose of the package is to provide simple and quick insights by visualizing qualitative data.
Streamlit: Sentiment analysis system is customized as an application with the help of streamlit module with various UI.

Classified posts

The posts are divided into three categories based on the expressed/observed sentiments: favourable, negative, and neutral. In the labelling procedure, the following guidelines are followed:

Positive: If the entire post has a positive/happy/excited/emotion or if something is mentioned with positive annotations. Else if the sentence has positive sentiment as a major constituent. Example: "Tonight, I introduced the American Families Plan — an ambitious, once-in-a-generation investment to rebuild the middle class and invest in America's future."
Negative: If a negative/sad/displeased emotion pervades the entire post, or if something is referenced with negative annotations. Otherwise, if the text has a significant amount of unpleasant or bored attitude. Example: "America

won't back away from our commitment to human rights and fundamental freedoms. No responsible American president can remain silent when basic human rights are violated."

Neutral: If the posts express no personal sentiment and barely presents information. Example: "Madam Speaker. Madam Vice President. No president has ever said those words from this podium — and it's about time."

RESULTS AND DISCUSSION

The accuracy of the model earned about 93 % without involving much complexity by extracting more features. Screenshots for Twitter sentiment analysis of Mr.Donald Trump are shown below which classification of tweets and generated word cloud with graph visualization.

CONCLUSION

Sentiment Analysis is one of the fascinating applicability of Natural Language Processing which produces automated qualitative conclusions about the posts and texts Feeling automated is the current trend of the human race. The place of utilization of sentiment analysis includes social media trend analysis, marketing purposes and customer review texts. The use of social media sentiment analysis opens a slew of new possibilities. The ability to evaluate posts in real time and understand the sentiment that underpins each message gives social media monitoring a whole new meaning. This adds an additional layer to standard analytics, making it easier to analyze the performance of specific brands on social media and other platforms, resulting in powerful prospects for developing enterprises. Improvement of specific aspects what makes them stand out among the competitors in a business can be found using aspect-based sentiment analysis. Sentiment analysis tools can be connected directly to social platforms, so the posts out there can be monitored and analyzed as when they come in all time. The tasks of real time sentiment analysis in social platforms specifically in the domain of microblogging are still in the stage of progress and not in a completed form. The proposed system accuracy and predictability can be improved by adding more features like closeness of the sentence etc, Negation concepts associated with the texts can also be analyzed and classified in the upcoming days accurately. Failing to pay attention to the negation words may result in inconsistency and inappropriate polarity computation. Also, the effect of relative position of a word in a sentence is quite an interesting factor for improving the system performance further.

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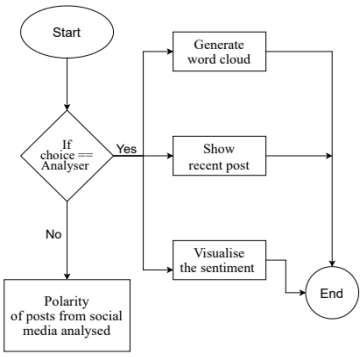

Fig 1: User Interface for sentiment analysis using streamlit

Fig 2: User Interface with Tweet Analyzer as a choice of activity

Fig 3: User Interface for generating twitter data

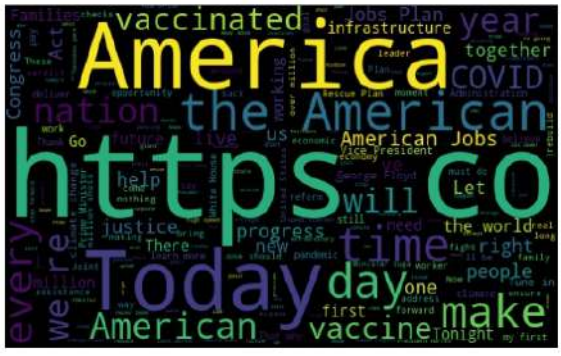

Fig 4: Visualizing sentiment as wordcloud

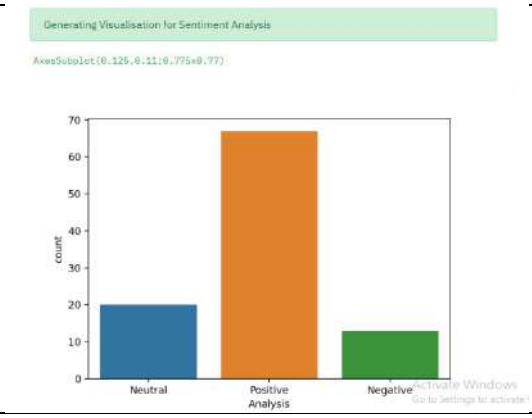

Fig 5: Visualizing sentiment as a graph

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RESEARCH ARTICLE

Computation of Weighted PI and Szeged Indices of Conical Graph

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ABSTRACT

The weighted (node) Padmakar-Ivan index and weighted (node) Szeged index are degree and distance based topological indices in which reflect certain structure feature of molecular graph. In this paper, we present explicit formulae for weighted Padmakar- Ivanindex and weighted Szeged index of conical graph.

Keywords: Conical graph, Weighted szeged index, Weighted Padmakar-Ivan index.
MSC: 05C12, 05C35

INTRODUCTION

Topological index of numerical values to determined from the connective patterns graph and used to distil and compress the material belonging that patterns. Using topological indices one can obtain the benefits from saving time and money to estimate basic physical chemical characteristics of the molecule, including density, critical pressures, evaporation enthalpy etc.. Several topological indices have been introduced by academics working in mathematical chemistry and in the so-called chemical graph theory and then used for the QSAR/QSPR research in which physicochemical characteristics of substances are linked with their molecular structure. Therefore, such indices are typically referred to as molecular descriptors [22]. Molecular descriptors (or Topological indices) are used in organic chemistry to represent physicochemical, pharmacologic, toxicological, biological, and other aspects of chemical compounds [8] even some of them have been shown to be effective in other areas where connectivity patterns are significant, such as inter-processor connections and communication. The Wiener index are among the most intensely researched topological indices and due to its many applications which is considered to be one of very important, it's a distance-based

$$W(G)=\sum_{(u,v)\in V(G)} d(u,v).$$

There are some net-worthy classes of topological indices are exist in the literature. These indices can be classified according to the structural characteristics of the graph such as, the Wienerindex, Harray index which are based on the distance, the Zagreb index, Randic index which are based on degree, the Estrada index, second Mohar index which are based on the spectrum of a graph, the Hosaya index which is based on the matching. Apart from these, (Vertex) node-additive indices and distance-based indices are two more intriguing classes of indices that aim to capture certain significant features of entire graphs by adding contributions from particular nodes (vertices) and/or edges. There has been a lot of attention paid to chemical graph theory, both in the setting of complex networks and in more conventional applications. Some of the familiar bond-additive distance-based indices like Szeged index put forward by Gutman [7] and Padmakar-Ivan (PI) index introduced by Khalifeh et al. [13], as well as a global measure of peripherality of a particular graph, which is defined as

$$Sz(G)=\sum_{e=uv\in E(G)} n_e^G(u)\,n_e^G(v)$$

$$PI_v(G)=\sum_{e=uv\in E(G)} \left(n_e^G(u)+n_e^G(v)\right)$$

Where $n_e^G(u)$ denotes the number of nodes(vertices) of G closer to u than to v and $n_e^G(v)$ is similar way. After the introduction of Szeged and PI index which are attracted much attention in the mathematical chemistry community. Nowadays there is a vast literature presenting scientific researches deeply related to the Szeged and PI index (e.g. for some recent results see [1, 3, 5, 10, 12] and references cited therein). "Inspired by an extension of the Wiener index, Ili˘c and Milosavljevi´c proposed a" modification of the Szeged index and the vertex PI index" [9]. Defined as this quantatity is named as weighted Szeged index and weighted PI index,

$$PI_w(G)=\sum_{e=uv\in E(G)} \left(\deg_e^G(u)+\deg_e^G(v)\right)\left(n_e^G(u)+n_e^G(v)\right)$$

$$Sz_w(G)=\sum_{e=uv\in E(G)} \left(\deg_e^G(u)+\deg_e^G(v)\right)\left(n_e^G(u)+n_e^G(v)\right)$$

Many mathematical findings are known for these indices as a consequence of considerable research. Recently Tratnik [21], "determine the weighted szeged and PI indices of quotientgraphs". For a list of current and pertinent studies, see [6, 17, 15, 18, 19, 20] Chemical graph theory relies heavily on graph operations. By applying graph operations to some general or specialised graphs, different molecular graphs may be created. Taking the linear polynomial chain as an example, nanotube, nanotorus, tetrameric 1,3-adamantane,truncated cube etc..

The various graph operations must thus be studied in order to understand how they are connected with the topological index of their source graphs. Several papers were produced and dealing with" weightedszeged and PI indices on graph operation and for various molecular graphs have appeared" [14]. More results on various topological indices under different graph operations like Cartesian product, Corona, Tensor and Hierarchical product, etc. see[6, 16, 18]. Recently, Gopika et al.[6] obtained "weighted PI index of tensor product and strong product of graphs". We consider throughout the text that G is a simply un directed connecting graph with node and edge sets, $V(G)$ and $E(G)$, respectively. A simple graph the distance between nodes u and v of G is denoted $d(u,v)$, the minimal distance connecting them the two nodes of graph and the degree of the node $u\in V(G)$ is the number of edges incident with u denoted by $\deg_e^G(u)$ or simply $\deg^G(u)$.

In 2020, Ayache et al.[4] recent asked to a graph called the Conical graph (Generalized wheel graph) that consists of focus u_0 and (l,k)-Cycles $C_k^1, C_k^2, ..., C_k^l$ interposed" as it's illustrated in Figure 1 given below, and denoted by

$G(l,k) = C(l,k)$. In his paper weinerindex and its polynomial are obtained for conical graph. Then Alameri et al.[2],calculated Zagerb, F-index and its related indices of conical graph. Motivated by this structure, Kandan and Subramanian [11] recently obtained the explicit formula of bond additive indices like PI, szeged and Mostar index to the conical graph.

Definition 1.1 [11]. The Conical graph $C(l,k)$ is a graph which is obtained by taking adjacency from a centre vertex u_0 to the first layer of the Cartesian product of C_k and P_l with $l \geq 1$ and $k \geq 3$.(see Figure 1.)

Let node set of $C(l,k)$ can be written as

$$V(C) = \left\{u_0, u_1^1, u_2^1, ..., u_k^1, u_1^2, u_2^2, ..., u_k^2, ..., u_1^l, u_2^l, ...u_k^l\right\}$$

and for the edges set of $C(l,k)$ into four sets such that $E(C) = \bigcup_{n=0}^{3} E_n(C)$, where

$$E_0(C) = \left\{u_0u_1^1, u_0u_2^1, ...u_0u_k^1\right\},$$

$$|E_0| = k, E_1(C) = \left\{u_1^{l-1}u_1^l, u_1^{l-1}u_2^l, ...u_1^{l-1}u_k^{l-1}u_1^{l-1}u_1^l, u_k^l\right\},$$

$$|E_1| = k. E_2(C) = \left\{u_1^lu_2^l, u_2^lu_3^l, ..., u_k^lu_1^l\right\},$$

$$|E_2| = k, E_3(C) = E^+(C) \cup E'(C) \cup E^*(C),$$

Where

$$E^+(C) = \left\{u_1^1u_2^1, ..., u_k^1u_1^1\right\},$$

$$E'(C) = \left\{u_1^ju_2^j, u_2^ju_3^j, ..., u_k^ju_1^j \mid j = 2,3,....,l-1\right\},$$

$$E^*(C) = \left\{u_1^ju_1^{j+1}, u_2^ju_2^{j+1}, ..., u_k^ju_k^{j+1} \mid j = 1,2,3,....,l-2\right\},$$

$$|E_3(C)| = k + k(l-2) + k(l-2).$$

It's clear for a Conical graph $C(l,k)$, we have

$$|V(C(l,k))| = kl+1, |E(C(l,k))| = 2kl.$$

Observe that

$$\deg^C(u_0) = k, \deg^C(u_i^j) = 4,$$

if $i = 1,2,3,....,k$ any $j = 1,2,3,...,l-1, \deg^C(u_i^l) = 3$, if $i = 1,2,3,...,k$.

MAIN RESULTS

The primary goal of this research is to get the explicit form of the computed formulae for the conical graph of the P-I weighted and Szeged weighted.

Conical Graph

The following lemma, which is first noticed by [11], is important to the main results of the paper. The proof of the Lemma follows easily from the above defined edge partitions and structure of the conical graph $C(l,k)$.

LEMMA 2.1 [11]. For a conical graph $C(l,k)$, with $l \geq 1$ and $k \geq 4$, we have

(i) if $e = u_0 u_i^1 \in E_0(C)$, then $n_e^C(u_0) = l(k-3)+1$ and $n_e^C(u_i^1) = l$, for $i = 1,2,3,...,k$ **(ii)** if $e = u_i^{l-1} u_i^1 \in E_1(C)$, then $n_e^C(u_i^{l-1}) = (kl+1)-k$ and $n_e^C(u_i^l) = k$, for $i = 1,2,3,...,k$ **(iii)** if $e = u_i^l u_{i+1}^l \in E_2(C)$, for $i = 1(=k+1),2,....k$ then,

(a) if $n_e^C(u_i^l) = \frac{kl}{2} = n_e^C(u_{i+1}^l)$, for k is even **(b)** if $n_e^C(u_i^l) = \frac{(k-1)l}{2} = n_e^C(u_{i+1}^l)$, for k is odd **(iv)(a)** for $i = (=k+1),2,....,k$, if $e = u_i^1 u_{i+1}^1 \in E^+(C)$, then $n_e^C(u_i^l) = 2l = n_e^C(u_{i+1}^l)$.

(b) for $j = 2,3,....,l-1$ and $i = 1(=k+1),2,3,...,k$, if $e = u_i^j u_{i+1}^j \in E'(C)$, then

(i) if $n_e^C(u_i^j) = \frac{kl}{2} = n_e^C(u_{i+1}^j)$, k is even

(ii) if $n_e^C(u_i^j) = \frac{(k-1)l}{2} = n_e^C(u_{i+1}^j)$, k is odd

(c) for $j = 1,2,3,...,l-2$ and $i = 1,2,...,k$, if $e = u_i^j u_i^{j+1} \in E^*(C)$, then

$n_e^C(u_i^j) = \sum_{j=1}^{l-2}(jk+1)$ and $n_e^C(u_i^{j+1}) = \sum_{j=1}^{l-2}(l-j)k$. $C(l,k)$.

THEOREM 2.1. For a conical graph $C(l,k)$ with $l \geq 1$ and $k \geq 4$, we have

$$PI_w(C(l,k)) = \begin{cases} k(k+4)(l(k-2)+1)+(kl+1)(8l-9) \\ +2kl(k(4l-5)+16) & \text{if } k \text{ is even} \\ k((k+4)(l(k-2)+1)(kl+1)(8l-9)) \\ +2kl(16+(k-1)(4l-5)) & \text{if } k \text{ is odd} \end{cases}$$

By the definition of weighted Padmakar-Ivan index, to obtain it for the conical graph $C(l,k)$, we have $PI_w(C(l,k)) = \sum_{e=uv \in E(G)} (\deg_e^C(u) + \deg_e^C(v))(n_e^C(u) + n_e^C(v))$.

Using the edge partition E_0, E_1, E_2 and E_3 of the conical graph $C(l,k)$, as defined in the introduction and by the Lemma 2.1. We have the following four cases,

Case (i): For $i = 1,2,3,....,k$, if $e = u_0 u_i^1 \in E_0(C)$, then

$$\sum_{e \in E_0(G)} (\deg_e^C(u_0) + \deg_e^C(u_i^1))(n_e^C(u_0) + n_e^C(u_i^1)) = k(k+4)(l(k-2)+1).$$

Case (ii): For $i = 1,2,3,....,k$, if $e = u_i^{l-1} u_i^l \in E_1(C)$, then

$$\sum_{e \in E_1(G)} (\deg_e^C(u_i^{l-1}) + \deg_e^C(u_i^l))(n_e^C(u_i^{l-1}) + n_e^C(u_i^l)) = 7k(kl+1)$$

Case(iii): For $i = 1(=k+1),2,...,k$, if $e = u_i^l u_{i+1}^l \in E_2(C)$, then

$$\sum_{e\in E_2(G)}\left(\deg_e^C\left(u_i^l\right)+\deg_e^C\left(u_{i+1}^l\right)\right)\left(n_e^C\left(u_i^l\right)+n_e^C\left(u_{i+1}^l\right)\right)$$

$$=6k\begin{cases} kl & if\ k\ is\ even \\ (k-1)l & if\ k\ is\ odd \end{cases}$$

Case(iv): For $e\in E_3(C)=E^+(C)\cup E'(C)\cup E^*(C)$, then the three sub-cases are

sub-case(a): For $i=1(=k+1),2,3,....,k$, if $e=u_i^1u_{i+1}^1\in E^+(C)$, then

$$\sum_{e\in E^+(G)}\left(\deg_e^C\left(u_i^1\right)+\deg_e^C\left(u_{i+1}^1\right)\right)\left(n_e^C\left(u_i^1\right)+n_e^C\left(u_{i+1}^1\right)\right)=32kl$$

sub-case (b): For $i=1(=k+1),2,...,k$ and $j=2,3,....,l-1$, if $e=u_i^ju_{i+1}^j\in E'(C)$, then

$$\sum_{e\in E'(G)}\left(\deg_e^C\left(u_i^j\right)+\deg_e^C\left(u_{i+1}^j\right)\right)\left(n_e^C\left(u_i^j\right)+n_e^C\left(u_{i+1}^j\right)\right)$$

$$=8k(l-2)\begin{cases} kl & if\ k\ is\ even \\ (k-1)l & if\ k\ is\ odd \end{cases}$$

sub-case(c): For $i=1,2,3,....,k$ and $j=1,2,3,....l-2$, if $e=u_i^ju_i^{j+1}\in E^*(C)$, then

$$\sum_{e\in E^*(G)}\left(\deg_e^C\left(u_i^j\right)+\deg_e^C\left(u_i^{j+1}\right)\right)\left(n_e^C\left(u_i^j\right)+n_e^C\left(u_i^{j+1}\right)\right)=8k(l-2)(kl+1)$$

By summing the above four cases, we have the explicit formula of weighted Padmakar-Ivan index to the conical graph $C(l,k)$ is

$$PI_w(C(l,k))=PI_w(E_0(C))+PI_w(E_1(C))+PI_w(E_2(C))+PI_w(E_3(C))$$

Case (i): For k is even

$$PI_w(C(l,k))=k(k+4)(l(k-2)+1)+7k(kl+1)+6k^2l+32kl+8k^2(l-2)l+8k(l-2)(kl+1)$$

$$=k\left((k+4)(l(k-2)+1)+(kl+1)(8l-9)+2kl(k(4l-5)+16)\right)$$

Case (ii): For k is odd

$$PI_w(C(l,k))=k(k+4)(l(k-2)+1)+7k(kl+1)+6kl(k-1)+32kl+8kl(l-2)(k-1)+8k(l-2)(kl+1)$$

$$=k\left((k+4)(l(k-2)+1)+(kl+1)(8l-9)\right)+2kl\left(16+(k-1)(4l-5)\right)$$

Observe that the weighted Padmakar-Ivan index can be expressed interms of Padmakar- Ivan index of the conical graph $C(l,k)$ as follow

$$PI_w(C(l,k))=(k+4)PI(E_0(C))+7PI(E_1(C))+6PI(E_2(C))+8PI(E^+(C))+8PI(E'(C))+8PI(E^*(C))$$

Using the above Theorem 2.1, we have following corollary.

COROLLARY 2.1 [19]. For $l=1$ and $k\geq 4$, the wheel graph W_k whose weighted Padmakar-Ivan index $PI_w(W_k)=k^3+2k^2+21k$.

Next we determine the explicit formula of weighted Szeged index to the conical graph $C(l,k)$.

THEOREM 2.2. For a conical graph $C(l,k)$, with $l \geq 1$ and $k \geq 4$, we have

$$Sz_w(C(l,k)) = \begin{cases} k\left(\left(l(k+4)\left(l(k-3)+1\right)\right)7k\left(k(l-1)+1\right)\right. \\ \left.+kl^2\left(32+\left(2l-\frac{5}{2}\right)k^2\right)\right) \\ +8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{l-1}{2}\left(\frac{k(2l-3)}{3}+1\right)\right) & \text{if } k \text{ is even} \\ k\left(l(k+4)\left(l(k-3)+1\right)+7k\left(k(l-1)+1\right)\right) \\ +\left(kl^2\left(32+\left(2l-\frac{5}{2}\right)(k-1)^2\right)\right) \\ +8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{l-1}{2}\left(\frac{k(2l-3)}{3}+1\right)\right) & \text{if } k \text{ is odd} \end{cases}$$

By the definition of weighted Szeged index, to obtained it for the conical graph $C(l,k)$, we have $Sz_w(C(l,k)) = \sum_{e=uv \in E(C)} \left(\deg_e^C(u) + \deg_e^C(v)\right) n_e^C(u) n_e^C(v)$.

Using the edge partition E_0, E_1, E_2 and E_3, of the conical graph $C(l,k)$ as defined in the introduction and by the Lemma 2.1, we have the following four cases.

Case (i): For $i = 1,2,3,\ldots,k$, if $e = u_0u_i^1 \in E_0(C)$, then

$$\sum_{e \in E_0(C)} \left(\deg_e^C(u_0) + \deg_e^C(u_i^1)\right) n_e^C(u_0) n_e^C(u_i^1) = kl(k+4)\left(l(k-3)+1\right).$$

Case(ii): For $i = 1,2,3,\ldots,k$, if $e = u_i^{l-1}u_i^l \in E_1(C)$, then

$$\sum_{e \in E_1(C)} \left(\deg_e^C(u_i^{l-1}) + \deg_e^C(u_i^l)\right) n_e^C(u_i^{l-1}) n_e^C(u_i^l) = 7k^2\left(k(l-1)+1\right)$$

Case(iii): For $i = 1(=k+1), 2\ldots,k$, if $e = u_i^l u_{i+1}^l \in E_2(C)$, then

$$\sum_{e \in E_2(C)} \left(\deg_e^C(u_i^l) + \deg_e^C(u_{i+1}^l)\right) n_e^C(u_i^l) n_e^C(u_{i+1}^l) = 3k \begin{cases} \frac{k^2l^2}{2} & \text{if } k \text{ is even} \\ \frac{(k-1)^2l^2}{2} & \text{if } k \text{ is odd} \end{cases}$$

Case(iv): For $e \in E_3(C) = E^+(C) \cup E'(C) \cup E^*(C)$, then the three sub-cases are

sub-case(a): For $i = 1(=k+1), 2, 3, \ldots, k$, if $e = u_i^1u_{i+1}^1 \in E^+(C)$, then

$$\sum_{e \in E^+(C)} \left(\deg_e^C(u_i^1) + \deg_e^C(u_{i+1}^1)\right) n_e^C(u_i^1) n_e^C(u_{i+1}^1) = 32kl^2$$

Sub-Case(b): For $i = 1(=k+1), 2,3,\ldots,k$ and $j = 2,3,4,\ldots,l-1$, if $e = u_i^j u_{i+1}^j \in E'(C)$, then

$$\sum_{e\in E'(C)}\left(\deg_e^C\left(u_i^j\right)+\deg_e^C\left(u_{i+1}^j\right)\right)n_e^C\left(u_i^j\right)n_e^C\left(u_{i+1}^j\right)$$

$$=2k(l-2)\begin{cases}k^2l^2 & if\ k\ is\ even\\ (k-1)^2l^2 & if\ k\ is\ odd\end{cases}$$

Sub-Case(c): For $i=1,2,3,\ldots,k$ and $j=1,2,3,\ldots,l-2$,if $e=u_i^ju_i^{j+1}\in E^*(C)$, then

$$\sum_{e\in E^*(C)}\left(\deg_e^C\left(u_i^j\right)+\deg_e^C\left(u_i^{j+1}\right)\right)n_e^C\left(u_i^j\right)n_e^C\left(u_i^{j+1}\right)$$

$$=\sum_{e\in E^*(C)}8\left(jkl-j^2k+l-j\right)k=8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{(l-1)}{2}\left(\frac{k(2l-3)}{3}+1\right)\right)$$

By summing the above four cases, we have the explicit formula of weighted Szeged index to the conical graph $C(l,k)$ is

Case(i): For k is even

$$Sz_w\left(C(l,k)\right)$$

$$=kl(k+4)(l(k-3)+1)+7k^2(k(l-1)+1)+\frac{3l^2k^3}{2}+32kl^2+2(l-2)k^3l^2$$

$$+8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{(l-1)}{2}\left(\frac{k(2l-3)}{3}+1\right)\right)$$

$$=kl(k+4)(l(k-3)+1)+7k^2(k(l-1)+1)+3kl^2\frac{(k-1)^2}{2}+32kl^2+2(l-2)k^3l^2$$

$$+8k^2\left(l-2\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{l-1}{2}\left(\frac{k(2l-3)}{3}\right)+1\right)\right)$$

Case(ii): For k is odd

$$Sz_w\left(C(l,k)\right)$$

$$=k\left((k+4)\left(l\left(l(k-3)+1\right)\right)7k\left(k(l-1)+1\right)\right)+3kl^2\frac{(k-1)^2}{2}$$

$$+32kl^2+2(l-2)k\left((k-1)l\right)^2$$

$$+8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{l-1}{2}\left(\frac{k(2l-3)}{3}+1\right)\right)$$

$$=k\left(l(k+4)\left(l(k-3)+1\right)+7k\left(k(l-1)+1\right)\right)$$

$$+kl^2\left(32+\left(2l-\frac{5}{2}\right)(k-1)^2\right)+8k^2(l-2)\left(l\left(\frac{k(l-1)}{2}+1\right)-\frac{l-1}{2}\left(\frac{k(2l-3)}{3}+1\right)\right)$$

Observe that the weighted Szeged index can be expressed in terms of Szeged index for the conical graph $C(l,k)$ as follow:

$$Sz_w(C(l,k))$$
$$=(k+4)Sz(E_0(C))+7Sz(E_1(C))+6Sz(E_2(C))$$
$$+8Sz(E^+(C))+8Sz(E'(C))+8Sz(E^*(C)).$$

Using the Theorem 2.2 above, we can get the following conclusion.

COROLLARY 2.2[17]. For $l=1$ and $k\geq 4$ the wheel graph W_k whose weighted Szeged index $Sz_w(W_k)=k^3+k^2+18k$.

CONCLUSION

In this paper, we have evaluated the exact formula of vertex weighted version of Padmakar-Ivan and Szeged indices of Conical graph. Our exploration kept on determining new consequences of these graphs. In order to shed light on the relationship between these diverse ideas, several topological indices were given emphasis on the mathematical side.

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REVIEW ARTICLE

Vitamin E on Endometrial and Cervical Cancers: A Mini Review

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ABSTRACT

Vitamin E is a well-known antioxidant agent that comprises of two essential compounds, tocopherols(TOC) and tocotrienols (TCT). Each of these compounds is present in four subtypes, which are in the alpha (α), beta (β), delta (δ) and gamma (γ) forms. Vitamin E is widely reported to exhibit the anticancer action through multiple mechanisms. Although studies have been conducted to study in detail on the anticancer activity of vitamin E, there are still limited studies reported on particular types of cancer such as the endometrial and cervical cancers. Hence, this paper was written to summarize on the reported effects of vitamin E on the endometrial and cervical cancers from the available publications. This paper is anticipated to provide a reference for future researches on vitamin E and endometrial & cervical cancers.

Keywords: Vitamin E, tocopherols, tocotrienols, endometrial cancer, cervical cancer

INTRODUCTION

Vitamin E, a fat-soluble vitamin, is well-known for its numerous beneficial roles that are important to preserve the normal physiological function of the human body. Specifically, the knowledge and understanding on the mechanisms of action by vitamin E on cancer cells are essential in order to find a possible alternative of anticancer approach. Thus, progressive researches are being conducted to study the effect of vitamin E on cancers. Despite many reported studies available on vitamin E and cancers, the published articles are considerably limited on a certain types of cancers, including the endometrial and cervical cancers. Various reported effects of vitamin E on different cancer types suggested that vitamin E possesses an anticancer effect against the endometrial and cervical cancers, but the exact mechanisms of actions remain unclear and require further studies. Hence, this paper was

written with aims to provide a review on vitamin E effect on the endometrial and cervical cancers on the reported studies between the years of 2010 – 2020. This paper is aimed to contribute to more organised findings on the effects of vitamin E on endometrial and cervical cancers.

Vitamin E and Endometrial & Cervical Cancers

In all phases of the oncogenic progressions, oxidative stress has been reported to be the main culprit that causes the process of initiation, promotion, angiogenesis and invasion. Therefore, the reduction in oxidative stress is often targeted as a useful treatment strategy for cancer prevention. Despite its antioxidant function, vitamin E has been widely reported to modulate anticancer action against the cancer cells [1], which involves multiple mechanisms.

Anticancer Effect of Vitamin E on Endometrial Cancer

As vitamin E is an antioxidant that also possesses the anticancer property, the available reports on the effect of vitamin E on endometrial cancer published between the years of 2010-2020 were reviewed. However, the retrieved results showed that the reported studies on vitamin E and endometrial cancer are limited [2-6].For instance, a major study on the alpha-tocopherol transfer protein (αTTP) and Ishikawa endometrial cancer cells found that αTTP possibly exerted the protective effect against endometrial cancer cells progressions and replication[2]. In this study, the Ishikawa endometrial cancer cells were treated with (2-amidinopropane) dihydrochloride (AAPH) and l-buthionine-(S, R)-sulfoximine (BSO) to mimic the oxidative condition. AAPH functions to induce oxidative stress by generating free radicals in the cultured media, while BSO halts the major cellular antioxidant system through the inhibition of the *de-novo* glutathione synthesis. Findings from this study found that both AAPH and BSO were found to induce αTTP expression in Ishikawa endometrial cancer cells [2], suggesting that endometrial cells up-regulated the expression of αTTP in the attempt to protect from oxidative stress. This study highlighted the role of vitamin E supplementation that could potentially effective in treating endometrial cancer. Other published studies on vitamin E and endometrial cancer are as summarized in Table 1.

Anticancer Effect of Vitamin E on Cervical Cancer

Several studies have been conducted to study the anticancer effect of vitamin E on cervical cancer. These include the *in-vitro*, clinical, and epidemiological studies. The findings of these studies demonstrated that vitamin E mainly acts through the induction of apoptosis. The epidemiological studies also showing similarities of the findings, which are summarised in Table 2. Based on the reported *in-vitro* and *in-vivo* studies as shown in Table 2, most of the studies indicated that the molecular mechanisms used by vitamin E are mainly the inhibition of cervical cancer cells proliferations and apoptosis initiation. For example, gamma-TCT was shown to induce apoptosis in human cervical cancer HeLa cells [7] through the down regulation of the proliferative cell nuclear antigen (PCNA) and *Bcl-2*, upregulation of *Bax*, cytochrome release from mitochondria. It also showed to activate caspase-9 and caspase-3 as well as the poly (ADP-ribose) polymerase (PARP) cleavage, where the authors reported on demonstrating the ability of gamma-TCT to induce apoptosis through the mitochondrial apoptotic pathway [7].

The initiation of apoptosis was also reported by [8], in which alpha-TCT and gamma-TCT arrested the cell cycle at G2/M phase and induced apoptosis to inhibit the HeLa cells proliferations. The mechanisms used were possibly the upregulation of the IL-6. IL-6 is one of the pro-inflammatory cytokines that plays an important role in cervical cancer pathogenesis. Therefore, the upregulation of IL-6 is possibly related to the action of alpha-TCT and the gamma-TCT in inducing apoptosis [19]. However, both showed no effect on the IFN-gamma, IL-2 and IL-10 [8]. The role of TCT in inducing apoptosis in tumour cells lacking the ER-β by triggering the signals associated with endoplasmic reticulum (ER) was reported by [9]. The study findings showed that the administration of γ-TCT and δ-TCT may associate with Ca^{2+} release. The release of calcium from ER to cytoplasm confirms the role of ER in apoptotic pathway. This was further confirmed when the TCT administration increased the caspase-12 and caspase-8 activity after 24-hour administration. Besides, TCT also regulated specific gene expression related to the ER, including the modulation of the *XBP-1*, *CHOP* mRNA expression and *IRE-1*, which lead to apoptosis [9].

Another study on the human cervical cancer cell line, the CaSki cells, by administering palm vitamin E, gamma-TCT, and alpha-TCT was also reported [10]. This study finding demonstrated that compared to alpha-TOC, gamma-TCT exhibited the anti-proliferative effect through suppression of the of cervical cancer cells proliferation at a significant level [10]. Meanwhile, apoptosis was induced through the decrease in the protein expression of MEK-2 and ERK-2 at 12-hours and 18-hours of gamma-TCT administration suggesting that the anti-proliferative effect of gamma-TCT might involve alteration of the proliferative signalling cascade [10]. These findings also correlate with the author's previous study on the palm oil vitamin E, gamma-TCT, and alpha-TCT in exhibiting the anti-proliferative effect through selective induction of apoptosis in CaSki cell [20].

CONCLUSION

The understanding on the mechanisms of action by vitamin E in both endometrial and cervical cancers is essential to identify its anticancer potential. The information obtained from the reported findings should be used to establish research strategies targeting the eradication of endometrial and cervical cancer cells. For that, many future studies should be conducted to study vitamin E's anticancer action in these types of cancers.

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Table 1: Reported studies of vitamin E in endometrial cancer (between 2010- 2020)

Type of Vitamin	Results	References
Alpha-TOC	Alpha-TOC concentration decreased to a differently in endometrial cancer patients underwent open laparotomy (LT), laparoscopic (LS) or robot-assisted surgery (RS)	[3]
Dietary vitamin E and supplementation	The risk of endometrial cancer in a population in Canada was found to reduce when vitamin E intake from food and supplementation was high.	[4]
Vitamin E supplement	There is no association between the intakes of vitamin E from food or supplement with the risk of endometrial cancer.	[5]
Dietary vitamin E and supplementation	Data of 669 invasive adenocarcinoma cases analyzed from the prospective Nurses' Health Study from 1980 to 2006found no association between intakes of dietary or supplements vitamin E or carotenoids with the endometrial cancer risk.	[6]

Table 2: Reported studies of vitamin E in cervical cancer (between 2010- 2020)

No.	Type of Vitamin E	Mechanism of Action	References
1.	Gamma-TCT	Gamma-TCT significantly inhibited cell proliferation through G0/G1 cell cycle arrest, and induced apoptosis via the mitochondrial apoptotic pathway in human cervical cancer HeLa cells.	[7]
2.	Alpha- and gamma-TCT	Alpha-TCT and the gamma-TCT inhibited the HeLa cell proliferation through the upregulation of the IL-6, downregulation ofcyclinD3, p16 and CDK6 expression.	[8]
3.	TCT	Delta- and gamma-TCT stimulates the apoptosis pathway in tumour cells that lack the ER-β by triggering the signals associated with endoplasmic reticulum.	[9]
4.	Palm Oil Gamma-TCT	Palm oil gamma-TCT induced apoptosis and exerted the anti-proliferative effect in CaSki cells through the decrease intheMEK-2andERK-2proteinexpression.	[10]
5.	Alpha-Tocopherol	Study using the Roasted Pili Nut Oil (RPNO) (known to contain a higher amount of vitamin E and less β-carotene compared to Unroasted Pili Nut Oil (UPNO)). The *in-vitro* cytotoxicity test indicated that both RPNO and UPNO did not affect against HeLa cervical cancer cells.	[11]
6.	Vitamin E(serum)550–1700 μg/dL.	Study found a significant reduction in the antioxidant level of vitamin E in the carcinoma cervical cancer patientsduring chemo radiotherapy and immediately after the brachy therapy.	[12]
7.	Dietary vitamin E	Study results indicated that in Chinese women, antioxidant vitamins including vitamin E could potentially reduce the risk of invasive cervical cancer, particularly in passive smokers.	[13]
9.	Dietary vitamin E (meta-analysis)	In an overview, there were preventive effects of vitamins or antioxidant intake on cervical neoplasms such as cervical intraepithelial neoplasia (CIN) and invasive cervical cancer.	[14]
10.	Dietary vitamin E(meta-analysis)	There was an inverse relationship between vitamin E and the risk for cervical neoplasia.	[15]
11.	TOC	There is an inverse relationship between the concentration of TOC and the risk for cervical cancer among Chinese women.	[16]
12.	Dietary vitamin E	• This was a prospective study of 299,649 women participating in the European Prospective Investigation into Cancer and Nutrition study. • There was no significant inverse association found between vitamin E and the incidence of carcinoma *in situ* (CIS) and invasive squamous cervical cancer (ISC)	[17]
13.	Dietary vitamin E	The higher serum concentration of alpha-and gamma-TOCs has inverse association with the risk for cervical invasive neoplasm grade 3 (CIN3) among low-income Brazilian women.	[18]

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REVIEW ARTICLE

A Review on Misuse of Over the Counter Drugs and Prescription Drugs

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ABSTRACT

Substances taken for nonmedical reasons, usually for their mind-altering effects, are called drugs of abuse. The use of psychoactive plants as drugs of abuse has had a long tradition. Most commonly abused drugs extracted from or based on natural products are illicit substances, such as cannabis products, morphine, or cocaine, but other herbal products used to produce a "high" are becoming increasingly popular drugs of abuse. Unfortunately, these "new herbal drugs" are falsely labelled as safe and legal. Health care professionals must be cognizant of this emerging problem as increased media coverage and marketing have made these products accessible and recognizable to many young adults and teenagers. This article gives a brief view of some herbal drugs of abuse, and their current trends of use.

Keywords: no recipe, behind dtecounter, self-medication

INTRODUCTION [1-8]

Medicines obtained from patients for the treatment of common diseases, without a doctor's prescription, are known as Surversay (OTC) or prescription drugs. OTC drugs provide prevention and treatment for a wide range of conditions, including, among others, headaches, common cold, musculoskeletal pain, allergies, tobacco dependence, and stomach acidity. However, there is always a risk involved in the use of OTC drugs. These include inadequate self-diagnosis, inadequate dose, prolonged addiction problems, adverse reactions, and medicine interactions, since most patients do not discuss their bench-top drugs with a doctor, they are not aware. OTC Drug abuse for this review is defined as the use of drugs not subject to prescription for non-material purposes. Abuse is often intentional, unlike the abuse of bench-top drugs, which can be the drug used for medical purposes, but in an

incorrect way used, for example, lack of knowledge of interactions, inappropriate use of drugs, and the incorrect duration of use.

AIM AND OBJECTIVES

Drugs, auto destos The sale of drugs on pharmacies' sober (OTC) drugs can help people's self-management symptoms. However, some OTC drugs can be abused, with dependence and damage becoming more recognized.

Places in use [9-13]

People use potentially addictive prescription or OTC drugs in the following ways:

- As an additional drug to use when DOC is not available on the streets.
- How reinforcement for intense high.
- They tend to be younger (when the stimulants are doc).
- They tend to use opiates.
- They tend to use prescription and OTC medications in combination with alcohol as a vehicle for suicide.

Buse drug During the Covid19 pandemic [14-23]

The Covid19 epidemic has questioned public health policies due to additional concerns about drug addicts and people with foam. Individuals of this vulnerable category could be exposed to additional risks, such as physical problems without fissa dwelling; imprisonment; Price increases for consumers in the black market; and purity reductions. These problems, in combination with a general economic loss, can promote changes to more risky drug behavior, such as:

- use of substances produced at national level;
- The use of prescription/drugs OTC;
- Mix with less expensive drugs and synthetic cannabinoids.

Access to drugged services is interrupted by quarantine, social spacing and other restrictive measures adopted to stop the propagation of Covid19.

Partmacists Document on drug prevention, education and assistance of drug abuse [24-27]

Since multiple users revolve from drugs gradually prescribed / OTC products, pharmacists must increase their surveillance by providing drugs and be both to both potential medicines In the black market.

Pharmaceuticals must participate in open communication to provide tranquility to patients and develop a relationship of trust, especially in vulnerable populations that could be less confident to communicate the entertainment and improper use of health professionals. Pharmaceuticals can help identify patients who can have problems related to substance abuse, and will send them to the appropriate service .

Adolescents and Young Adults [86,88-92]

Prescription drug abuse is highest among 1825-year-olds, with 14.4% reporting non-medical use in the past year. Among adolescents between the ages of 12 and 17, 4.9% said they had not taken any prescription drugs in the past year. The NIDA's Future Survey on Drug Use and Attitudes for Adolescents found that about 6% of high school graduates reported using the prescription stimulant Adderall® last year, and 2% reported abuse of the opioid painkiller Vicodin last year, although this is not the last medical year. Teens who abuse prescription drugs are also more likely to report using other drugs. Multiple studies have shown that there is a link between prescription drug abuse and higher levels of smoking; heavy intermittent drinking; and the use of marijuana, cocaine, and other illegal drugs among American teenagers, young adults, and college students.

Older Adults [34,35]

More than 80% of elderly patients (57 to 85 years old) take at least one prescription drug per day, and more than 50% of older people take more than five drugs or supplements per day. This can lead to health problems due to unintentional use of prescription drugs over the counter or intentional non-medical use.

Data from the National Institute on Drug Abuse.[39]

Commonly abused prescription drugs difficult to detect these drugs because the Federal Department of Transp5ortation's drug testing team or standard forensic drug testing teams may not be able to detect some of these substances, including oxycodone. Sedatives Barbiturates are commonly used as sedatives and anticonvulsants, but they play an equal role in reducing the likelihood of seizures and other symptoms during withdrawal from alcohol, heroin, and other types of drugs. They are addicted, but they develop tolerance; withdrawal syndromes include agitation, headache, psychomotor retardation, confusion, and possible seizures. One response to inadequate treatment is drug addiction in people with legal non-cancerous pain. A recent doctor survey on drug abuse showed that doctors regarded the 4 distraction mechanisms as

(1) Visits to a doctor to find a cooperating doctor;
(2) Purchasing controlled drugs from multiple doctors;
(3) Doctors to deceive or manipulate the patient;

What is prescription (Rx) drug abuse? [40]

Prescription drug abuse occurs when someone takes a drug in an improper way, for example:

- Without a prescription
- With a non-prescription
- Being induced to be "excited"

Every day 2,000 teenagers in the United States abuse Rx drugs for the first time.

- Rx drugs are the most commonly abused drugs by teenagers, second only to alcohol, marijuana and tobacco.

Commonly Abused Rx Drugs[41]

- Opioids-usually used to treat pain
- Stimulants-most commonly used to treat ADHD
- Central nervous system "CNS" sedatives-used to treat anxiety and sleep disorders.

Myth: Using stimulant drugs such as Adderall or Ritalin can help teenagers perform well in school by improving concentration and energy.

Stimulants [42]

Is particularly noteworthy for stimulants used to treat attention deficit hyperactivity didsorder. In 2004, patients diagnosed with poisoning due to non-medical use of amphetamine, dextroamphetamine or methylphenidate, underwent 7,873 emergency hospital admissions. The incidence rate in the 12 to 17-year-old age group is higher than that in the 18-year-old and older age group. More than two-thirds (68%) of the visits involved the non-medical use of these two drugs and another substance .

Sedatives and muscle relaxants [43,44]

Benzodiazepines are often diverted for non-medical purposes. They are commonly used as sleeping pills or anti-anxiety drugs. They can also be used to detoxify alcohol or other substances, and can be used to treat spastic diseases. Addicts use high doses of benzodiazepines to increase the euphoric effects of opioids; increase fascination with methadone or heroin; energetic cocaine; increase the effects of alcohol; or reduce withdrawal from heroin, methadone and The effects of other drugs.

Relaxing muscles can also be compelling. Carisoprodol, a muscular relaxing acting relaxing sold centrally under the name of soma, is an example. Ingestion can cause addiction and a slight sense of euphoria.There is tolerance and there is an anxiety, tremor, muscle contraction syndrome, insomnia, hearing and visual hallucinations, and strange behavior.

Drugs for erectile dysfunction [45-52]

Sildenafil became the drug of Centinel erectile dysfunction in drug culture; There is an underground network of false recipes, foreign imports and Internet purchases. Both men and homosexual women and heterosexual women are documented to have demonstrated drug administration behavior with this substance. In a sexually active male survey aged 18 to 25, 13% reported erectile dysfunction and 6% used drugs, but rarely under medical control and often mix With recreational drugs.

Between men and homosexual women, the sildenafil, together with cocaine, glass metanfetamine, amile nitrate poppers, ecstasy, gamanahydroxibute, and ketamine has become a "drug drug".

Effects.Recent Anecdotic Evidence suggests that Sildenafil abuse is becoming increasingly popular among Ecstasy's consumers to try to cancel the side effects of erectile dysfunction or drugs to improve the drug experience through the use of concomitant drugs for the Erectile dysfunction.

The most commonly used prescription drugs are divided into three classes [53-55]

opioids

Examples: Ossicodone (OxyContin), IDROCODONE (Vicodin) and Meridina (Demerol)

Medical Use: Opioids are used for pain or for Relieve cough treatment or diarrhea.

How they work: opioids are united to opioid receptors in the central nervous system & # 40; The brain and spinal cord & # 41;, avoiding the brain to receive pain messages.

Central nervous system & # 40; CNS & # 41; Deprestors

Examples: phenobarbital (Luminal), diazepam (Valium), and alprazolam (Xanax)

Medical Uses: Snc Dressants are used to treat anxiety, tension, panic attacks and sleep disorders.

How to work: Deprentive Depreent Snc decreasing decreasing brain activity by increasing the activity of a neurotransmitter called Gaba. The result is a sleepy or relaxing effect.

Stimulants

For example: methylphenidate (Ritalin) and amphetamine/dexamphetamine (Adderall)

Medical use: Stimulants can be used to treat narcolepsy and hyperactivity.

How they work: Stimulants increase brain activity, thereby increasing alertness, alertness and energy.

What is the danger of drug abuse? [56-58]

If someone abuses drugs, whether it is street drugs, he is more likely to commit a crime, become a victim of a crime, or have an accident. As with any drug abuse, taking prescription drugs for the wrong reasons can pose serious health risks.

Abuse of opioids can cause vomiting, mood swings, decreased thinking ability, and even decreased respiratory function, coma or death.

The abuse of central nervous system inhibitors is also risky. Stopping suddenly or reducing too quickly can cause seizures. Taking central nervous system depressants and other drugs, such as prescription pain relievers, some over-the-counter cold and allergy medicines, or alcohol can slow down a person's heartbeat and breathing—or even kill them.

Abuse of stimulants can cause heart failure or seizures. When stimulants are mixed with other drugs, these risks increase—even over-the-counter drugs like cold medicines.

Abused OTC drugs [59-64]

OTC drugs are easy to obtain and difficult to detect in routine drug tests. Once the main drugs of drug users are removed from OTC drugs and herbal medicines, they can be used as a substitute for DOC. In a study of 511 men who participated in 5 gyms, it was found that 18% reported using and rosteredione or other anabolic steroids to increase muscle mass; 25% used them Ephedrine acts as a stimulant.

The most commonly misused over-the-counter drugs [69,70]

The availability of over-the-counter (OTC) drugs provides a wealth of benefits to the public-but it also has drawbacks, including the possibility of abuse and addiction. Compared with prescription drugs, OTC drugs are more readily available and generally affordable, and therefore are widely used to treat common diseases.

Oxybutynin transdermal system [77,78]

Although it is anOTC, this medicine can only be used after consulting a doctor. Oxybutynin is used to treat overactive bladder in women with urge incontinence and urination/frequency ≥ 3 months. If abused, this anticholinergic drug will reduce symptoms of depression, euphoria and relaxation.

Salvia divinorum

Salvia is an important genus consisting of about 900 species in the family Lamiaceae. Some species of Salvia have been cultivated worldwide for use in folk medicine and for culinary purposesSalvia divinorum is a member of the Lamiaceae family and contains the psychotropic diterpene and kappa-opioid receptor agonist salvinorin-A. Originally a shamanic inebriant used by the Mexican Mazatec Indians, the plant and its preparations are becoming increasingly popular among non-traditional use. The plant grows to more than 3 feet tall with hollow square stems and large green leaves. Smoking the extract was the preferred form of administration. Subjective effects were described as intense but short-lived, appearing in less than 1 min and lasting 15 min or less. They included psychedelic-like changes in visual perception, mood and somatic sensations, and importantly, a highly modified perception of external reality and the self, leading to a decreased ability to interact with oneself or with one's surroundings. Activation of the κ opioid receptor, one of three major types of opioid receptors, produces many effects including analgesia, dysphoria, antipruritis, water diuresis, and hypothermia. The primary cause of morbidity associated with the use or abuse of S. divinorum occurs from contaminated preparations or settings in which the intoxicating effects lead to accidents and injury. Routine urine drug screening does not detect the use of this plant. Patients who develop mild anxiety may benefit from calm re-assurance in a quiet environment. More severe reactions or agitation can be treated with benzodiazepines.

Lysergamide-containing plants

Lysergamides are a class of hallucinogens including LSD (D-lysergic acid diethylamide) and LSA (lysergic acid hydroxyethylamide). With LSD, a potent psychoactive chemical, a dose of 25 mg is capable of producing effects. LSD is not found in nature, but LSA, an analogue that is one-tenth as potent, exists naturally. LSA is a tryptamine found in the seeds of *Ipomoea violacea* (morning glory) and *Argyreia nervosa* (Hawaiian baby woodrose). 5-HT2A receptor agonism likely contributes to hallucinogenic effects, but a complete neuro chemical understanding of these substances has not been elucidated. Morning glory seeds, known as tlitlitzin, were traditionally used in Aztec rituals in ancient Mexico. In the 1960s, popularity increased when the seeds were used as a substitute for LSD. Seeds are taken orally, and doses may consist of pulverized seeds or an extract of the psychoactive alkaloids. Threshold effects are noted with ingestion of 25 to 50 seeds. Visual imagery and hallucinations are prominent following ingestion of 150 to 200 seeds (3-6 g), whereas dosages of 200 to 500 seeds produce intense hallucinations in addition to nausea, vomiting, and abdominal pain. Hawaiian Baby Woodrose (*Argyreia nervosa*), not to be confused with the Hawaiian woodrose (*Merremia tuberosa*), is a perennial climbing vine, also known as Elephant Creeper and Woolly Morning

Glory. Native to the Indian subcontinent and introduced to numerous areas worldwide, including Hawaii, Africa and the Caribbean, it can be invasive, although is often prized for its aesthetic value. The plant is a rare example of a plant whose hallucinogenic properties have only recently been discovered by non-Hawaiians. Its properties were first brought to attention in the 1960s and the seeds contain the highest concentration of psychoactive compounds in the entire family. The plant contains LSA in large seeds surrounded by pods. Nutmeg Nutmeg comes from the evergreen tree *Myristica fragrans*. This tree grows in the Molucca Islands and elsewhere in Indonesia. It is now cultivated commercially in the West Indies, especially Grenada, for its spices. The fruits of the tree look like apricots but when split open yield a single brown kernel, the nutmeg. Its various unproven clinical uses include treatment of gastrointestinal disorders, musculoskeletal problems, and psychiatric conditions. In low doses, nutmeg produces no noticeable physiological or neurological response. Large doses can be dangerous potentially causing dizziness, flushes, dry mouth, accelerated heartbeat, temporary constipation and difficulty in urination, nausea, and panic. In addition, experiences usually last well over 24 hours making recreational use rather impractical. A risk in any large-quantity ingestion of nutmeg is the onset of 'nutmeg poisoning', an acute psychiatric disorder marked by thought disorder, a sense of impending doom/death, and agitation. Although nutmeg has a high incidence of unpleasant side effects, it is preferred by some users in search of a legal and easily obtainable euphoric drug with hallucinogenic effects. Nutmeg contains myristicin, a weak monoamine oxidase inhibitor. Speculative comparisons between the effects of nutmeg intoxication and MDMA have been made. However, nutmeg contains amphetamine derivatives and such are formed in the body of a significant number of people from the main chemical components of nutmeg. These and other active components (eugenol, borneol, and linalol) likely combine to produce psychotropic and sympathomimetic effect. There is no specific antidote for nutmeg poisoning. Symptomatic and supportive care is required to treat nausea and vomiting, agitation, hallucinations, and to address any sympathomimetic effects.

Anticholinergics

Datura stramonium, known by the common names jimson weed, angel's trumpet, devil's weed, thorn apple, tolguacha, Jamestown weed, stinkweed, datura, moonflower, and, in South Africa, malpitte and mad seeds is, along with *Datura metel* (zombie cucumber), a common weed in the Solanaceae (the nightshade family). It contains tropane alkaloids that are sometimes used as a hallucinogen. The active ingredients are atropine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics. Due to the elevated risk of overdose in uninformed users, many hospitalizations, and some deaths, are reported from recreational use. Adolescents today most commonly use jimsonweed recreationally by ingesting, smoking, or brewing a tea from the seeds. Jimsonweed (*Datura stramonium*) grows to approximately 1.5 m tall and has a solitary white, trumpet-shaped flower. In autumn, a spiny capsular fruit is produced that contains up to 50 small black seeds. Although all parts of the plant are toxic, the seeds contain the highest concentration of atropine. Other Solanaceae known to contain belladonna alkaloids include *Datura inoxia, Datura aurea, Datura sanguinea*, and *Brugmansia arborea*. Several other plantsdeadly nightshade (*Atropa belladonna*), henbane (*Hyoscyamus niger*) and mandrake (*Mandragora officinarum*) contain hyoscyamine and scopolamine alkaloids that are recreationally abused for their anticholinergic properties. Although these plants are abused for their hallucinogenic and euphoric effects, anticholinergic intoxication can result in classic anti muscarinic symptoms because of competitive blockade of acetylcholine at the central and peripheral muscarinic receptor sites. Symptoms of Jimson weed toxicity usually occur within 30-60 minutes after ingestion and may continue for 24-48 hours because the alkaloids delay gastrointestinal motility. Ingestion of Jimson weed manifests as classic atropine poisoning. Initial manifestations include dry mucous membranes, thirst, difficulty swallowing and speaking, blurred vision, and photophobia, and may be followed by hyperthermia, confusion, agitation, combative behaviour, hallucinations typically involving insects, urinary retention, seizures, and coma.[16] Treatment consists of supportive care, gastrointestinal decontamination (i.e., emesis and/or activated charcoal), and physostigmine in severe case.

Sympathomimetics

Most plant or herbal recreational drugs touted as stimulants have sympathomimetic properties. Sympathomimetics are naturallyoccurring and synthetically- produced chemicals that either increase the activity of the adrenergic nervous system or mimic its effect. Included in this class are direct-acting and indirect-acting agents. Direct-acting

agents bind and stimulate a- or b-adrenergic receptors. Indirect-acting agents, such as amphetamines, cause increased presynaptic release and concentration of synaptic neurotransmitters, such as norepinephrine. Some substances cause both direct- and indirect-acting adrenergic effects, and certain herbal stimulants have sympathomimetic effects in combination with psychoactive and euphoric properties.

Ephedra alkaloids

Ephedra, from the plant *Ephedra sinica*, has been used as a herbal remedy in traditional Chinese medicine for 5,000 years for the treatment of asthma and hay fever, as well as for the common cold. Known in Chinese as ma huang, ephedra is a stimulant which constricts blood vessels and increases blood pressure and heart rate. The Ephedra plant is a leafless shrub with a horsetail appearance that grows throughout the desert regions of Asia and North America. Mormons have historically been avid consumers of the ephedra-containing beverage ''Mormon tea,'' which affords them the stimulatory effects of coffee or tea without the caffeine. More recent use has been in the form of dietary supplements for the purposes of increased energy and alertness, enhanced athletic performance, or weight loss. Ma-huang contains the psychoactive alkaloids ephedrine, norephedrine. These alkaloids can cause rapid or irregular heartbeat, very similar to the effects of adrenaline. Blood pressure rises. There have been reported cases of liver injury and hepatitis, and users experience aggressiveness, anxiety, and tremors. Complications from these side effects can result in cerebral hemorrhage, cardiac arrest, and, of course, death. Prolonged use of the drug can be the cause of weakened adrenal glands, nervousness, and insomnia. Other side effects include nausea, vomiting, fever, depression, seizures, and headaches. Treatment of ephedra-related toxicity is similar to other CNS stimulants, with close attention paid to addressing the central sympathomimetic stimulation, cardiovascular toxicity, and secondary complications as a result of the heightened adrenergic state.

Betel nut

Betel nut (*Areca catechu*) is the fourth most commonly abused substance worldwide, after caffeine, alcohol, and nicotine, chewed for hundreds of years and is currently being used by 10% of world population. Betel nut is a coconut like fruit of areca catechu palm that is harvested in India, Vietnam, Srilanka, Philippines, Bangladesh, Americans and many more countries where it is chewed for its relatively mild stimulant effect. Commonly betel nut is mixed with various chewing materials like Betel leaf, lime and may include clove, cardamom, catechu (kattha).The areca nut contains tannin, gallic acid, a fixed oil gum, a little terpineol, lignin, various saline substances and three main alkaloids: Arecoline, Arecain and Guvacine which have vasoconstricting properties. The pharmacologic effect of betel nut is largely attributable to the alkaloid arecoline, which acts as a cholinomimetic agonist at nicotinic and muscarinic receptors and as an acetylcholinesterase inhibitor. Symptoms of dizziness, vomiting, and flushing are more pronounced in first-time users. Stimulation of glandular secretions, pupillary constriction, and bradycardia may follow. Adverse events include asthmatic exacerbation, cardiac arrhythmias, acute psychosis, dystonias, and seizures. The calcium hydroxide component of the betel quid can be responsible for causing milk-alkali syndrome and an increased risk for nephrolithiasis.The International Agency for Research on Cancer includes betel quid as a known human carcinogen even without combined tobacco use. Chronic use causes characteristic red staining of the teeth and gums and increases the risk for oral leukoplakia, oral squamous cell carcinomas, and submucous fibrosis. Most cases of acute toxicity are mild. The primary treatment is symptomatic and supportive care because no specific antidote exists.

Yohimbe

Yohimbine is a psychoactive drug of the tryptamine chemical class with stimulant and aphrodisiac effects obtained from the bark of *Pausinystalia yohimbe* and *Corynanthe yohimbe*. It is also found naturally in *Rauwolfia serpentina* (Indian Snakeroot), along with several other active alkaloids. Yohimbine is marketed to the general public as a supplement for body-building and sexual enhancement and is consumed for its aphrodisiac and hallucinogenic properties It is also available by prescription for the treatment of erectile dysfunction, despite limited evidence regarding its efficacy. Yohimbine has high affinity for the α2A-adrenergic, α2B-adrenergic, and α2C-adrenergic receptors, moderate affinity for the 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2B, and D2 receptors, and weak affinity for the

D3 receptor. Yohimbine behaves as an antagonist at all receptors except for the 5-HT1A, 5-HT1D, and 5-HT2A receptors, where it acts as a weak partial agonist. Yohimbine has significant side effects, such as anxiety reactions. Higher doses of oral yohimbine may create numerous side effects, such as rapid heart rate, high blood pressure, overstimulation, insomnia and/or sleeplessness. Some effects in rare cases were panic attacks, hallucinations, headaches, dizziness, and skin flushing. More serious adverse effects may include seizures and renal failure. Yohimbine should not be consumed by anyone with liver, kidney, heart disease, or a psychological disorder. Acute management is largely supportive. Although no specific antidote exists, clonidine has been recommended for reversal of yohimbine toxicity in adolescent and adult patients.

Analgesics/euphorics

Kava

The drug Kava lactones, also known as kava pyrones, are derived from the dried root and rhizome of Piper methysticum. Kava extract is used in traditional recreational drinks in many South Pacific countries. In Western societies, kava is used as an overthe-counter anxiolytic, muscle relaxant, mood enhancer, sedative or treatment for premenstrual syndrome. Several pharmacologic effects of kava have been observed, including platelet inhibition, difficulties with visual accommodation and photosensitivity, and possible dopaminergic antagonist activity. Weakness, numbness, and sedation may follow. Kava may also enhancethe effects of other centrally acting agents such as benzodiazepines and alcohol. Longterm use of kava, especially in high doses (400 mg of kava pyrones daily), has been associated with the development offlaky, dry, yellow skin (kava dermopathy) through an unknown mechanism; the effect may be reversible upon cessation of thedrug. Other possible adverse effects include ataxia, hair loss, hearing loss and anorexia.

Cloves

Containing a mixture of approximately 30% ground or shredded cloves (*Syzygium aromaticum*) with 70% tobacco, clove cigarettes deliver twice as much tar, nicotine, and carbon monoxide as the average cigarette. Many users have the mistaken belief that clove cigarettes are an herbal, "natural," nontobacco alternative. Others are attracted to their use because of the association of clove cigarettes with the image of surfing, new wave music, and the search for"exotic" and unusual experiences. On inhalation, eugenol, an ingredient in cloves, has a topical anesthetic effect and causes numbness in the throat. In the United States, deep inhalation and increased retention of the smoke, or toking, is the most common technique in which these cigarettes are smoked in an effort to enhance effects. There is concern that clove cigarettes may be a gateway drug for adolescents.[29] Case reports of severe illness have been reported with clove cigarette use, including bronchospasm, hemoptysis, and pulmonary edema. Treatment of any related toxicity is supportive.

Absinthe

Absinthe is an alcoholic drink made with an extract from wormwood (*Artemisia absinthium*) which is a native of Europe and has been naturalized in the United States. It is an emerald green drink which is very bitter (due to the presence of absinthin) and is therefore traditionally poured over a perforated spoonful of sugar into a glass of water. The drink then turns into an opaque white as the essential oils precipitate out of the alcoholic solution. Absinthe was once popular among artists and writers and was used by Van Gogh, Baudelaire, and Verlaine, to name a few. It appears to have been believed to stimulate creativity. However, in the 1850's, there began to be concern about the results of chronic use. Chronic use of absinthe was believed to produce a syndrome called absinthism, which was characterized by addiction, hyperexcitability, and hallucinations. It can also cause gastrointestinal disorders, sleeplessness, tremors, convulsions, auditory and visual hallucinations, brain damage, and death Thujone, a terpene found in oil of wormwood with α and β isomers is the suspected source of hallucinations and psychosis from absinthe use . Thujone has been shown to antagonize the g-aminobutyric acid (GABA) receptor, and this may explain absinthe's excitatory and seizure producing effects. Treatment of ingestion is symptomatic and supportive because no specific antidote exists.

CONCLUSION

The abuse of prescription drugs and OTC drugs has aroused increasing public interest worldwide. Current medication regimens pose major challenges for healthcare providers and pharmacists, especially during the COVID19 pandemic With the market full of herbal products focused at teenagers and young adults, with advertisement, of providing a "safe, natural high", control must be forced to detect and regulate the drugs of abuse. Often despite the potential for abuse, addiction, and serious adverse effects, false perception exist that ,these products are all safe, legal, and organic. In conclusion, herbal medicinal products are regularly associated with serious adverse affects but the size of this problem cannot be estimated at present. Vigilance and research seem to be the best way forward.

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RESEARCH ARTICLE

Research Studies on *Chitiniphilus shinanonensis* towards Anticancer Activity

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ABSTRACT

In nature, various marine organisms are available, which possess different bioactive molecules. The organisms have macro and micro molecules including various polysaccharides. Major polysaccharide is cellulose. In addition, one more substance in these marine organisms, namely, chitin is also available which exhibits various functions and activates. Among these functions, one of the most important functions is to produce oligosaccharides. These chitins related produce oligosaccharides are used to prepare single cell protein. And useful to prepare medium to culture cancer cells which are helpful to prepare protoplasts of fungi. After identifying the research gap from the literature review. in the present study, five organisms were used to detect the degradation ability of this polysaccharide chitin. The potent microorganism was subjected to detect and enhance the secondary metabolites using media optimization. Different solvent extract systems were used in our study to detect novel bioactive molecule. The bioactive molecules in crud extract was also subjected to detect antimicrobial and anticancer activity. The bioactive molecules Asiatic and triterpene which were isolated from second microorganism showedantibacterial and anticancer activity. These organisms will have a vital role in pharma applications.

Keywords: Polysaccharides: Bioactive molecules: Pharma applications: Solvent extract.

INTRODUCTION

Chitin, the N-acetylglucosamine (GlcNAc) β-1,4-linked homopolymer, is the most abundant polymer in the marine environment and the second after cellulose in nature [1]. Chitin is the fundamental structural constituent of the cell

wall of most fungi, but is susceptible to innumerable bacterial and fungal species acting as antagonists, due to their synthesizing of chitinases. About 75% of the whole weight of shellfish, such as crabs and shrimp are considered as waste, and chitin forms 20-58% of its dry weight. The use of chitinous residues often results in corrosive chemicals resulting in high cost, low product yields and ecological toxicity. So chitinivorous micro-organisms may present a most frugal and eco-friendly approach to treat these chitinous wastes. Chitin is the structural basis for crustacean and insect exoskeletons and a part of the cell wall of the fungus. Global chitin production is estimated at 10tons per year, but chitin does not accumulate as marine microorganisms hydrolyze it [2,3,4]. Hydrolysis is mediated by chitinolytic enzymes and allows chitin to be used by microorganisms as a source of carbon and nitrogen, and chitin turnover is essential for the biogeochemical C and N cycles [5]. Also of biotechnological interest are chitin and chitinolytic enzymes with potential applications in the food, medical and agricultural sectors [6,7]. Chitin in the form of shellfish waste can also be considered a commodity that can potentially be used in microbial fermentations as a carbon source. Shellfish waste is an increasingly large environmental problem and the discovery of cheap processes that can degrade chitin into chitooligosaccharides, chitosan and GlcNAc can address this problem. Chitinases can be used in the production of functional chitin-oligosaccharides; in making of single cell protein and in the preparation of fungal protoplasts. Chitinases are predominately helpful in agriculture as biocontrol agents against fungal phytopathogens because they cause lysis of the fungal cell wall and they offer a nontoxic alternative to chemical fungicides. Marine organisms are well known for their high bioactive secondary metabolite production and its complex cell life [8]. These secondary metabolites are of high biological activity given its potential in food and pharmaceutical industries. Moreover, the ability to degrade chitin appears to be an important attribute of marine bacteria. In the present study, five chitinase positive marine isolates were used and evaluated their chitinase producing potentials. In addition, their secondary metabolite production was optimized and were tested for anti-bacterial and anti-cancer properties.

MATERIALS AND METHODS

CONFIRMATION OF CHITINASE PRODUCTION

Five cultures obtained from Marina beach, Chennai were named as M1, M2, M3, M4 and M5. The cultures were streaked as a perpendicular single line in the middle of the petriplate containing nutrient agar medium with 1% chitin and incubated for 48 hours in a 37 °C incubator. The plates were then stained using 1% Congo red stain and incubated for 5-10 minutes. The stain was drained and 1% NaCl was flooded over the surface for 5-10 minutes of the culture for detaining and then drained off. The appearance of a zone visible on the backside of the Petri plate indicates the ability of the culture to produce chitinase.

DETECTION OF SECONDARY METABOLITE PRODUCTION

The marine cultures were screened for their secondary metabolite production by inoculating the cultures into 50 ml of sterile production medium. After four days of incubation, the broth was centrifuged at 10000 rpm to separate cell debris from the broth. An equal quantity of ethyl acetate was added to the broth and kept in shaking for 24 hours. This was followed by removal of ethyl acetate fraction using a separating funnel and the solvent was concentrated under rotor vacuum to finally yield the crude metabolites. The presence of metabolites was confirmed by loading the crude samples in a prepared thin layer chromatography (TLC) plates and run in a mobile phase of hexane: ethyl acetate solvent (1:1). After running the TLC, the plates were dipped in vanillin to visualize the bands.

MEDIA OPTIMIZATION

Media optimization was performed to identify the suitable conditions for the growth of the cultures and the maximum secretion of secondary metabolites. DoE Software based model was drafted and the condition was optimized initially. Based on the software output, experimental setup was done where in the culture conditions were varied and the condition yielding maximum secondary metabolites was chosen for further studies. The culture

parameters such as different carbon sources at 1% (w/v), pH (acidic-alkaline), temperature (20-45 °C), aeration and days of culture were tested individually**.**

SCALE-UP PRODUCTION OF SECONDARY METABOLITES AND ANTIBIOTICS

The effective strain was grown in 100ml of nutrient medium (seed medium) in 250ml conical flask and then the inoculums was used in the fermentation for the bulk production of secondary metabolites. All the fermentation process was carried out in batch culture mode of operation using the optimized culture conditions. For the production of secondary metabolites with anti-microbial activities, a two-step culture was performed. Initially, bacteria M5 were first grown at 38°C for 24 hours in seed medium. The resulting culture were harvested, rinsed and transferred to optimize secondary production medium. The product recovery was achieved by centrifugation to get the broth free of cell debris or solid particles. This was followed by partitioning thrice with hexane, ethyl acetate and chloroform, respectively. The respective solvent fractions were concentrated to remove the solvents. The dried crude extracts were then used for bioactivity studies.

BIOACTIVITY STUDIES

Bioactivity studies were carried out using the bacterial cultures subcultures at 1% (v/v) in nutrient agar media for 24 hours at the optimized temperature of 35°C. The final crude extracts, after concentration, were weighed and dissolved in DMSO (1mg in 1ml) was screened using agar well diffusion method with a sterile cork borer of size 6.0 mm. The 24 hours old cultures, grown on nutrient broth were used for inoculation of bacterial strain on Muller Hinton agar plates. Each microorganism (*E. coli and Bacillus*) was diluted in sterile saline solution and adjusted to 0.1 OD reading and a dilution of 1000ppm was used. The diluted microbial cultures were then flooded on the surface of the pre sterilized Muller Hinton agar plate. Three wells, each 10mm in diameter, were cut from the agar and crude extracts of same dilution was loaded in to each well. The plates were incubated for 24 hours at 37°C and the zones of inhibition were measured with the zone scale in mm**.**

BULK PRODUCTION, PURIFICATION AND CHARACTERIZATION OF SECONDARY METABOLITES

Bulk production of the secondary metabolites were carried out in 20 litre bioreactor and the extraction of secondary metabolites was done using ethyl acetate which showed prominent yield of secondary metabolites. At the 14th day of incubation, the whole broth was taken for processing. The broth was separated from the cells by filtration and the cells were submitted to three times partition with ethyl acetate. The resulting solvent fractions were combined and concentrated respectively, to eliminate the organic solvent. Then, the finished dried crude extracts were purified by column chromatography technique.

THIN LAYER AND COLUMN CHROMATOGRAPHY

Identification of metabolites using Thin layer chromatography (TLC) was carried out with the crude extract on silica gel (TLC silica gel 60,20X20 ,0.5mm, Merk and co, Inc) with Ethyl acetate: hexane (7:3) solvent system. The crude extract was spotted, and the solvent front was allowed to run for approximately 16 cm. The running lane was then dried thoroughly; elution of compound was detected at 365nm. The effective bioactive metabolite was purified by column chromatography, in the glass column (50x2cm) packed with slurry of silica gel (60-120 mesh) pre activated at 120^{o}C for 4 hours. After the column was successfully eluted with gradients of hexane, then hexane /ethyl acetate, then ethyl acetate and finally washed with methanol. The fractions collected were distilled on water bath and monitored by TLC. The fractions of similar compositions were combined together and concentrated. The concentrated fractions were subjected to further mini column to get pure compound. Two pure, major compounds were isolated using column chromatography and processed for NMR spectrum for structure prediction.

CELL CULTURES AND PASSAGING OF CELL LINES

Osteoblast-like MG63 cells, human breast cancer MDA-MB-231 cells and A549 human lung adenocarcinoma cells (ATCC) were cultured in T-25 flasks containing Dulbecco's modified Eagle medium (DMEM, Gibco, USA) supplemented with 10 % fetal bovine serum (FBS, ATLAS, Dae Myung Science Co., Ltd., Korea) and 1 % penicillin-

streptomycin (Pen-Strep, Gibco, USA). Cultures were incubated at 37°C in a humidified atmosphere of 95 % air, and 5 % CO2, and the medium was changed every 2 days. The HT-29 cells were maintained in McCoy's 5A complete growth medium (CGM) supplemented with 10% FBS and 1% antibiotics (penicillin/streptomycin). HT-29 cells (1 × 106 cells/ml) were plated in T-25 flasks containing 5 mls of CGM and grown in a humidified incubator under an atmosphere of 95% air and 5% CO2 at 37°C to sub-confluence (90 - 95%). The culture medium was replaced at every 48 hours. Passages of cells were done at 80–90% confluence generally every 2–3 days. All experiments were performed on exponentially growing cells.

MTT Assay

The cytotoxic effect of the extracts was assessed *in vitro* using thiazolyl blue tetrazolium bromide (MTT), an indicator of metabolic activity of viable cells. MG63, MDA-MB-231, HT-29, and A549 cells ($5x10^3$) were seeded in 96-wells plate. Logarithmic concentrations (1, 10, 100 ng/ml and 1,10,100 µg/ml) of test samples were added and incubated for 24 h. After incubation, 10 µL of MTT reagent (5 mg/mL) was added and incubated for 4 h at 37°C. Subsequently, insoluble formazan was dissolved using DMSO and absorbance was measured at 570 nm spectrophotometrically.

AO/EB FLUORESCENT STAINING

MG63, MDA-MB-231, HT-29, and A549 in the logarithmic growth phase were digested with 0.25% trypsin. culture medium was deposited in each well of a 96-well plate (150 µl/well). Twenty five microliters of cell suspension ($5×10^6$ cells/mL) was stained with 1 µl of acridine orange and ethidium bromide dye mix (100 µg/ml of acridine orange and 100 µg/ml of ethidium bromide prepared in PBS) (Winnicka et al. 2007). Suspensions (25 µl) were transferred to glass slides. Dual fluorescent staining solution (1 µl) containing 100 µg/ml AO and 100 µg/ml EB (AO/EB, Sigma, St. Louis, MO) was added to each suspension and then covered with a coverslip. Then the samples were examined under fluorescent microscopy (OLYMPUS, Japan). The following criteria were used to identify the differences in the cellular states, viable cells with normal nuclei (bright green chromatin with organized structure) viable cells with apoptotic nuclei (bright green chromatin which is highly condensed or fragmented) and nuclei with necrotic cell (bright orange chromatin with organized structure).

CASPASE ACTIVITY

Caspase - 3 like activity was measured as described previously (Sutter et al. 2003). The activity of caspase-3 was calculated from cleavage of the fluorogenic substrate AC-DEVD-AMC. After 24 h incubation, the test sample treated cell lysates were incubated with substrate solution (caspase-3 substrate AC-DEVD-AMC 20 mg/ml, HEPES 20 mM, glycerol 10%, dithiotheritol 2 mM, pH 7.5) for 1 h at 37^0C and cleavage of the caspase-3 substrate was measured at an excitation wavelength of 390 nm and emission wavelength of 460 nm. Activity was expressed as Relative Fluorescence Unit (RFU).

STATISTICAL ANALYSIS

All the analysis was performed using GraphPad software. The statistical t-test was carried out for the analysis.

RESULTS

MARINE ORGANISM SCREENING YIELDED CHITINASE PRODUCING STRAINS

Screening of five marine isolates for the production of chitinase revealed that chitinase production was high in M2 and M4 cultures, medium in M3 and M5 and low in M1 as revealed by Congo red staining. From the cultures isolated, it was found that M2 is said to be highly efficient in producing chitinase production. The Best culture M2 was then subjected to genetic sequence to confirm its molecular identification.

OPTIMIZED MEDIA FOR THE PRODUCTION OF SECONDARY METABOLITES

The selected marine isolate was confirmed as *Chitiniphilus shinanonensis* using genetic sequence method . The culture was further tested for their ability towards secondary metabolite production by testing the metabolites from the broth after extraction and concentrations in different solvents. The optimization studies showed that a pH of about 8-9 and temperature 25-40°C and nutrient broth favours the secondary metabolite production. Further, the extraction with ethyl acetate showed the maximum yield and was used for further studies (Table 1). Two pure, major compounds are isolated using column chromatography and processed for structure prediction. The structures of the bioactive compounds were determined using NMR as Asiatic acid and triterpene.

MARINE SECONDARY METABOLITES INDUCED CELL DEATH IN CANCER CELLS

Treatment of crude fractions from ethyl acetate extraction of marine organisms induced significant cell death in MG63, A549, MDA-MB-231 and HT-29 cancer cells as determined using MTT assay. The presence of cell death was evident with 24 hours of treatment with the crude extracts. Doxorubicin was used as a positive control for the analysis of cell death

MARINE SECONDARY METABOLITES INDUCED LYSOSOMAL ACIDIFICATION IN CANCER CELLS

Lysosomes are nodal mediators of growth and proliferation through their surface localization of mTORC1 signalling, a key mediator of cellular anabolic responses, whose responses require lysosomal acidification. Hence the changes in lysosomal acidification were determined using acridine orange staining of the cells treated with crude extract from marine organism and compared it with control and doxorubicin positive control. The staining revealed that both extract and positive controls induced significant lysosomal acidification noted by the shift in green channel intensity within the cells (Figure 1).

MARINE SECONDARY METABOLITES INDUCED APOPTOTIC PATHWAY

In order to validate the mode of cell death in cancer cells by the marine extract, we evaluated the markers of apoptosis viz Caspase 3, BCL2 and BAX. We found a significant elevation of caspase 3 with the treatment with extract and positive control doxorubicin in all the cell types investigated. The presence of significant increase in the downstream activation of caspase 3 towards apoptosis signifies the apoptotic mode of cell death through the marine secondary metabolites.

DISCUSSION

In both marine and terrestrial environments, degradation of chitin is an important process. Chitin is also an important resource in various industrial and medical processes, and it is of interest to degrade chitin enzymes or micro-organisms, e.g. as antifungal agents or as bio-insecticides. As a result, new enzymes with chitin-modifying properties are increasingly in demand. Because of the prevalence of chitin-producing species in the ocean, chitin may be expected to kill most marine bacteria. However, crop studies suggest that relatively few marine bacteria degrade chitin, ranging from 0.4% to 19% of total crop bacteria . Our estimates for estuarine and coastal waters were within this range. Although chitinase activity can be much higher on particles than in the surrounding seawater , including particle-associated bacteria in the coastal library did not greatly increase the estimate of the portion of bacteria that degrade chitin. Even though culture-based methods retrieve only a small fraction of the total bacteria. Our work demonstrates the chitiniase producing abilities of marine isolates which could have direct implications on testing for chitin degradation applications in industrial scale. Further, we have optimized the growth and production of secondary metabolites by the marine isolates. Optimum extraction of the secondary metabolites is essential for its application in the industrial scale. We have determined ethyl acetate extraction to be of high yielding in the marine isolate cultures.

In addition to chitin degrading abilities, the secondary metabolites from the marine isolates exhibited prominent anti-bacterial and anti-cancer properties. The anti-cancer effects of the compounds were observed by the activation of caspase 3, a marker for the apoptosis pathway activation. This supports its application as a co-treatment with existing anti-cancer drugs for clinical interventions without adding any additional side-effects. The compounds identified using NMR were Asiatic acid and triterpene. Asiatic acid is a naturally found pentacyclic triterpenoid that is known to be present in medicinal herb *Centella asiatica*. Asiatic acid is known to exhibit a broad spectrum of biological activities such as anti-cancer , antioxidant , anti-inflammatory and wound healing , anti-diabetic and neuroprotective effects . Specially, Asiatic acid has been noted for its anti-cancer effects though its antioxidant effects and interaction with nuclear factor erythroid-derived 2-like 2 (Nrf2), nuclear factor kappa B (NF-κB) and protein kinase C (PKC). Asiatic acid has also been reported to induce apoptotic cell death by altering the expression of apoptotic regulators such as caspases, B-cell CLL/lymphoma 2 (BCL-2) family members . It has been shown that Asiatic acid induced cancer cell apoptosis involves the increase in mitochondrial membrane permeability and release of cytochrome c from mitochondria into cytosol. Moreover, Asiatic acid also induces the activity of caspase-9 which further stimulates caspase-3 cleavage resulting in irreversible apoptotic death in cancer cells . In our study, we found an upregulation of caspase 3 under treatment with pure extract which can be accounted for the presence of Asiatic acid in the extract. In addition to Asiatic acid, NMR analysis revealed the presence of Triterpene.Triterpene acid compounds have many excellent physiological and pharmacological effects, including anti-inflammatory,antiviral, antibacterial, and nerve calming. In addition, the literature studied its immune control, blood sugar management, blood pressure reduction and antitumor activity. The mechanistic effect of triterpene compounds are known to be associated with the signaling pathway of the nuclear factor kappa B(NF-κB)/phosphatidylinositol3-kinase (PI3K)/protein kinase B (Akt). Furthermore, studies have also shown that pentacyclic triterpenoid anti-tumor lupane type by regulating the Bax / Bcl-2 ratio in nude mice transplanted with high metastatic human melanoma.Our observations with the induction of apoptotic death in cell extract also matches with the potential anti-cancer effects of triterpenes. These observations from our study make secondary metabolites from marine organisms an attractive therapeutic agent for developing novel treatment protocols, and possibly for combining with other antibiotics and chemotherapeutics to overcome drug resistance and achieve better outcomes. Though we have evaluated the overall effects of cancer cell death, we have not evaluated the signalling mechanisms responsible for the synergistic effect of the compounds Asiatic acid and triterpene present in the extract. This has to be also extended to the anti-bacterial effects exhibited by the extract. It is clear that further studies are required to elucidate the full spectrum of direct and downstream cellular targets of the isolated compounds and is of potential interest in improving chemotherapeutics.

$(GlcNAc)_4$ is found to have strong stimulating activity toward natural killer cells [Bezouska et al., 1997]. $(GlcNAc)_5$ is an important building block for NOD factor synthesis [Samain et al., 1997]. $(GlcNAc)_6$ and $(GlcNAc)_7$ show antitumor activity against mice sarcoma 180 [Suzuki et al., 1986] and antimicrobial activity against fungal pathogens [Roby et al., 1987].

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Table 1. Yield of secondary metabolites using different extraction solvents

Nutrient broth	Quantity of the secondary metabolites(100ml)								
	Hexane extract(mg)			Ethyl acetate extract(mg)			Chloroform extract (mg)		
Replicates	R1	R2	R3	R1	R2	R3	R1	R2	R3
Batch 1	0.100	0.1244	0.111	0.154	0.1733	0.122	0.034	0.061	0.056
Batch 2	0.154	0.2927	0.134	0.211	0.294	0.215	0.083	0.096	0.433
Batch 3	0.056	0.0576	0.0467	0.121	0.1172	0.110	0.081	0.082	0.083
Batch 4	0.074	0.0793	0.0652	0.102	0.1076	0.110	0.045	0.148	0.135

Table 2. Zone of inhibition using different crude extracts

	Zone of Inhibition (mm)		
	Hexane	Chloroform	Ethyl acetate
Culture			
E.coli	10mm	11mm	11.5mm
Bacillus	1omm	11.5mm	11.5mm

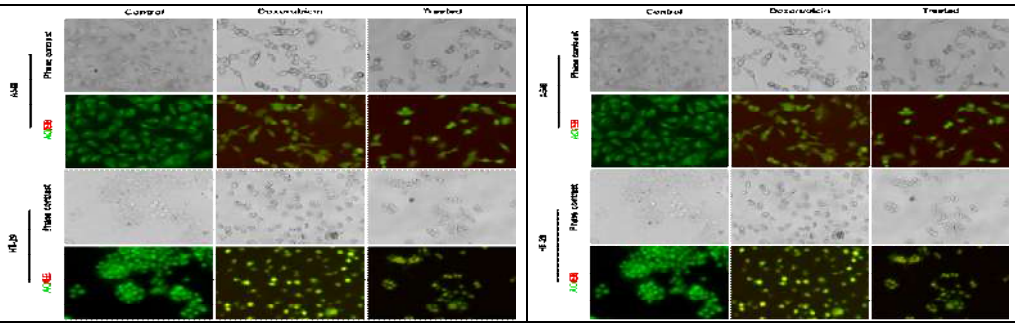

Figure 1. green channel intensity within the cells

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RESEARCH ARTICLE

Toxicity Analysis of Cardio Protective Homeopathy Drugs *Aconitum napellus* and *Digitalis purpurea* using Zebrafish Embryo

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ABSTRACT

Homeopathy practice is one of the age-old alternative medical practices followed worldwide. In this study, we determined the toxic potential of two homeopathy drugs namely *Aconitum napellus* and *Digitalis purpurea* using zebrafish embryos, which are used to treat cardiovascular disorders by homeopathic procedures in humans. Our study implies that both drugs are potentially toxic at lower dilution such as 10^{-1}, which even showed absolute mortality. Reduced heartrate and pericardial edema was observed at 10^{-2} dilutionin *A.napellus*, and at 10^{-2} and 10^{-3} dilutions in *D purpurea*. However, other developmental abnormalities, such as reduced heart rate, heart malformation, and hatching rate were not observed at higher dilutions ranging from 10^{-6} to 10^{-12}. Based on the results we conclude that higher dilutions of these two drugs could be used to investigate the cardioprotective role in zebrafish embryos. Hence, at higher dilutions, as given in homeopathic practices these drugs may have possible anti-cardiovascular effects.

Keywords : *Aconitum napellus*, *Digitalis purpurea,* pericardial edema, cardioprotective, zebrafish

INTRODUCTION

Homeopathy is an alternative system of medicine to treat a range of illness. It was developed by Samuel Hahnemann (1755–1843) from Germany almost 200 years ago [1]. The drugs used in homeopathy are obtained from the extracts of plants, animals, and mineral sources. In this method, drugs will be used to activate the body's self-healing power by

stimulating appropriate reactions in the body in response to the symptoms. Homeopathy is based on two fundamental principles: "Like cures like"- a substance that causes specific signs and symptoms in a healthy individual can be utilized as a medicine for patients who have comparable disease symptoms [2]."Law of minimum dose" – according to this law, the drug retains biological activity after repeated dilution beyond Avogadro's number, that is the highly potentized formulations give observables effects on healthy individuals, but with relatively higher doses it may aggravate the ailment by enhancing the parallel symptoms and adverse effects [3]. Globally, homeopathy is practiced in more than 85 countries. In India, the system was first practised in Bengal in the early 19th century and has spread across the country [4]. Now it is quite popular and has evolved as an alternative medical system in India. The homeopathy drug market is growing 25% annually and more than 100 million people are following this practice for various health-related complications including HIV, Asthma, skin diseases, cancer, heart diseases, and diabetes [5]. Moreover, administration of homeodrugs is simple, its accumulation is limited inside the body, and are environmentally sustainable [6].

Homeopathy is also well-known for its critics and controversies, which arise from skepticism about the drug's efficacy due to the lack of scientific evidences [7]. The main concern is that the homeopathic drugs are extremely diluted, so they may not contain a considerable quantity of active ingredients. It is also uncertain arguemented that the biological activity of these drugs is still being maintained at higher dilutions [8]. The presence of heavy metals, and toxic nature of drug source, may have negative consequences among some patients when administered with high concentrations of the drugs. Moreover lack of scientific evidence to establish the mechanism of homeopathy drugs limited their applications to treat diseases. In this study, we used two homeopathy drugs namely *Aconitum napellus* and *Digitalis purpurea*, which are already used as a remedy for cardiac and other common ailments. Both of these drugs are made from plant extracts which are highly toxic by nature [9].A study with alkaloids extracted from the Aconite plant shows that it induces cardiotoxicity along with yolk sac edema in zebrafish embryos at the concentration of 2.5µg/L [10]. However, *Aconitum napellus* and *Digitalis purpurea* are used to treat heart related problems in homeopathy and other traditional medicines [11]. In fact, *D. purpurea* has been used as a drug for emetics and heart diseases among Egyptians and Romans from ancient time to till date [12].

In the present study, we used zebrafish embryo as a model to evaluate the toxicity induced by the above mentioned homeopathy drugs. Zebrafish is an excellent model organism for toxicological studies and is used widely to elucidate the underlying molecular mechanisms of toxicity and to predict risk on humans and for preclinical drug discovery and screening [13,14]. To study the biological activity of the drugs, it is important to determine the non-lethal concentrations of these two drugs. Therefore the non-lethal concentrations were determined prior to study the efficacy of the drugs for the treatment of treat heart diseases. To determine the non-lethal concentrations of *A.napellus* and *D. purpurea,* we analysed the parameters such as percentage of mortality, hatching rate, heart rate, and teratogenic potential with different dilutions

MATERIALS AND METHODS

Zebrafish Maintenance

Adult wild-type Zebrafish were purchased from a local aquarium and carefully transferred to the laboratory in oxygenated polythene bags. In the laboratory, the fishes were maintained in glass tanks (35 X 17 X 18 cm LBH). The fishes were acclimatized for two weeks. The male and female fishes were then kept in separate tanks with adequate aeration. The room temperature was maintained at 26 ± 1°C and 14:10 h light-dark cycle was provided. The fishes were fed with dry floating pellet feed (Toyo brand red and green floating pellet) and frozen brine shrimp (*Artemia*). Tank water was changed once in two days at the regular time point and water quality was checked regularly.

Breeding and Collection of Embryo

Male and female fishes were placed in breeding tanks with an equal ratio and kept in the dark to induce spawning. The fish were exposed to light for 40 minutes the next morning after the barrier was removed. The fertilized embryos

were collected with a filter and placed in a petri dish containing E3 medium. The collected embryos were washed twice with E3 medium and unfertilized and undeveloped embryos were removed with pasture pipette by observing under a stereomicroscope (NIKON stereo microscope).

Drug Dilutions and *In vitro* Embryo Toxicity Assay

The embryotoxicity test was carried out according to the OECD guideline 236 [15]. The mother tincture of the drugs *Aconitum napellus* and *Digitalis purpurea* were purchased from Bhandari Homeopathic laboratories, Faridabad, India. The mother tincture was briskly shaken and diluted serially in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM, $MgSO_4$) on X scale (Homeopathy decimal scale) ranged from 10^{-1} to 10^{-12}. Embryos with the four-cell stage (n=20/dilution) were chosen and exposed to diluted drugs for 96h in 12 well plates with 3ml of drug solution from appropriate dilution. E3 medium without drugs was used as control. Plates were incubated at 26 ± 1ºC with a 14:10 LD cycle. The solutions were changed every day until the end of the experiment.

Mortality and Hatching Rate of Treated Embryos

The number of dead embryos was recorded and removed once in every 24hours. The percentage of mortality for different dilutions of the drug was computed based on the number of dead embryos at each time interval. The hatching rate was calculated at the following three different time points: 48hpf, 72hpf, and 96hpf.

Analysis of Heartbeat Rate

The heartbeat rate was counted manually in both control and drug-treated groups under a stereomicroscope at 96hpf. Embryos were immobilized using 3% methylcellulose in a glass slide and positioned using an embryo loop. The number of heartbeat per 30sec was calculated.

Analysis of Developmental Abnormalities

The developmental stages were monitored every 24h, until 96hpf under a stereomicroscope (NIKON Eclipse), and compared to the stages described by Kimmel *et al* [16]. The developmental stages were documented pictorially and analyzed for possible abnormalities.

Statistical Analysis

All experiments were carried out in triplicates. The mortality and hatching rate was calculated and represented in percentage. The heartbeat rate of embryos with various concentrations (10^{-1} to 10^{-12}) of homeopathy drugs was analyzed by one-way ANOVA with Dunn's multiple comparisons. Statistical significance was accepted at $p<0.05$. All statistical analyses were performed using GraphPad Prism 6 (Version 6.01).

RESULT

Mortality

Mortality was recorded in every 24h and the cumulativepercentage of mortality was determined after 96hpf.The result showed that 100% of mortality was observed in 10^{-1} dilution in both drugs and both resulted in immediate mortality. In addition, *Digitalis purpurea* (DP) resulted in 100% mortality with 10^{-2} and10^{-3} dilutionsat 6hpf and 96hpf respectively (Fig 1).

Hatching Rate

In zebrafish, hatching starts from 48hrs and maximum hatching occurs in 72hpf [16]. In this study, the hatching rate of embryos was noted from 48hpf to 96hpf. It was found to be 100% at 96hpf with 10^{-5} to 10^{-12} dilutions. Comparison between concentrations of drugs and hatching rate didn't show any significant variation in both drugs. However, the percentage of hatching rate was more than 50% with *A. napellus* at 48hpf. In case of *D. purpurea,* observed hatching rate was less than 50% at 48hpf (Fig.2).

Heartbeat Rate

Heartbeat rate was monitored at 96hpf. Compared with control group, A. *napellus* treated groups showed significantly reduced heart rate in 10^{-2} dilution (Fig.3.a) whereas with *Digitalis purpureait was observed* in 10^{-4} dilution (Fig.3.b). However, no significant variation was observed with remaining dilutions of both drugs.

Developmental Deformities

Developmental deformities such as pericardial edema, tail and jaw deformation, and cornea development were observed at lower dilutions. Among them, pericardial edema was predominatly observed on embryos treated with *A. napellus* at 10^{-2}dilution and at 10^{-2} and10^{-3} dilutions with *Digitalis purpurea* (Fig 4 A and B.). With lower dilutions, no significant malformations were observed.

DISCUSSION

The toxicity testing of pharmacological products gives a better understanding about the non-lethal concentration of substances and also gives the conceivable toxic effects of those products [17]. In the present study, the toxicity potential of homeopathy drugs *A.napellus* and *D. purpurea* was investigated using zebrafish embryos, which is the best model for screening toxicity potential for drugs and toxic compounds [18]. Though the drug is used to treat cardiac ailment, the toxicity levels should be standardized for different model organism because the toxic level and efficacy of the drug depends on species, size and age of the testing organisms [19]. Identifying effective concentrations allows researchers to investigate the molecular aspects of the drug and as well as to treat the heart diseases in the model organism.

In this study, *A. napellus (AN)* caused 100% mortality athigher concentration (10^{-1}), whereas treatment with*D. purpurea* (DP) showed 100% mortality with 10^{-1}, $10^{-2}$and 10^{-3}dilutions in zebrafish embryos. Aconite present in the *A. napellus* interacts with the voltage-dependent sodium channel present on cell membranes of excitable tissues, including myocardium, striated and smooth muscle, and neurons, altering membrane depolarization and repolarization. It showed high affinity to the voltage-sensitive sodium channel in its open state and inhibit the conformational change to the inactive state [20]. It delays repolarization by prolonging sodium influx and membrane depolarization[21]. Moreover, aconite increases the strength of muscle fibre contraction by increasing acetylcholine release from nerve endings at lower concentrations. At higher concentrations, aconite depress the muscle contraction by maintain the sodium channels in its open state and reduced the release of acetylcholine at axonal end [20, 22].Recently the cardio protective role of aconite was studied in rats and implies that aconite enhances the heart function by exerting anti myocardial ischemia effect through the PI3K/Akt signalling pathway and also reducing myocardial fibrosis, inflammatory responses [23]. In the present study, as expected reduced heart rate was observed with lower dilutions (10^{-2} to 10^{-5}) and normal heartbeat rate was observed with higher dilutions.

D. purpurea contains a cardiac glycoside, digoxin which has the potential to modulates the heart functions and acts as an anti-inflammatory, anti-oxidant, anti-tumor, and hepatoprotective agent [24,25]. Digoxin inhibit the Na+ /K+ -ATPase pump by binding the K+ binding site in the myocardium, which results in increase in the intracellular sodium concentration at the same time decrease in potassium concentration. This elevated sodium level results in increased intracellular calcium level, which raises the action potential of cardiac cells [26].

Aconite and Digitalis have long been explored for their cardio protective properties [27]. Antioxidant and anti-inflammatory potential of these drugs are important in preventing cardiovascular diseases [24,25,28]. Meanwhile, a case study reveals that both the drug induces cardiotoxicity when ingested at higher concentration; in both cases patients took the medicine without proper advice from the physicians [29, 30]. In the present study lower dilutions of drugs reduce the heart beat rate, whereas normal heart rate was not affected with higher dilutions of the drugs. Among the developmental deformities, we found that *A. napellus (AN), and D. purpurea* (DP) resulted in 100% pericardial edema at 10^{-2} and 10^{-2} and 10^{-3} dilutions respectively. As a response to cardiotoxicity induced by the drug

at higher concentrations, pericardial edema, reduced heart rate, and high mortality were observed. In addition, cardioavascular functions were not affected at lower concentrations of the drugs. It is clear from the above study that lower dilutions of the both drugs causes morphological and physiological changes in the heart, but in higher dilutions, no such effects were observed.

CONCLUSION

Homeopathy drugs are prepared from material which is highly toxic by nature. From this study, we conclude that higher dilutions' ranging from 10^{-6} to 10^{-12} doesn't show any significant effects on developing zebrafish embryos. Hence, higher dilutions of *A. napellus (AN),* and *D. purpurea* (DP) might be utilised to investigate the cardio protective roles against the cardiac problem induced by toxic substances using zebrafish embryos..

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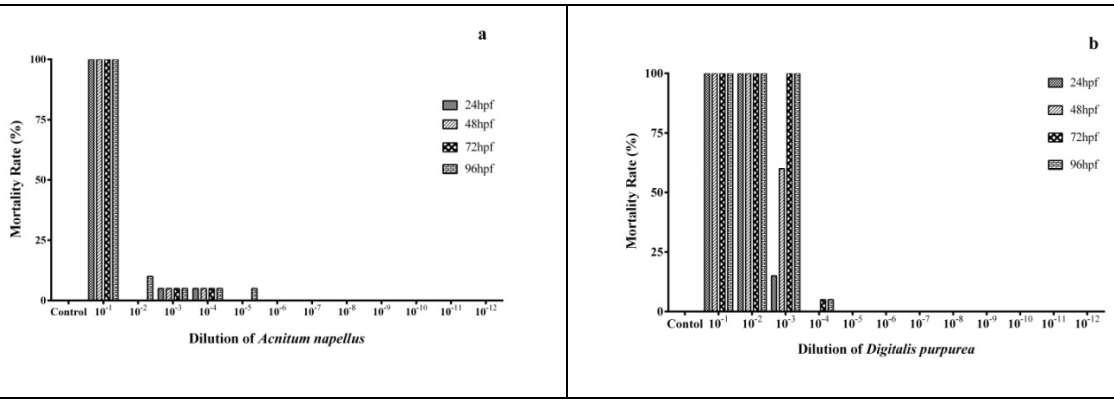

Fig.1. Mortality rate of zebrafish embryos treated with homeopathy drugs (a) *Aconitum napellus* (AN) and (b) *Digitalis purpurea* (DP)after 96 hpf.

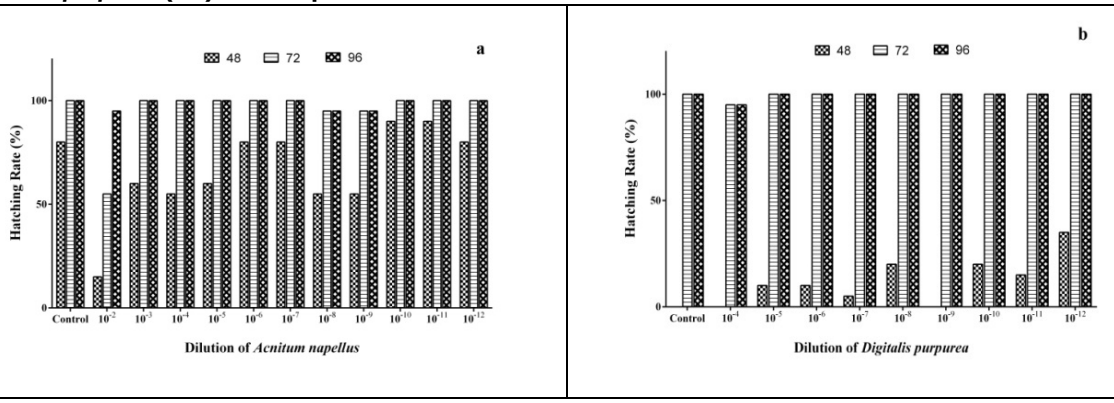

Fig.2. Hatching rate of zebrafish embryos treated with homeopathy drugs (a) *Aconitum napellus* (AN) and (b) *Digitalis purpurea* (DP)after 96 hpf.

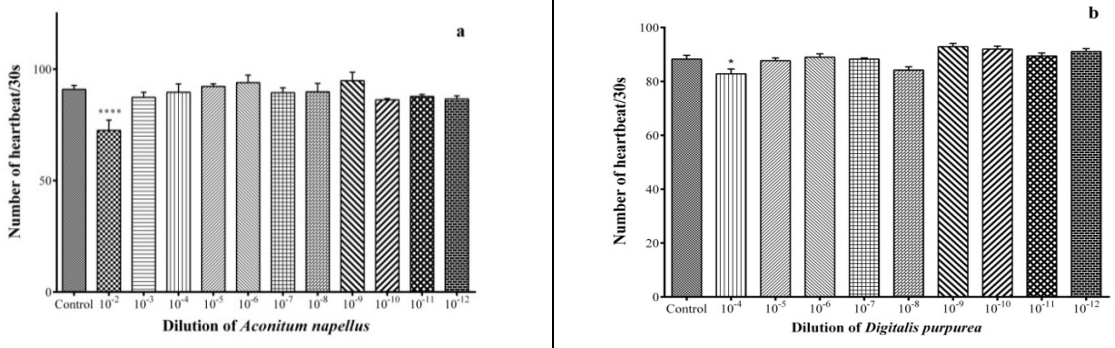

Fig. 3. Heartbeat rate of embryos treated with homeopathy drugs after 96 hpf(a) *Aconitum napellus* (AN), and (b) *Digitalis purpurea* (DP). Data are expressed as the mean ± SEM and asterisks (*) indicate statistically significant differences between groups, *p<0.05; **p<0.01; ***p<0.001; ****p<0.001 (One-way ANOVA with Dunn's multiple comparison).

Fig. 4. Developmental and malformations of zebrafish embryos treated with (A) *Aconitum napellus* (AN), and (B) *Digitalis purpurea* (DP). Number represents the concentration of used drugs. Pericardial edema (PE) was observed with the lower dilutions of both drugs.

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RESEARCH ARTICLE

An Overview on Nanocrystals in Pharmaceutical Drug Delivery Systems

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ABSTRACT

Drug nanocrystals offer a beautiful approach for improving the solubility and dissolution rate of poorly soluble drugs which accounts for nearly 40 % newly discovered drug molecules. poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. Drug nanocrystals are nano sized particles of pharmacologically active substances. They're used as a physical approach to change and improve the pharmacokinetic and pharmaco-dynamic properties of varied sorts of drug molecules. They need been utilized in vivo to guard the drug entity within the circulation. The tactic of preparations of nanocrystal is top down and bottom up, spray drying then new techniques. There are several important advantages of nanocrystal formulations like, enhanced oral bioavailability, improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration thanks to decreased particle size range. Selection depends upon the location s and to deliver the drug at a controlled and sustained rate to the site of action. Here, we review various aspects of nanocrystals formulation, characterization, effect of their characteristics and their pharmaceutical applications in delivery of drug molecules and therapeutic genes.

Keywords: Drug nanocrystals, solubility, bioavailability, drug delivery.

INTRODUCTION

Nanocrystals are crystalline nanoparticles with size starting from 200 to 500 nm stabilized by surface stabilizers. They increase the saturation solubility, dissolution rate and doubtless the muco-adhesion leading to improved oral bioavailability of medicine exhibiting dissolution rate dependent bioavailability [1].

The concept of nanocrystal was first introduced within the nineteenth century, and despite fantastic efficacy, their availability as market products is limited. Currently, nanocrystal drugs gained attention as a challenging approach due to an increasing number of poorly soluble drugs within the drug developmental process, safe formulation, and pharmaco-economic value. Pharmaceutical industries also can gain benefit by possibly redesigning a line of a previously existed formulation by nanocrystal technology. There are various techniques involved to organize nanocrystals which may be categorized as bottom-up, top-down, and combination technique [2]. The bottom-up technique isn't commonly utilized in the manufacturing of economic nanocrystals. There are various problems just like the got to remove the solvent, challenges in process optimization, and lots of poorly soluble drugs neither soluble in aqueous media nor organic media related to this system [3]. The number of poorly soluble drugs increasing day by day requires innovative formulation approaches to succeed in a sufficiently high bioavailability after oral administration or a minimum of form available intravenously injectable forms. There are number of formulation approaches for drugs being poorly soluble in water, e.g., the utilization of solvent mixtures, solid dispersion, micro-emulsion, cyclodextrins, or o/w emulsions for intravenous administration, and salt formation etc. A meanwhile classical formulation approach to process poorly soluble drugs is micronation meaning converting the coarse drug powder to an ultrafine powder with a mean particle size within the range of 2–5 μm and particle size distributions normally range from approximately 0.1 to 25 μm [4].

Micronation may be a very simple technology achieved by jet milling or wet milling. Micronation may be a technology for sophistication II drugs of the biopharmaceutical arrangement (BCS), i.e., drugs having an honest permeability but a coffee oral bioavailability thanks to their poor solubility and low dissolution velocity. The principle for enhancement of solubility was to extend the dissolution velocity by enlarging the area of the drug powder. Nano-crystalline drug technology improves the solubility of hydrophobic drugs, an increased area to volume ratio and improved dissolution rates (i.e., dissolution velocity) related to nanosizing [5].

Properties of Nanocrystals

Increase of Dissolution Velocity by Surface Area Enlargement

The size reduction results in an increased area and thus consistent with the Noyes-Whitney equation (Noyes and Whitney 1897) to an increased dissolution velocity. Therefore, micronation may be a suitable thanks to successfully enhance the bioavailability of medicine where the dissolution velocity is that the rate limiting step. By moving from micronation further right down to nanonization, the particle surface is further increased and thus the dissolution velocity increases too. In most cases, a coffee dissolution velocity is correlated with low saturation solubility.

Enhanced Saturation Solubility

The saturation solubility may be a constant value and depends upon the character of the compound, the medium of dissolution, and therefore the temperature conditions. This fact is applicable to powder drugs within the size range of micrometre/nm. However, the dimensions range below 1–2 μm is additionally depended upon particle size and crystalline structure. The saturation solubility is inversely proportional to particle size. The dissolution rate of nanocrystals is often understood by the Noyes Whitney equation. For nanocrystal drugs, the saturation solubility (C) and area (A) are directly proportional to the speed of dissolution. For e.g., the rise in saturation solubility (C) and area of the surface (A) results in increase the speed of dissolution (dx/dt).

Increased Adhesiveness

Nanocrystals have natural adhesiveness for biological mucosa including gastrointestinal mucosa. The nanocrystals present in suspension or generated after the disintegration of the solid dosage form, get attached to the gastrointestinal mucosa thanks to the formation of hydrogen bonds and van der Waals bonds between the surfaces of particles and mucus. The prolonged retention of nanocrystals generates a better concentration gradient across the GIT (Gastrointestinal tract) which ultimately improves the absorption of the drug and hence the bioavailability**.**

Improved Stability

Being a simple formulation, composed of mainly of the drug and stabilizers (in the case of nano suspension, it also includes an appropriate dispersion medium), the probability of reaction within the drug nanocrystals system is far less compared to other approaches during which many other excipients are required to possess a stable final formulation.

Improved Bioavailability

An improvement within the dissolution velocity and saturation solubility of a drug generates a high concentration gradient across the membrane to possess rapid diffusion and hence improves the bioavailability.

The Versatility of Final Dosage Form

The flexibility in adjusting surface properties and regulating the dimensions along-side simple post-production enables the nanocrystals to be incorporated in several dosage forms like tablets, pellets, capsules, dry suspension and hydrogels [6-8].

Nanocrystal Preparation Methods

Several preparation methods devolved today, implemented preparation methods of Nanocrystal formulations are often classified as “bottom up”, “top-down”, “top down and bottom up” and “spray drying”. “Bottom up” technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation. “Top down” technology applies dispersing methods by using differing types of milling and homogenization techniques. “Top down” technology is more popular than “Bottom up” technology; it's referred to as “nanosizing”. In other words, it's a process which breaks down large crystalline particles into small pieces. In “top down and bottom up” technology, both methods are utilized together. Spray drying is additionally a way for preparing drug nanocrystals, which is quicker and more practical compared to the opposite methods.

Bottom-up Technology

- Anti-solvent precipitation
- Supercritical fluids
- Spray-drying

Top-down Technology

2.1 Media milling
2.1.1. Bead milling
2.1.2. Dry co-grind
2.2 High pressure homogenizations
2.2.1. Homogenization in Aqueous media (Disso cubes)
2.2.2. Homogenization in Non-Aqueous Media (Nano-pure)
2.2.3 Nano jet technology
2.3 Emulsion solvent diffusion method

Combination technology

- NANOEDGE® Technology
- Smart-Crystal® Technology

Other methods

- Solvent evaporation
- Sono-crystallization
- Melt emulsification
- Bottom-Up Nano-CrySP Technology.9

Bottom-up Technology

Principal of this technology is based on precipitation by dissolving the drug in a solvent and adding the solvent to a non-solvent that cause precipitation of the fine drug particle.

Precipitation Methods

The drug is dissolved during a solvent and subsequently added to a non-solvent, resulting in the precipitation of finely dispersed drug nanocrystals. nanocrystals got to be stabilized so as once they aren't allowed to grow to the micro-meter range. The drug must be soluble in a minimum of one solvent, which creates problems for newly developed drugs that are insoluble in both aqueous and organic media. an answer of the carotenoid, along-side a surfactant in digestible oil, is mixed with an appropriate solvent at a selected temperature. to get the answer a protective colloid is added. This results in an O/W two phase system. The carotenoid stabilized by the colloid localizes within the oily phase. After Lyophilisation X-ray analysis shows that approximately 90% of the carotenoid is in an amorphous state.

Supercritical Fluid Methods

Nanoparticles are produced by various methods like rapid expansion of supercritical solution (RESS) process, supercritical anti-solvent process, and precipitation with compressed Anti-solvent (PCA) process. In RESS technique, drug solution is expanded through a nozzle into supercritical fluid, leading to precipitation of the drug as fine particles by loss of solvent power of the supercritical fluid. Young et al. prepared cyclosporine nanoparticles having diameter of 400 to 700 nm by using this system. within the PCA method, the drug solution is atomized into the CO2 compressed chamber. because the removal of solvent occurs, the answer gets supersaturated and eventually precipitation occurs. In supercritical Anti-solvent process, drug solution is injected into the supercritical fluid and therefore the solvent gets extracted also because the drug solution becomes supersaturated. the essential disadvantages of this methods are use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques.

Spray Drying

This method is typically used for drying of solutions and suspensions. during a conical or cylindrical cyclone, solution droplets are sprayed from top to bottom, dried within the same direction by hot air and spherical particles are obtained. Spraying is formed with an atomizer which rapidly rotates and provides scattering of the answer thanks to centrifugal effect. the answer, at a particular flow, is shipped to the tube with a peristaltic pump, nitrogen or air at a continuing pressure is shipped to the outer tube. Spraying is provided by a nozzle. Droplets of solution become very small thanks to spraying; therefore, area of the drying matter increases resulting in fast drying. Concentration, viscosity, temperature and spray rate of the answer are often adjusted, and particle size, fluidity and drying speed are often optimized. The dissolution rate and bioavailability of several drugs, including hydrocortisone, Cox-2 inhibitor (BMS-347070) were improved utilizing this method.[10]

Top-down Technology:

The "Top-down Technologies" are the disintegration methods and are preferred over the precipitation methods.

Media Milling (Nanocrystals or Nano systems)

Bead Milling

The method is first developed by Liversidge et. al. during this method the Nano-suspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. Shear forces of impact, generated by the movement of the milling media, cause particle size reduction the milling medium is formed from glass, zirconia or highly cross-linked polystyrene resin. The milling chamber is fed with the milling media, water, drug and stabilizer then milling media or pearls are then rotated at a high shear rate. The milling process is performed under controlled temperatures. The Nano-suspension or nanoparticles are form as results of high energy and shear forces generated thanks to the impaction of the milling

media with the drug which give the energy input to interrupt the micro particulate drug into Nano-sized particles. The media milling procedure can successfully process micronized and non-micronized drug crystals. to scale back the quantity of impurities caused by erosion of the milling media, the milling beads are coated. There are two basic milling principles. Either the milling medium is moved by an agitator, or the entire container is moved during a complex movement leading consequently to a movement of the milling media. The milling time depends on many factors like the surfactant content, hardness of the drug, viscosity, temperature, energy input, size of the milling media. The milling time can last from about half-hour to hours or several days.11

Co-grinding Stable

Nano-suspensions are formulated using dry grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing during a liquid media. it's the colloidal particles formation of the many poorly water-soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with poly vinyl pyrrolidone (PVP) and sodium dodecyl sulfate (SDS). Various soluble polymers and co-polymers like PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrins derivatives are used. By using this method, the physicochemical properties and dissolution of poorly water-soluble drugs were improved due to an improvement within the surface polarity and transformation from a crystalline to an amorphous drug. Dry co grinding are often administered easily and economically and may be conducted without need of organic solvents.12

High Pressure Homogenization:

When producing nanocrystals using homogenization methods, there are three important technologies namely: Microfluidizer technology (Nano jet technology), Piston gap homogenization in aqueous media (Dissocubes® technology) and in water mixtures or in nonaqueous media (Nanopure® technology).

Micro-Fluidizer Technology (Nano- Jet Technology)

This technology called opposite stream or Nano-jet technology. This method contains Micro-fluidizer which uses a chamber where a stream of suspension is split into two or more parts, which colloid with one another at high. This results in particle collision, shear forces and cavitation forces. The high shear force produced during the method due particle collision and high leads to particle size reduction. Equipment using this principle includes the M110L and M110S micro fluidizers. Dearn prepared nano-suspensions of atovaquone using the micro fluidization process. the main disadvantage of this system is that the high number of passes through the micro fluidizer which the merchandise obtained contains a comparatively larger fraction of micro-particles.

Piston Gap Homogenization In Aqueous Media (Disso-Cubes)

This technology was developed by R.H.Muller in 1999 and first patent was taken by DDS Gmbh and afterward the patent was transferred to Skype pharmaceuticals. Commonly used homogenizer is the APV Micron Lab 40 (APV Deutschland Gmbh, Lubeck, Germany) and piston-gap homogenizers. During this method, the suspension containing a drug and surfactant is forced struggling through a Nano-sized aperture valve of a high homogenizer. during this method the particle size reduction depends on cavitation principle. The dispersion present in 3cm diameter cylinder is suddenly skilled a narrow gap of 25µm. consistent with Bernoulli's law the flow volume of liquid during a closed system per cross section is constant. It results in increase in dynamic pressure and reduce of static pressure below the boiling point of water at diameter from 3cm to 25µm.Then water starts boiling at temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal atmospheric pressure is reached. The particles cavitation forces are sufficiently high to convert the drug micro particles into nanoparticles. during this the ultimate particle size of drug nanocrystals is predicated on power density of homogenizer, number of homogenization cycles, temperature and homogenization pressure.

Homogenization in Non-Aqueous Media (Nano-Pure)

during this technology suspension is homogenized in water-free media or water mixtures. within the Disso-cubes technology the cavitation is that the principle determining factor of the method oils and oily fatty acids have very

low vapour pressure and a high boiling point as compared to water. Hence, the drop of static pressure won't be sufficient to initiate cavitation. Patents covering disintegration of polymeric material by high homogenization mention that higher temperatures of about 80°C promoted disintegration, which can't be used for thermo- labile compounds. In nano- pure technology, the drug suspensions within the non- aqueous media were homogenized at 0°C or maybe below the melting point and hence are called "deep-freeze" homogenization. The results obtained were like Disso-cubes and hence are often used effectively for thermo- labile substances at milder conditions.13-16

Emulsion Solvent Diffusion Method

The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents are often used because the dispersed particles of the emulsion. An organic solvent or mixture of solvents loaded with the drug is dispersed within the aqueous phase containing suitable surfactants with stirring to make an emulsion. The obtained emulsion was further homogenized by high homogenization. After homogenization cycles the emulsion was diluted with water, homogenized by homogenizer to diffuse the organic solvent and convert the droplets into solid particles. Since one particle is made in each emulsion droplet, it's possible to regulate the particle size of the Nano-suspension by controlling the dimensions of the emulsion. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading within the emulsion. Originally methanol, ethanol, ester, chloroform used as organic solvents. However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Nano-suspension of ibuprofen, diclofenac, and acyclovir were prepared by this method.17

Combination Technology

Nanoedge™

The basic principles of NANOEDGE are an equivalent as that of precipitation and homogenization. a mixture of those techniques leads to smaller particle size and better stability during a shorter time. the main drawback of the precipitation technique, like crystal growth and future stability, are often resolved using the NANOEDGE technology. during this technique, the precipitated suspension is further homogenized, resulting in reduction in particle size and avoiding crystal growth. Precipitation is performed in water using water miscible solvents like methanol, ethanol and isopropanol. it's desirable to get rid of those solvents completely, although they will be tolerated to a particular extent within the formulation. For an efficient production of Nano-suspensions using the NANOEDGE technology, an evaporation step often included to supply a solvent-free modified starting material followed by high-pressure homogenization**.**

Smart Crystal® technology

This technology was first developed by Pharma Sol GmbH and was later acquired by Abbott. it's a toolbox of various combination processes during which process variations are often chosen depending upon the physical characteristics of the drug (such as hardness). the method H42 involves a mixture of spray-drying and HPH. Within few homogenizations cycles the nanocrystals are prepared. Process H69 (Precipitation and HPH) and H96 (lyophilization and HPH) yield nanocrystals of amphotericin B within a size range of about 50 nm. S. Kobierski et al. (2008) produced nanocrystals during a two-step process i.e., pre- milling followed by high homogenization (HPH). Nano-suspensions of cosmetic active hesperidin were produced by ball milling process and with combination process. Prepared nano-suspensions were kept for storage. Nano- suspension prepared using Smart Crystal® technology was found to be of a smaller size indicating better physical stability.18-19

Other Technologies

Solvent Evaporation

During this method, the solutions of polymer are prepared in volatile solvents and emulsions. But from previous couple of years dichloromethane and chloroform were used which was now replaced by ester which features a better profile of toxicology. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continual phase of the emulsion. within the conventional methods,

two main strategies are getting used for the formation of emulsions, the preparation of single emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods require high-speed homogenization or ultra-sonication, followed by evaporation of the solvent, either by continuous magnetic stirring at temperature or under reduced pressure. By ultracentrifugation the solidified nanoparticles are collected which was washed with water to get rid of the additives like surfactants, then it had been lyophilized. The particle size was influenced by the concentration of polymer, stabilizer and therefore the speed of homogenizer.

Sono-Crystallization

The novel approach for particle size reduction on the idea of crystallization by using ultrasound is Sono crystallization. Sono-crystallization utilizes ultrasound power characterized by a frequency range of 20-100 kHz for inducing crystallization. It not only enhances the nucleation rate but also an efficient means of size reduction & controlling size distribution of the active pharmaceutical ingredient (API). Most applications used ultrasound within the range 20 kHz -5 MHz, Sono-crystallization technique or technology has also been studied to switch the undesirables of NSAID'S i.e., poor solubility and dissolution rate and consequently the poor bioavailability**.**

Melt Emulsification Method

Solid lipid nanoparticles are mainly prepared by melt emulsification method. Kipp and co-workers firstly prepare Nano-suspensions of ibuprofen by using melt emulsification method. It is a four-step procedure. Drug is first added to aqueous solution having stabilizer. The solution is heated at temperature higher than the melting point of the drug and then homogenized by high-speed homogenizer for the formation of emulsion. The temperature is maintained above the melting point of the drug during overall process. Finally, the emulsion is cooled to precipitate the particles. The particle size of Nano-suspension mainly depends on parameters like drug concentration, concentration and type of stabilizers used, cooling temperature, and homogenization process.

Bottom-Up Nano-Crysp Technology

Introduced a more modern method to get Nano crystalline solid dispersion (NSD) of "hesperetin" using Nano-CrySP technology. a completely unique bottom-up process supported spray drying to get solid particles containing drug nanocrystals dispersed within the matrix of small molecule excipients the aim of their study to improved oral bioavailability and pharmacodynamics activity of hesperetin nanocrystals generated employing a novel bottom-up nano-CrySP Technology. Hesperetin and mannitol were utilized in 1:1 ratio and NSD was generated using spray drying. the method of NSD formation is predicated on classical nucleation theory wherein mannitol contributed to crystallization of hesperetin by acting as plasticizer, crystallization inducer and by providing heterogeneous nucleation sites. Hesperetin was found to exist as nanocrystals dispersed within the matrix of mannitol with average crystallite size of 137 nm within the NSD.20-22

Stabilization of Drug Nanocrystals

Drug nanocrystals are nano-sized solid drug particles surrounded by a stabilizer layer. Often nanocrystals are considerably easy to supply, but the steadiness and therefore the selection of stabilizer is that the most challenging and important step. Stabilizers stabilize the newly formed drug nanocrystals, but they even have a crucial role in further formulation and that they even affect the drugs bioavailability. Mostly, the stabilizer selection is predicated purely on the need of physical stability, e.g., maintaining the nano-sized particle size if possible after the formation of drug nanocrystals. The massive area of nanocrystals leads to sufficiently high free energy or surface charge which may cause attraction or agglomeration. Small sized nanocrystals sometimes raise the solubility of drug beyond the saturation which promotes recrystallization into larger particles; also referred to as Ostwald ripening. These processes ultimately cause irreversible loss of formulation integrity. Agglomeration is prevented by the presence of stabilizers within the nano-formulation. Stabilizers can spontaneously adsorb on and canopy the newly formed particle surface to:

(a) decrease the free energy of system and interfacial (surface) tension of particles.
(b) form a dense hydrophilic layer around hydrophobic particles, provide steric hindrance and steep repulsions between the particles (steric stabilization).
(c) charge the particle surface if the stabilizer has ionisable groups, which increase the repulsive force (electrostatic stabilization).
(d) combine the steric and electrostatic stabilization.23-26

Mechanism of the Stabilization

Electrostatic Stabilization: -- Adsorption of the ions to the surface leads to mutual repulsive forces between the particles. Increase within the ionic strength influence the repulsion and reduce the thickness of electrical double layer which results in a decrease within the repulsion potential of the particles**.**

Steric Stabilization: Adsorption of the non-ionic amphipathic particles on the surface of the particles. within the case of the steric stabilization, the adsorbed polymer doesn't possess charge.

Electro-steric Stabilization: Electro-steric stabilization is that the combination of both above mentioned stabilizations. Electro-steric stabilization also can be provided by employing a combination of two different stabilizers, an ionic surfactant and a polymer, respectively.[27]

Characterization of Nano Crystals

Particle Size Analysis: Size and size distribution of the crystals in dried form was determined following redispersion in water containing 0.1% polyvinyl alcohol (PVA&403) by dynamic light scattering through particle size analyser Nanotrac 150 (Japan) with a wet sampling system and the diameters reported were calculated using mean particle size distribution.

Determination of Drug Content: The drug content of freeze-dried samples was checked by UV & spectrophotometer to confirm the purity of the prepared samples. For quantitative determination of drug content in formulations aqueous dispersions of formulations (25mg/10ml distilled water) were passed through 0.8 <m filter.The filtrates containing fine particles smaller than 0.8<m was dissolved in 4% sodium lauryl sulphate solution and the concentration of drug was determined by spectrophotometry at a wavelength of 291 nm. The amount of drug in filtrate relative to the total amount of drug in the dispersion was calculated and expressed as nanocrystal yield.28

Scanning Electron Microscopy: The surface morphology of the commercial drug powder and the freeze-dried formulation samples was examined by SEM. Before examinations the samples were mounted on top of double-sided sticky carbon tape on metal discs and coated with 80 nm Gold/palladium in Blazers 120B sputtering device.

Powder X-Ray Diffraction (PXRD): The PXRD was carried out using Philips Analytical XRD B.V. at the scanning rate of 40 /min 2θ range of 10&70°C.

Differential Scanning Calorimeter: DSC, equipped with a liquid nitrogen cooling system was used to measure the thermal behaviour of the commercial griseofulvin powder and the freeze-dried samples. In DSC analysis, 2&5 mg of sample was put in aluminium pan and examined at a scanning rate of 100 C/min from 25 to 300°C.[29]

Solubility: Saturation solubility measurements were assayed through ultraviolet absorbance determination at 291 nm using an UV spectrophotometer. An excess amount of griseofulvin powder and formulations were added to 150 ml of 4% SLS solution the mixture was stirred in mechanical shaker for 24 hours at a temperature of 37+ .05 0 C using GLF 1086 shaker. Visual inspection was carefully made to ensure there was excess sample in solid state indicating that saturation had been reached. The mixtures were filtered using 0.2<m filter and filtrates were diluted suitable to determine the solubility of griseofulvin from each formulation.

Dissolution Test: A dissolution test for commercially available griseofulvin and formulations was carried out by filling them in hard gelatine capsules (Zydus Cadilla, Goa, India). The prepared samples and the drug powder were filled in capsules (125mg) and subjected to dissolution studies with 900 ml 4% SLS solution as dissolution medium preheated and maintained at 37 + 0.5 0 C. The baskets were rotated at a speed of 75 rpm/min. 10ml samples were withdrawn at specified time intervals, filtered through 0.2<m filter, and the concentration of was determined by UV& spectrophotometer.

Stability Studies: All the formulations were subjected to stability study as per ICH guidelines the formulations were divided into two parts and stored at 30 o ± 2 o C and 65% ± 5% RH and 40 o ± 2 o C and 70% ± 5% RH. The drug release and the drug content were estimated after specified intervals of time.30

Nanocrystals (Nano-suspensions) and Bioavailability

The bioavailability of a drug depends on its ability to dissolve in biological fluids, cross membranes, and efficiently reach its pharmacological target. within the biopharmaceutical classification of medicine, drugs of the category II group are characterized by poor solubility but have an honest ability to cross membranes. Thus, to enhance the bioavailability of a category II drug, it's necessary to extend drug solubility and/or the drug dissolution rate. Especially, for nanocrystals, it's possible to think about the subsequent scenarios:

1. A decrease in particle size results in a rise in area available for the interaction with the dissolution media, and thus a rise within the particle dissolution rate, in accordance with the modified Noyes-Whitney law.
2. a rise within the particle curvature (particularly pronounced for colloidal particles) results in a rise in dissolution pressure, consistent with the Kelvin's equation.
3. Increased solubility results in an increased concentration gradient at membranes, and thus subsequently to higher penetration or permeation through membranes.
4. High penetration through membranes is additionally favoured by high adhesion to biological membranes of nanocrystals, favoured by their size, but adhesion is often also improved by the coating with mucoadhesive polymer.
5. consistent with several authors, the transcellular uptake of nanocrystals through epithelial cells is one more reason for the enhancement of bioavailability.
6. Nanocrystals are often administered by injection (nano suspensions) and are ready to efficiently reach the target tissue or organ with 100% bioavailability.
7. Targeting are often promoted by coating nanocrystals with molecules that are ready to interact with specific substrates.31-33

Advantages of Nanocrystals

1. It are often given by any route of administration.
2. Enhanced solubility and bioavailability of drug.
3. Reduced tissue irritation just in case of subcutaneous/intramuscular administration.
4. Rapid dissolution & tissue targeting are often achieved by IV route of administration.
5. Oral administration of Nano suspension provide rapid onset, reduced fed/fasted ratio& improved bioavailability.
6. The absorption form absorption window are often increased, thanks to reduction within the particle size.
7. It are often incorporated in tablets, pellets, hydrogel & suppositories are suitable for various routes of administration.
8. Increasing the amorphous fraction within the particles resulting in a possible change within the crystalline structure & higher solubility.
9. Possibility of surface-modification of Nano-suspension for site specific delivery.
10. Higher drug loading are often achieved.
11. future physical and chemical stability (due to absence of Ostwald ripening).34-35

Disadvantages of Nanocrystals

a) Physical stability, sedimentation & compaction can cause problems.

b) its bulky sufficient care must be taken during handling & transport.
c) Uniform & accurate dose can't be achieved.
d) High-cost instruments are required for production of drug nanocrystal that increases the value of dosage form.
e)High-cost instruments are required for production of drug nanocrystal that increases the value of dosage form.36

Application

Oral Drug Delivery

Oral delivery is that the first choice in drug therapy, due to safety, patient compliance, simple production, and scalability, though the principal limitations are associated with drug bioavailability. Nanocrystals may improve bioavailability through a rise in solubility and particle dissolution, and thru an increased gradient concentration at membranes and adhesion to the gastrointestinal wall.The primary works highlighting this idea was administered on danazol, which may be a poorly soluble drug exhibiting poor bioavailability and was formulated as three different formulations: an aqueous nano-suspension (169 nm), a danazol-hydroxypropyl-β-cyclodextrin complex, and an aqueous micro suspension (10 µm). Under the curve (AUC) after oral administration in beagle dogs revealed that the nano suspension and therefore the cyclodextrin complex had similar levels of bioavailability, while the bioavailability of the micro-suspension was lower. the higher performance of the aqueous nano-suspension compared to the aqueous micro suspension was explained through the previous overcoming the limited dissolution rate normally observed with conventional suspensions. The authors thus proposed nanoparticles because the appropriate formulation for dissolution-rate limited absorption. In a lutein nano-suspension prepared by high homogenization, nanocrystals exhibited a fold increase in saturation solubility compared thereto of coarse powder. The in vitro release of nanocrystals delivered in pellets and hard gelatine capsules for nutraceutical use was three to fourfold greater than that of coarse particles.37

Intravenous Drug Delivery

Due to their particle size, nanocrystals (nano-suspension) have the good advantage of being intravenously injectable, reaching 100% bioavailability. Nanocrystals within the range of 100–300 nm are often injected intravenously with unwanted effect, like the obstruction of small capillaries. Consequently, nanoparticles circulate within the bloodstream and dissolve consistent with their dissolution properties, then are ready to reach the target tissue.One of the foremost powerful applications of the injection of drug nanocrystal suspensions is that the delivery of anticancer drugs, nanocrystal formulations seem to permeate tumours tissues showing effective anticancer activity and fewer toxic effects than conventional formulations.The improved permeability and retention effect (EPR) of paclitaxel nanocrystals was like that of conventional formulation, but the distribution into the tumours and organs (brain, liver, spleen, hearth) was different, probably due to a rapid accumulation of paclitaxel nanocrystals into the macrophages. Thus, albeit in both formulations the drug accumulation didn't exceed 1% into the tumours, which is that the dose that's considered efficacious to treat the tumours, the build-up of paclitaxel in health organs was inferior thereto of conventional formulation, explaining the lower systemic toxicity. Another potent breast and lung anticancer drug, amacrine, which is an inhibitor of topoisomerase II, was formulated as nanocrystals by high-pressure homogenization and subsequent lyophilization.38

Pulmonary Drug Delivery

Poorly soluble drugs are often delivered on to the lungs by nebulizing the aqueous nano-suspensions using mechanical or ultrasonic nebulizers. Using nanoparticles, drug is more evenly distributed in droplets. All aerosol droplets are likely to contain drug nanocrystals. Budesonide, poorly water-soluble corticosteroid, has been successfully prepared as a nano-suspension for pulmonary delivery. It showed future stability. No particle growth and aggregates formed over a period of 1 year. Additionally, Buparvaquone nano-suspension was formulated for an alternate treatment of lung infection (pneumonia) to deliver the drug at the location of lung infection using nebulization. Administration to infected guinea pigs of nebulized rifampin, isoniazid and pyrazinamide encapsulated in nutriment agglutinin functionalized PLG nanoparticles was far more effective. Three doses

administered fortnightly for 45 days were sufficient to supply a sterilizing effect in lungs and spleen. Drug nanocrystals showed an increased muco-adhesiveness resulting in a protracted duration at the lung mucosa.39

Ocular Drug Delivery

Drug delivery to eye tissues is especially problematic due to generally poor drug bioavailability, drug instability, short duration, poor drug solubility, a coffee amount of aqueous humour, and therefore the loss of the drug with tears. Nanocrystals (nano-suspensions) can enhance ocular drug permeation, favour controlled release, and promote targeting, also guaranteeing fewer or more attenuated side effects than traditional formulations.An example is obtainable by the formulation of nano-suspensions of three practically insoluble glucocorticoid drugs (hydrocortisone, prednisolone, and dexamethasone). They showed an enhanced rate and extent of ophthalmic drug absorption, and an increased intensity within the action of the drug. A rise in bioavailability has the important advantage of reducing the risks of adverse side effects related to large doses of those drugs, like cataracts, glaucoma, and nervous opticus injury. Nanocrystal suspensions of brinzolamide a poorly soluble drug, were prepared to scale back the pressure. At both tested PH7.4 and 4.5, 100% of the drug dissolved in one minute. The lowering of pressure was investigated in vivo in rats and proved to be particularly effective.40

Dermal Drug Delivery

Dermal nano- suspensions are mainly of interest if conventional approaches fail. Nanocrystals can increase the penetration of poorly soluble cosmetic and pharmaceutical substances into skin. This happens because increased saturation solubility increases the concentration gradient. Juvenal launched first four Nanocrystal cosmetic products with rutin. Petersen reported that rutin Nanocrystal formulation possesses 500 times higher bioactivity (measured as Sun Protection Factor, SPF) compared to water-soluble rutin-glycoside. Dermal application of nanocrystals is protected by a US and PCT application. Shaal et al. prepared apigenin nanocrystals and reported that UV skin protective potential are often significantly increased by decreasing the particle size from micrometre to the nano meter range.Nanocrystals of the flavonoid apigenin produced by a mixture of bead milling and subsequent high-pressure homogenization were formulated into a hydrogel for topical application.41

CONCLUSION

Drug nanocrystals are considered together of the foremost important formulation approaches for poorly soluble dru1s. Nanocrystals are often adopted by all poorly soluble drugs to defeat. Their solubility and bioavailability issues. The reduction in particle size to nano-meter range adds to the improved particle surface, curvature, saturation solubility, dissolution velocity and further reasonable bioavailability. it's a universal formulation principle but limited to BCS class II drugs. The striking advantage is that the drug nanocrystals are often applied to varied administration routes, meaning oral but also parenteral, especially iv. administration. Other administration routes are dermal delivery to make supersaturated systems with high thermodynamic activity, ophthalmic administration to make systems with prolonged retention times, nasal administration to stay nanocrystals to the nasal mucosa, vaginal administration to make systems evenly spreading throughout the therapeutic area, and aerosols containing drug nanocrystals for pulmonary delivery.

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RESEARCH ARTICLE

Synergistic Effect of Resveratrol and CPP-ACP on Remineralization of Demineralized Dentin – An *In vitro* Study

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ABSTRACT

Progression or reversal of dental caries is determined by the equilibrium between demineralisation and remineralisation. Natural extracts and derivatives to prevent demineralisation and promote remineralisation are gaining attention these days. This in vitro study was aimed to assess the comparative remineralising potential of Resveratrol, CCP- ACP and their combined form when used in demineralised dentin. Thirty dentin blocks of extracted human teeth after initial preparation were then submitted to 8 alternating demineralization and remineralization cycles. The amount of degraded collagen was assessed from remineralizing solution by TOC analysis and the amount of calcium released in the demineralizing solution was assessed using Atomic Absorption Spectrophotometry. Data was entered in a MS excel sheet and the descriptive statistics were computed. The amount of calcium released was less in the demineralizing solution when resveratrol was used along with CPP-ACP. And also the degraded collagen in the remineralising solution also significantly reduced when resveratrol was used with CPP-ACP in the test solution. The study concluded that incorporation of resveratrol in CPP-ACP containing paste had a better on remineralization of artificial root caries as compared to single paste of CPP-ACP and single solution of resveratrol.

Keywords: demineralisation, remineralisation, CPP-ACP, resveratrol, cyclic pH.

INTRODUCTION

Due to gingival recession and exposure of root surfaces, geriatric population is more prone for the development of root caries. In the first stage of its development, minerals are dissolved by acid produced by bacterial biofilms whereas in the second stage, further degradation of demineralized dentin matrix and bacterial infiltration into the inter-tubular area take place [1]. Progression or reversal of lesion be determined by the equilibrium between pathological factors favouring demineralization and the protective factors promoting remineralization [2]. In remineralization, a new surface is rebuilt mainly by calcium, phosphate and fluoride ions on existing crystal remnants in subsurface lesions of demineralization. These remineralized crystal structures are more acid durable than the original mineral. It is documented that calcium availability remains the singular limiting factor in enamel remineralization [3]. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) a natural compound found in many plant extracts is a member of the stilbene family. Resveratrol is recognised to have multiples biological functions such as antimicrobial activity, antiviral, antioxidant, anti-inflammatory, and anticancer activities [4]. The anti-cariogenic activity of the nanocomplexes formed between casein phosphopeptides (CPPs) and amorphous calcium phosphate (ACP) is well documented. CPPs are phosphorylated casein-derived peptides made by proteolytic breakdown of the milk products αS1-, αS2-, and β-casein. The main action of CPP is dependent on its ability to buffer free calcium and phosphate ions that promotes ACP super saturation relative to the tooth enamel, thus reducing demineralization and enhancing remineralization [5]. Many studies have been documented the anti-cariogenic capacities of CCP-ACP.[6,7] But studies comparing the remineralising potential of Resveratrol and CCP-ACP are scarce in literature. Hence this in vitro study was aimed to assess the comparative remineralising potential of Resveratrol, CCP- ACP and their combined form when used in demineralised dentin.

MATERIALS AND METHODS

An in vitro study was conducted on root dentin samples obtained from thirty extracted human permanent teeth. Single rooted, non-carious human permanent teeth were used. Teeth with caries, developmental defects, hypoplasia, restoration, wear and trauma were excluded. After the initial preparation of samples, rest of the steps were performed in a cyclic fashion to ensure the simulation of natural demineralisation and remineralisation cycles that occur in oral cavity. The pH cycling model was planned to mimicthe dynamics of mineral loss and gain involved incaries formation.[8] The response variables used in pH cycling to measure the extent of demineralisation and remineralisation potential were total calcium released and Total Organic Carbon (TOC) content (indicator of collagen degradation) in the solutions.

Preparation of samples

A total of 30 root dentin samples were obtained from 30 extracted single rooted teeth which were sectioned using diamond disk. The dentin specimen of 5mm × 5mm × 5mm were obtained from mid root section using airotor and long straight bur. Exposed root dentin surfaces were polished and made smooth using silicon carbide papers. The specimens were then soaked in 10%H_3PO_4 for 10 seconds to remove the smear layer.

Immersion in test solutions or pastes

The test solutions/pastes used were CPP-ACP and Resveratrol 10%.

10% resveratrol solution was prepared by dissolving 1g of resveratrol powder in 100ml distilled water.

Group I: Resveratrol 10% alone (n=10)

Group II: CPP-ACP (GC Tooth Mousse) alone (n=10)

Group III: Combination of both CPP-ACP and Resveratrol 10% (n=10)

After the initial preparation, samples were divided equally and immersed in the three test solutions/paste groups for 2 hours.

Immersion in demineralisation solution
After immersing in test solutions/paste, the samples were immersed into demineralisation solution which was prepared using

- 50 mmol/l acetic acid
- 1.5mmol/l calcium chloride
- 0.9mmol/l KH_2PO_4

The pH was adjusted to 5 using KOH. The specimens were immersed in this solution for14 hours.

Immersion remineralising solution
The next step was immersion of specimens in toremineralising solution which was prepared using

- 1.5mmol/l calcium chloride
- 0.9mmol/l KH_2PO_4
- 130mmol/l KCL
- 20mmol/l $NaHCO_3$

The pH of the solution was adjusted to 7 using KOH. The specimens were immersed in this solution for 8 hours. Thus the blocks were subjected to 8 alternating demineralization and remineralization cycles. pH cycling was performed in an incubator at 37°C for 8 consecutive days. The amount of degraded collagen was assessed from remineralizing solution by Total Organic Carbon (TOC) analysis and the amount of calcium released in the demineralizing solution was assessed using Atomic Absorption Spectrophotometry (AAS) on 3rd, 5th and 8th day.
Study armamentarium included gloves, mouth mask, extracted teeth, diamond disc, straight burs, dapen dish, carbon analyser, atomic absorption spectrophotometer and the above mentioned chemicals. Data was entered in a MS excel sheet. The descriptive statistics were computed using the Statistical Package of Social Sciences (SPSS) version 22.0 software.

RESULTS AND DISCUSSION

TOC level
In our study, the order of TOC estimated was as follows. It was observed that TOC level was found to be least in the solution when both CPP-ACP and Resveratrol 10% were used together whereas highest TOC level was obtained when Resveratrol 10% was used alone. The TOC level was least when measured on 8^{th} day as compared to 5^{th} and 3^{rd} day in all the groups.

Total calcium release
Similar results were obtained for total calcium release also. Least amount of calcium was released in the solution when both CPP- ACP and Resveratrol 10% were used together, followed by CPP-ACP alone and Resveratrol 10% alone. The current study compared the effectiveness of two remineralising agents Resveratrol 10% and CPP- ACP in individual as well as combined forms. The action of the CPP-ACP complex ranges from buffering pH, preventing demineralisation to enhancing remineralisation. CPP-ACP is a unique protein derived from milk, and therefore has a high safety level [9]. Atomic Absorption Spectrometry (AAS) was used for the comparative analysis of calcium loss from the remineralising solution. In literature although few studies have evaluated the effectiveness of CPP – ACP whereas no studies have been compared the remineralising potential of Resveratrol 10%. Hence comparisons are made wherever possible. It is reported that CPP-ACP was found to be more effective when combined with other agents rather than when used alone. Studies have observed the enhanced protective action of CCP-ACP when combined with fluorides and photo activated disinfection techniques [9].

A recent study on Resveratrol showed that it represents a promising anticariogenic agent because of its inhibitory effect on *S. mutans* cariogenic virulence properties. Another study reported that Resveratrol and curcumin are capable of reducing alveolar bone loss in an animal model of periodontitis. This occurred when these agents were added singly or in combination with one another, but there did not appear to be either synergistic or additive effects [10]. No studies have been so far reported the direct role of Resveratrol in demineralisation- remineralisation cycle. In our study, it was observed that combined action of CPP- ACP and Resveratrol 10% gave better results when they were used alone. This could be due to the synergestic biological action of the agents. The incorporation of resveratrol in CPP-ACP containing paste had a better on remineralization of artificial root caries. The study is not free from limitations. In this invitro study, role of micro organisms have not been assessed. Since dental caries is a multi-factorial disease, the study could not mimic the precise action of the new agents could not be clearly understood. Hence further studies in this regard is strongly recommended.

CONCLUSION

The study observed that the incorporation of synergistic action of Resveratrol 10% in CPP-ACP paste yielded better results when the components were used alone. The TOC level and total calcium release was obtained to be less when they were used together. We recommend further both in vivo and in vitro studies with these natural materials to explore their potential in preventing oral diseases.

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Table 1: Intergroup comparison of TOC level at 3rd, 5th and 8th day

Total Organic Carbon (TOC) mg/L	3rd day	5th day	8th day
Group I	8.296	8.248	8.206
Group II	8.085	7.992	7.902
Group III	7.288	7.221	7.191

Table 2: Intergroup comparison of total calcium release at 3rd, 5th and 8th day

Total calcium release (ppm)	3rd day	5th day	8th day
Group I	145.4	122.04	76.52
Group II	142.32	104.04	75.28
Group III	132.4	88.524	70.72

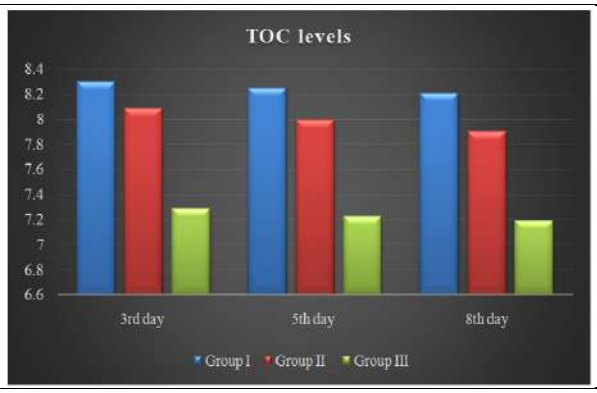

Figure 1: Intergroup comparison of TOC level at 3rd, 5th and 8th day

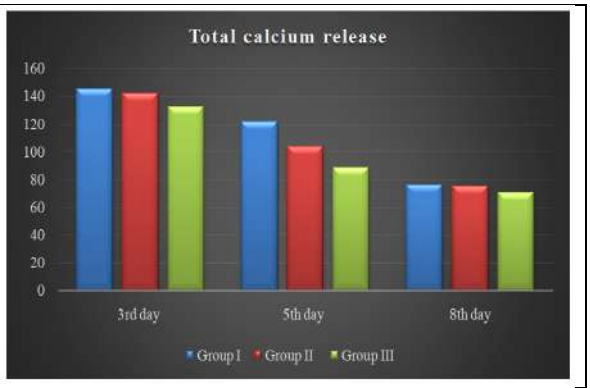

Figure 2: Intergroup comparison of total calcium release at 3rd, 5th and 8th day

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RESEARCH ARTICLE

Quantification of DNA from Normal Bloodstain and Fungal Growth Bloodstain

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ABSTRACT

DNA extraction has become an essential in cases of physical assault, murder, sexual assault cases, and considerably evolved since when it was initiated. Among all other biological evidence, blood evidence is the main source to obtain DNA. Blood is mainly attained in contaminated form with inhibitors during sampling and, it becomes a challenge for forensic scientists to deal with contaminated (soil, cement, concrete, other colored substances), badly preserved samples (plastics, non-porous container), fungal growth tissues that lead degradation of samples. This study was initiated to quantification of DNA from normal bloodstain and fungal growth blood stains. Quantification was performed by using Real time PCR from wet cloth with fungus, dry cloth with fungus and normal dry cloth piece of blood. Based on our research, we have determined that normal dry cloth piece provide the higher amount of DNA (56.43 ηg/µl.) while in dry cloth with fungus (0.34 ηg/µl.) and wet cloth with fungus (0.00ηg/µl.) couldn't generate a good profile of DNA. DNA profiling requires many different considerations such as allied factors, environmental factors and much research is required to determine profiling from degraded samples.

Keywords: Quantitation, DNA profiling, fungal growth, bloodstain, RT-PCR, environmental factors etc.

INTRODUCTION

A forensic science laboratory often deals with less ideal samples than the ideals one which may have been left on various surfaces and exposed to allied factors/environmental factors for a long time. Several biological samples i.e., blood, semen, vaginal fluids, saliva etc. are recovered in distinct forms from the scene of occurrence including physical violence like murders, assaults, rapes, abortion etc. Blood is often recovered in liquid form, blood gauze, dry blood stain. Examination of bloodstains has an immense value in reconstruction of the scene of crime and linking a criminal or the victim with the scene of crime1.Freshly dried bloodstains are reddish-brown in color, but whenever it is exposed by allied factors and environmental conditions, the color of blood changes to grey. Forensic analysis helps to reveal whether stain is blood or not? Does it belong to human origin or animal and blood grouping etc.?

DNA analysis has revolutionized the field of Forensic Science by allowing for unambiguous identification of the person from whom a biological sample has been obtained. DNA profiling helps the investigator to establish the identity and individuality whether perpetrator was involved or not. Blood samples have their own limitations such as, if it not stored accurately, it would degrade rapidly. Collection, storage and analysing of DNA is a vital part to achieve viable results. More often blood stain can be degraded/ may contain various substances be exceeding DNA that can inhibit PCR amplification [2,3]. Major concern in forensic cases is the extraction of sufficient amount of DNA from the sample for amplification.In the process of DNA extraction, dried blood doesn't require any constraint (temperature & time) likewise wet blood samples. It has longevity of several months for storage, hence it is collected more efficiently and stored for DNA extraction without any refrigeration4.

As it is very well known that decomposition plays an integral role that can start without any action of microbes, chemical decomposition at any extremely slow pace. Fungi colonize the decomposed dead bodies into moldy cadavers at the dry stage of decomposition. The formation of fungi is valuable for various aspects of forensic investigation, such as estimations of postmortem interval (PMI), post-burial interval (PBI), location of clandestine graves, and other efforts to characterize the environment in which the cadaver is located. When biological clues are processed for DNA isolation, all sources are extracted that may have non-human DNA (bacterial, fungal, animal material etc.) along with relevant human DNA of interest5. Thus, DNA Advisory Board (DAB) Standards that govern forensic DNA testing of forensic casework require human-specific DNA quantitation. Several methods have been developed to quantify DNA by using UV spectrometry, through gel-based techniques, dye staining, blotting techniques, and DNA amplification methods (PCR). In recent years, research in human DNA quantitation has focused on new "real-time" quantitative PCR (qPCR) techniques. Quantitative PCR methods enable automated, precise, and high-throughput measurements. This study was conducted to quantify DNA extracted from dried blood stains and was compared with the quantity of DNA from normal blood stain to Blood stain with fungal growth [6].

MATERIAL AND METHODS

Sample Collection

Biological samples collected from crime scenes, mass disasters, and missing person cases may have been exposed to harsh environmental conditions such as heat, direct sunlight and water that break down the chemical structure of DNA. Several environmental exposures and allied factors damage DNA by breaking its molecules into smaller pieces. Therefore, it is suggested to use legitimate provisions while collecting and storing these samples. In this study, all the samples were taken from the cases submitted in forensic science laboratory. Blood-stained samples were collected from the same cloth piece to analyze the disparity in the quality and quantity in DNA from normal bloodstain as well as bloodstain from fungal growth (Figure number 1 & 2). Blood-stained cloth was cut into small pieces and kept overnight in water-bath with solvent extraction buffer and proteinase K for cell rupture and proper lysis.

DNA Extraction

For DNA analysis, it is necessary that it should be extracted from cell and should be purified. For extraction, cell and nuclear membrane need to rupture and allow the DNA to be free in the solution. For this buffer solution and Proteinase K solution is used. The DNA been separated, and debris should be discarded. After extraction of DNA, it can be analyzed by quantification and PCR analysis. The DNA isolated from forensic biological evidence provides information related to the identification of the source. Isolation of good quality of DNA is a prime requirement in all the molecular genetic analysis. In human, blood is the most important material available for the isolation of DNA. The DNA from treated blood samples were extracted using the Phenol chloroform organic extraction method. Phenol chloroform technique is widely used for organic extraction from the specimen. In organic extraction, buffer, SDS and proteinase K are added, and mixture is incubated at 56^{o}. This break- down the proteins and cell membranes protecting the inner lying DNA[7]. The removal of protein is done by addition of phenol, chloroform and isoamyl alcohol followed by vortex and centrifugation. The pellet is washed several times and dried at room temperature. The other methods used for extraction of DNA are FTA card and Automated (mainly used for blood sample).

DNA Quantitation

In forensic samples, Quantification of DNA is of major importance for proper DNA amplification and STR profiling. Several methods have been developed to quantify DNA includes basic UV spectrometry, through gel-based techniques to dye staining, blotting techniques and DNA amplification methods (PCR). But recently RT-PCR or qPCR is most popularly used as it is reliable and accurate.Prior to PCR, it is necessary to know the exact amount of DNA present in the sample.As only narrow concentration range works best with short tandem repeats typing. Real-Time PCR is sensitive technique used in detection of contaminated DNA present in the sample and most used in laboratories for quantitation of DNA. Identifiler STR kit is used for unknown sample while Y-filer STR kit is used to confirm whether the sample belongs to male or not? RT-PCR has provenits significant use in detection of inhibitor of the sample[8], as inhibitors are impurities in DNA that can lead to inaccurate measurement of DNA concentration and multiple peaks can be observed.

RT-PCR or qPCR

The quantitation of DNA plays a central role in all areas and applications of forensic DNA analysis. The careful evaluation of the quantity of DNA extracted from biological samples is an imperative for DNA typing via the Polymerase Chain Reaction (PCR). The quantification of DNA by qPCR relies on the detection of amplified product (“amplicon”) at each cycle of the PCR. Detection of the PCR product is accomplished with the use of thermal cyclers that are capable of measuring real-time fluorescence changes due to amplicon production. The result is an “amplification curve” for each sample while the average quantity obtained from the sample has given in results. Duo kit was used for quantitation of samples. It implements the measurements of DNA extracted from forensic evidence andprovides the appropriate information to select STR profiling system[9]. This technique also helps to get appropriate STR profiles in very first attempt by using a minimal quantity of evidence/samples.

RESULT AND DISCUSSION

All samples including dry bloodstains, dry cloth with fungus and wet bloodstains with fungus and were analyzed to determine the quantity of DNA. Samples affected with fungus had potential challenge to determine the quantity of DNA. The obtained quantity is given below in table No.1-. The highest quantity of extracted DNA from wet cloth with fungus was 0.23ηg/µl while the lowest quantity was 0.00 ηg/µl. The quantity obtained from dry cloth with fungus was highest at 5.70 ηg/µl and lowest quantity was 0.34 ηg/µl. During the quantitation of DNA from dry cloth piece, it was observed that the highest quantity was 56.45 ηg/µl while 23. 45 ηg/µl was minimum quantity. During the analysis of fungal affected area of cloth piece (dark brown stain instead of damp fungal area) was pegged for DNA. As per the resultant of quantitative analysis, it was observed that dry fungal was able to give aplenty quantity to able to get the DNA for purpose of DNA profiling from the sample. However, the damp cloth with fungus were

not able to generate DNA profiling from the sample and if the profile was generated, in that case, most of the marker were affected due to degradation and profile will be incomplete form. The other clothes which were preserved in dry condition in porous packing like clothes or envelope, are able to give sufficient quantity of DNA may extend to 56ng DNA per microliter. Hence, it was very clear from the observation that blood-stained clothes should be preserved in porous container or if it has not done, then sample should be collected from the dried area of cloth in stead of damp fungal area. The obtained electropherograms of generated profiles no.1, 2 ,3 and 4 are given below. A few techniques of collection & preservation may push for expansion of fungus and mold over evidence. Fungal growth/ Bacteria detriment/ degrade DNA mixed along with evidence & inhibit dexterity to develop DNA profile. However, implementation of proper techniques helps to yield DNA. Being sensitive by nature, the potential contagion of evidence should be considered carefully. In old cases, evidence may have been possessed in such a manner that may have dearth contamination. It can help to make DNA less significant/misleading. Whenever such cases are investigated, determination of contributor of an additional profile can help to resolve whether DNA result can be used or not?

Qualitative and quantitative analysis of DNA extracted from blood samples is key feature and depend on the chosen protocol. It has potential to become more precise and accurate means of dating with bloodstained fungal growth samples and other biological evidence. In comparison between fungal growth samples and dry samples, dried blood sample yield significantly higher amount of DNA. Quantity of DNA from wet bloodstain cloth piece was approximately negligible which exhort that experts should handle the biological evidence carefully during amid chain of review and proper chain of custody should be followed. On another instance, DNA profile from dry blood with fungus suggest that any biological evidence should never be opened before sending in forensic laboratory if it has already sealed.

CONCLUSION

Long term exposure to environmental factors such as temperature, atmospheric-moisture, various bacteria's, fungus, and UV rays are common contributor and responsible factors for deterioration. Generally, it deteriorates biological fluids recovered from scene of occurrence which may result to generate partial/ no DNA profiling. During sampling, it is important to remove inhibitors and avoid degradation of the samples. Degradation and inhibition may complicate the PCR amplification. It is also suggested that blood samples should be collected in dried form or should preserved in proper manner to obtain higher quantity of DNA for accurate profiling. Implementation of Quantifler Duo kit is best way to determine the quality and quantity of samples for accurate DNA profiling from such samples. As per the resultant of this study, it is concluded that quantity of DNA was poor in wet cloth piece with fungus and was not capable to generate accurate profile.

Ethical Consideration: NA
Source Of Funding: Forensic Science laboratory
Conflict Of Interest: NA

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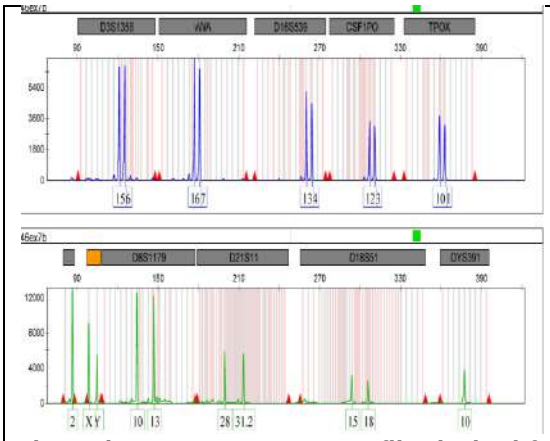
Table.1. Obtained quantity of DNA from dried bloodstained cloth piece, wet cloth piece with fungus & dry cloth with fungus.

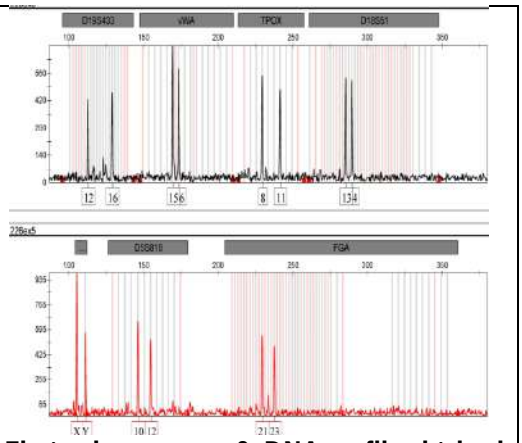
S.No.	Wet cloth with fungus (DNA in ηg./µl.)	Dry cloth with fungus (DNA in ηg./µl.)	Normal dry clothes (DNA in ηg./µl.)
1.	0.00	1.24	34.23
2.	0.00	0.34	44.98
3.	0.23	5.23	23.45.
4.	0.01	0.29	38.23
5.	0.09	4.23	56.45
6.	0.01	2.34	31.62
7.	0.00	1.82	23.98
8.	0.08	5.70	45.07
9	0.03	0.76	39.76
10.	0.21	2.71	38.12
11.	0.17	3.19	26.89
12.	0.06	0.98	41.19
13.	0.00	1.31	53.02
14.	0.00	1.98	31.67
15.	0.04	2.09	40.05

Figure no.1; blood stain cloth piece along with fungal growth.

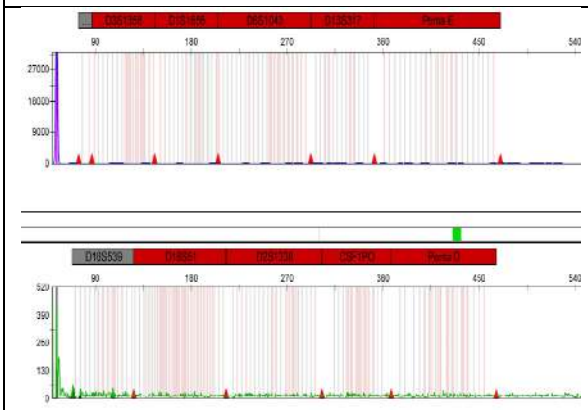
Figure no.2; dried blood stain cloth piece.



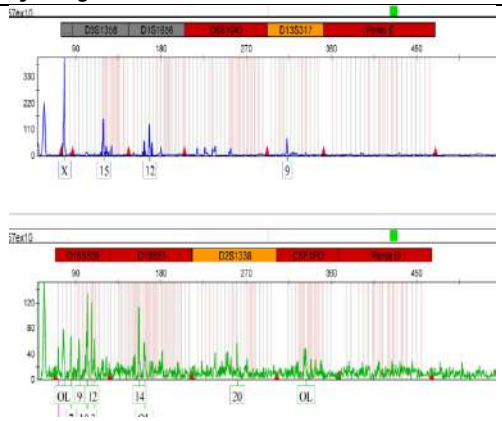
Electropherogram no.1; DNA profile obtained from blood-stained cloth piece without fungus.



Electropherogram no.2; DNA profile obtained from dried bloodstained cloth piece effected by fungus.



Electropherogram no.3; DNA profile obtained from wet cloth piece with fungus.



Electropherogram no.4; DNA profile obtained from wet blood sample.

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RESEARCH ARTICLE

Menopause: A Natural Process

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SHORT COMMUNICATION ARTICLE

The end of reproductive years of a woman is called as menopause. Menopause is not an abnormal sign it is a normal sign of reproductive aging of a woman. Menopause, perimenopause and postmenopause are the different stages a women may pass while she is attaining her menopause. Perimenopause starts 8 to 10 years before menopause; thus, this is the first stage. When a woman has no menstrual periods for atleast 12 months is called as Menopause. Last stage is called as Post menopause. Post menopause is the period called after menopause. Menopause is defined as natural stoppage of menstrual flow of a year without any medical or surgical condition. When a woman reaches the age of menopause ovaries produce less quantity of Estrogen Hormone. When this occurs, the menstrual cycle become irregular and gradually stops. Ovaries are the reproductive glands stores eggs and produce hormones like Estrogen, progesterone, and testosterone. During perimenopause ovaries stop release of eggs in the fallopian tubes.

Perimenopause is also known as "menopause transition" usually starts for the woman at the age of 30s and usually ends by 40s. During the last 2 to 3 years woman usually starts experiencing the symptoms of menopause. Few women who have menstrual cycle until this age there may be chance of getting pregnancy as there is an increase of estrogen level in the last 2 years. Menopause may be confirmed when a woman didn't get her menstrual cycle for a period of 12 consecutive months. Ovarian glands stop functioning in this stage. Menopause never occurs overnight. It's a gradual process. We cannot predict when exactly a woman will experience menopause. Symptoms of perimenopause, menopause and post menopause differs from woman to woman. Few common symptoms during menopause are hot flashes, stress tiredness, mood swings, fatigue, vaginal dryness and itching, and night sweats. Menopause is a normal and natural process and not requires any treatment. However, there is possibility of

treatment associated with symptoms in case of severity. Symptoms can be treated by Gynecologist, primary care providers, family medicine specialists and internists.

Hormonal Therapy (HT) or Menopausal Hormone Therapy (MHT) consists of estrogens or combination of both estrogen and progesterone (Progestin) and was formerly called as Hormone Replacement Therapy (HRT).This therapy usually reduces symptoms related to declined level of estrogen like hot flashes and vaginal dryness. As hormonal therapy has more side effects it should be taken like smallest effective dose for shortest duration. Antidepressants and other medications also used for symptomatic treatment during menopause. These medications are commonly used with prescription only as they have side effects. Most women were advised to use Soya beans, Chickpeas, and Lentils in their food as they reduce the symptoms occurs due to reduction of estrogen level. Certain lifestyle factors also help to lessen the symptoms of menopause and the medical complications that may develop due to postmenopausal women by leading a healthy lifestyle. Few life style modifications like regular exercise, avoiding smoking, proper nutrition, regular breast self-examination, practicing yoga etc. Postmenopause is the period after a woman stops bleeding for an entire year. In this period most of the menopausal symptoms will reduce. Few women may experience the symptoms for a decade or longer after the transition. Postmenopausal women are at a risk of number of health conditions such as cardiovascular diseases and osteoporosis due to lower level of estrogen. Hormone therapy or healthy lifestyles may reduce the risk.

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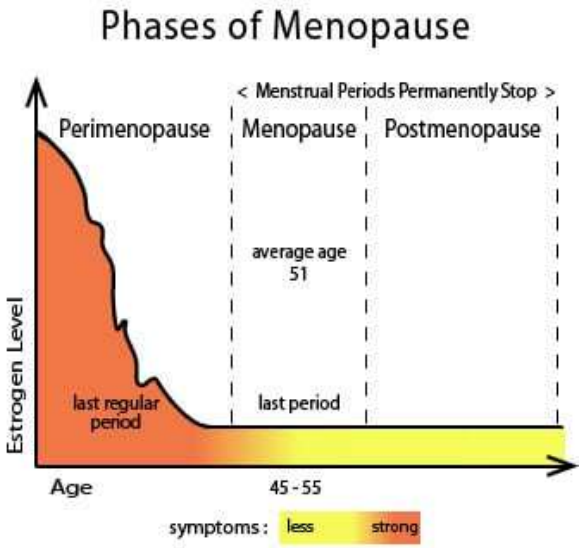
Phases of Menopause
< Menstrual Periods Permanently Stop >
Perimenopause
Menopause
Postmenopause
average age
51
Estrogen Level
last regular
period
last period
Age
45 - 55
symptoms :
less
strong

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REVIEW ARTICLE

Acquired Immuno Deficiency Syndrome – A Mini Review

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ABSTRACT

AIDS is caused by the human immunodeficiency virus (HIV), which is a lentivirus that spreads through the body (AIDS). AIDS is a human disease in which the immune system gradually fails, allowing life-threatening infections and malignancies to thrive and spread throughout the body. The transmission of HIV occurs through the transfer of blood, sperm, vaginal fluid, and breast milk. HIV is present in these physiological fluids as both free virus particles and virus within infected immune cells, indicating that the virus is both present and active. HIV infects critical cells in the human immune system, such as helper CD4 T cells and macrophages, and causes them to malfunction. Infection with HIV causes a reduction in the number of T cells in the body by a variety of mechanisms, one of which is pyroptosis of infected T cells. Conditions that do not ordinarily develop in individuals with healthy immune systems are the primary cause of the symptoms associated with AIDS in the first place. Even though there is currently no cure for AIDS, diligent adherence to antiretroviral regimens can significantly delay the disease's progression while also preventing secondary infections and consequences. This review aims to focuses on epidemiology, etiopathogenesis, diagnosis and management of AIDS in detail.

Keywords: HIV**,** AIDS, Immune system, Transmission.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) attacks the immune system, it reduces individual's ability to defend themselves against numerous illnesses and some types of cancer that people with healthy immune systems are able to combat. As a result of the virus's destruction and impairment of the function of immune cells, infected individuals gradually become immunodeficient. Immune function is commonly determined by the number of white blood cells known as Cluster of Differentiation 4 (CD4) cell count [2], which are a type of white blood cell. HIV kills these CD4 cells, reducing a person's ability to fight off opportunistic illnesses such as tuberculosis and fungal infections, as well

as serious bacterial infections and several malignancies [3]. There at end of 2020, it is anticipated that 37.7 million people were living with HIV, with more than two-thirds of those living in the African region. HIV/AIDS continues to be a major global public health concern, having claimed the lives of 36.3 million people to this day. Medicines can not only assist HIV-positive individuals in maintaining their health, but they can also prevent HIV from progressing to AIDS [4]. To avoid future sickness, it is essential that the medications are taken exactly as prescribed. HIV infection is a chronic condition that can be managed clinically. The World Health Organization (WHO) recommends that everyone who may be at risk of HIV should get tested [5]. HIV infection can be detected using easy and economical fast diagnostic tests, as well as self-tests, to determine whether or not a person has the virus. [6] It is critical that HIV testing services adhere to the 5Cs: consent; confidentiality; counselling; correct results; connectivity to treatment and other resources. The goal of this review is to provide an overview of the epidemiology, etiopathogenesis, diagnosis, and management of AIDS.

Epidemiology

HIV prevalence is highest in particular groups who have common risk factors in virtually all regions of the world, and this is true in nearly all countries. Males who have sex with men, intravenous drug users, persons in jails and other confined environments, sex workers, and transgender people are among the most vulnerable groups [7]. Each of these groups faces plenty of legal and societal challenges that arise as a result of their actions, which enhance their risk to HIV infection and hinder them from obtaining HIV prevention and treatment programs, respectively. In light of the high prevalence of HIV infection in these communities, they are seen as critical partners in the development of a comprehensive response to the epidemic. A third group at high risk of infection is infants born to HIV-positive mothers. However, one of the major success stories of HIV infection has been the near elimination of mother-to-child transmission when antiretroviral therapy (ART) has been utilized, as explained further below. Across the world, the prevalence of HIV infection among males who have sex with other men has remained consistently high over the past ten years, with no evidence of a drop reported in the majority of communities during this period. Among other factors, this risk is exacerbated by the comparatively high likelihood of transmission during receptive anal sexual intercourse as well as the greater number of exposures [8].

Aetiology of AIDS

AIDS is caused by the HIV virus, which is a member of the retrovirus family of RNA viruses. As opposed to other RNA viruses, they must reproduce through a DNA intermediate in order to be considered pathogenic. HIV-1 and HIV-2 are two HIV strains that are genetically distinct but closely related. HIV-1 is the most prevalent kind in the United States, Europe, and Central Africa; HIV-2 is the most common type in West Africa and India. According to the Centres for Disease Control and Prevention, the first cases of acquired immunodeficiency syndrome (AIDS) were documented in the United States in 1981. A short time later, it was discovered that the underlying pathogen was HIV, an RNA virus belonging to the Retroviridae family of viruses. HIV most likely entered the human population by cross-species transmission of the ancestral virus, which was discovered in wild chimpanzees in Central Africa and spread to humans. It is believed that the spread of HIV in Africa coincided with urbanization and happened before the discovery of AIDS [9]. HIV is a blood borne infection that can be acquired most easily through contact with mucosal membranes or parenteral administration through one of following five primary routes of transmission such as 1) Unprotected penetrative intercourse between two or more male partners, 2) Intercourse between heterosexuals that is not protected by law, 3) The use of drugs administered through injection, 4) Blood and blood by-products that are unsafe [10] (primarily in developing countries), 5) Transmission from mother to child through pregnancy, delivery, or breast-feeding.

Factors that may have an impact on AIDS are

Having sex in an unprotected environment: The majority of persons contract HIV through sexual contact [11]. The genitals, rectum, and mouth are the entry points for the virus into the body during sex. Usage of condom or other type of protection on a regular basis will reduce the risk.

The usage of illegal drugs: The use of needles to inject drugs increases risk of contracting HIV. This is especially true if the needles are unclean or if shared them with others. Even a small amount of HIV-infected blood can transmit the disease.

Suffering from certain medical conditions: Having a sexually transmitted infection (STI) increases the likelihood of contracting HIV.

Blood and Blood Products: It wasn't until 1985 that blood banks began testing for HIV. There was no means of knowing whether or not the blood contained HIV. The infection was spread by infusions of blood products. There is still some risk, however, because tests cannot detect HIV in a donor who has recently contracted the virus. Although it is uncommon, tissue or organ transplantation, as well as artificial insemination, can increase the risk of pregnancy.

Mode of transmission of HIV

HIV is transferred primarily through three routes: sexual contact, blood transfusion, blood products, or contaminated needles, and transit from mother to child [12]. Sexual contact is the most common route of transmission. Despite the fact that gay contact continues to be a significant cause of HIV infection in the United States, heterosexual transmission is the most important mode of HIV transmission around the world today. In developed countries, treatment of blood products and donor screening have virtually removed the risk of HIV transmission from tainted blood products; yet, the virus continues to spread among intravenous drug users who share needles.

HIV can be transmitted from an infected person to another through

Blood (including menstrual blood).
Semen.
Secretions from the vaginal canal.
Breast milk is an excellent source of calcium.

Activities that increase the risk of HIV transmission.

Unprotected sexual contact with a minor.
Direct blood contact, such as the use of injectable medication needles, blood transfusions, accidents in health-care settings, or the use of certain health-care goods.
From mother to child (before or during birth).

HIV is transmitted solely through sexual contact.

Blood, sperm, or vaginal and cervical secretions contaminated with the virus come into contact with the mucous membranes. Blood or blood products that have been infected with the virus are injected. Vertical transmission (that is, from an infected woman to a fetus) and horizontal transmission (that is, from a mother to a newborn through breast milk).

Ingestion of sexual fluids or blood that contact with the mucosal membranes.

The virus cannot move through skin that has not been harmed. HIV can enter the body through the mucous membranes that border the vaginal, rectum, urethra, and, in rare cases, the mouth, if the virus is transmitted by saliva. Virus transmission may be increased in the presence of damage to a mucous membrane, although it is not required for transmission to take place [14].

The administration of contaminated blood

Through intravenous injection, intramuscular injection, or subcutaneous injection.
HIV can be transferred by infected blood that enters the bloodstream without being processed.

There are several mechanisms in which blood-to-blood transfer happens.

The transfusion of contaminated blood and blood products, as well as the transfusion of other blood recipients.
The sharing of hypodermic needles and syringes that have not been sanitized.

The risk of HIV transmission is influenced by
The amount of HIV present in the contaminated fluid (concentration).
The amount of fluid that has been delivered into the body.
The ability of the contaminated fluid to gain access to the T4 cells.

Fluid with high concentration of HIV
Semen.
The presence of blood and blood components.
The flow of menstrual blood.
Secretions from the vaginal canal.
Pre-ejaculatory fluid
Breast milk is an excellent source of calcium.

Fluids containing a low level of HIV infection
Saliva.
Tears.
Urine.
Vomiting.
Mucosa of the nose

Pathophysiology of AIDS

The HIV, or human immunodeficiency virus, is responsible for the development of acquired immune deficiency syndrome. The infection results in the progressive destruction of the cell-mediated immune (CMI) system, which is predominantly accomplished by the elimination of CD4+ T-helper cells (T-helpers).Opportunistic infections and certain malignancies are caused by a weakened immune system. It is possible to contract an opportunistic infection from an organism that does not cause illnesses in healthy humans [15]. HIV can also cause direct harm to particular organs, such as the brain.

Seroconversion disease: This illness develops within 1 to 6 weeks of contracting the infection. The sensation is comparable to that of having the flu.

Asymptomatic infection: Following seroconversion, virus levels are low and replication is slow, resulting in no visible symptoms. The CD4 and CD8 lymphocyte counts are within normal limits. This stage is characterized by the absence of symptoms and the ability to last for years on end.

Persistent generalized lymphadenopathy (Pgl): Patients with persistent generalized lymphadenopathy (Pgl), in which the lymph nodes have been enlarged for three months or more and are not attributable to any other reason,

Symptomatic infection: This stage is characterized by the presence of symptoms.

AIDS: This stage is marked by profound immunodeficiency and is the most advanced stage. Life-threatening illnesses and uncommon tumors have been detected. It is distinguished by a CD4 T-cell count of less than 200 cells/mm3.

Nonprogressors: Only a small percentage of individuals develop AIDS extremely slowly are called Nonprogressors
The pathogenic spectrum of HIV infection is evolving as the infection expands into new societies with a variety of potential opportunistic diseases and as medical research develops medications to combat HIV replication [16].

Stages of the AIDS

The signs and symptoms of HIV infection can differ from person to person in terms of form and severity, and some people may not experience any symptoms for several years [17]. If HIV infection is left untreated, it spreads through

the body in the stages described below. In the absence of antiretroviral therapy, the HIV virus multiplies in the body, causing increasing harm to the immune system. With appropriate therapy, the infection will be under control and prevent it from spreading. It is critical to begin treatment as soon as possible when a positive test is obtained.

Stage 1: Acute primary infection.

Early symptoms of HIV can be similar to those of the flu. It begins with feel flu-like symptoms for one to four weeks after contracting HIV. Normally, these don't stay very long (a week or two). Some people only experience a few of the symptoms, while others have none at all. Symptoms can include fever, rash, throat discomfort, swollen glands and lymph nodes, Headache, discomfort in the stomach, aches and pains in the joints, muscle aches and pains.These symptoms occur as a result of body's response to the HIV infection. blood system is filled with HIV-infected cells that are circulating throughout it. As a result, immune system attempts to combat the virus by creating HIV antibodies; this is referred to as seroconversion. The length of time it takes for body to go through the seroconversion process varies, but once have HIV, it can take anything from a few weeks to several months.

Stage 2: Asymptomatic stage

It is common for people to feel better after going through the acute primary infection stage and the seroconversion process. In reality, HIV may not manifest itself with any additional symptoms for up to ten or even fifteen years (depending on age, background and general health). In spite of this, the virus will remain active, infecting new cells and replicating itself. During this period, HIV can still be transmitted. If HIV infection is left untreated, it will eventually cause serious damage to the immune system.

Stage 3: Symptomatic HIV infection

When a person is in the third stage of HIV infection, his or her immune system has been substantially compromised [18]. When kids reach this stage, they are more susceptible to contracting dangerous infections or diseases that their bodies would otherwise be able to fight off. Opportunistic infections are infections that occur as a result of a particular circumstance. Symptoms can include weight loss, chronic diarrhoea, night sweats, fever, persistent cough, Mouth and skin problems, regular infections, serious illness or disease.

Diagnosis of AIDS

Tests for HIV varies in terms of methodology as well as the purpose for which they are administered. In general, tests are performed for three purposes: individual diagnosis, protection of blood or tissue product safety, and public health surveillance. According to the testing objective, the most appropriate test is selected based on factors such as convenience, test characteristics, and the population to which the subject is assigned. The presence of antibodies to HIV proteins is now widely recognized as a reliable indicator of HIV infection, with the exception of exceptional conditions such as those following the administration of an experimental vaccine. False-positive HIV antibody testing can sometimes occur as a result of certain clinical disorders that are extremely rare. Testing algorithms with high sensitivity and specificity for clinical diagnosis have been refined through years of experience with serologic tests and knowledge of viral subtypes. In most cases, a very sensitive test is utilized for screening, followed by a highly specific test for confirmation.

HIV testing by serology

Different techniques can be used to detect antibodies to HIV proteins; the current gold standards are enzyme-linked immunosorbent assays (ELISA) for screening and Western blot (WB) for confirmation. As alternative screening methods, particle cell agglutination and immunofluorescence and radioimmunoblot assays for confirmation are suitable. Although ELISA is highly sensitive, it has been plagued by serious difficulties such as false positives caused by poor specificity and false negatives caused mostly by low antibody levels in the early stages of infection, known as the "window period"[19]. The rate of false positives has decreased with each consecutive generation of assays, and the window duration has reduced dramatically. This improvement can be attributed to the use of recombinant antigens in place of viral lysate, as well as the use of a double-antigen sandwich to increase the collection of IgM and

IgG antibodies. This test's overall performance qualities are extremely favourable when compared to the top diagnostic tests available in other areas of medicine.

ELISA (enzyme-linked immunosorbent assay): ELISA is the enzyme-linked immunosorbent assay (ELISA). This test is typically used as the first step in the detection of HIV infection [20]. It is customary to repeat the test to confirm the diagnosis if antibodies to HIV are detected (positive). If an ELISA test results negative, subsequent testing is usually not required.

Western blot analysis: The Western blot assay is a technique in which individual proteins from an HIV-1 lysate are sorted according to their size using polyacrylamide gel electrophoresis. Transferring the viral proteins on nitrocellulose paper and reacting with the patient's serum is next performed [21].

Pharmacotherapy of AIDS

While there is currently no cure for HIV, Medications are available that can stop the progression of the infection and reduce the risk of transmission are called antiretroviral therapy. It can also increase a person's life expectancy while simultaneously improving their quality of life. Many HIV patients enjoy long and healthy lives as a result of their treatments. These drugs are growing more effective and well-tolerated as time goes on. In this therapy, a person will only require one pill each day. The following sections discuss HIV therapies and drugs that can be used to prevent the virus.

Emergency HIV pills: PEP (Pre-exposure prophylaxis): It is a type of HIV medication that is available in an emergency.PEP should be discussed with a healthcare physician by anyone who believes they may have been exposed to the virus within the last 72 hours by a trusted Source. This treatment may be able to stop the infection, especially if it is taken as soon as possible after a person has been exposed to the virus. A person takes pre-exposure prophylaxis (PEP) for 28 days, after which a doctor checks the person for HIV. Due to the fact that PEP is not 100 percent effective, it is critical to employ preventative measures such as barrier protection and safe injection methods, even while taking PEP.

Antiretroviral medication: Antiretroviral drugs, which combat the infection and slow the spread of the virus, are used to treat HIV. High-dose antiretroviral therapy (HAART) or combination antiretroviral therapy (CART) are commonly used to treat HIV infection [23]. The method might be referred to as HAART or cART. There are several types of antiretrovirals available, including

Protease inhibitors: HIV requires the enzyme protease in order to replicate. These drugs bind to the enzyme and prevent it from doing its function, stopping HIV from replicating itself. It includes Atazanavir and Cobicistat (Evotaz), Lopinavir and Ritonavir (Kaletra) and Darunavir and Cobicistat (Prezcobix).

Integrase inhibitors: HIV requires the activity of integrase, another enzyme, in order to infect T cells, and these medications inhibit the activity of the enzyme. Because of their efficacy and lack of adverse effects, these medications are frequently used as the first line of treatment. Integrase inhibitors includes Elvitegravir (Vitekta), Dolutegravir (Tivicay) and Raltegravir (Isentress).

Nucleoside and nucleotide reverse transcriptase inhibitors: These medications, often known as nukes or nucleoside reverse transcriptase inhibitors (NRTIs), prevent HIV from replicating. Types include Abacavir (Ziagen), Lamivudine and zidovudine (Combivir), Emtricitabine (Emtriva) and Tenofovir Disoproxil (Viread).

Non-Nucleoside Reverse transcriptase inhibitors: Non-Nucleoside Reverse transcriptase inhibitors that do not contain nucleosides, also known as NNRTIs, these medications inhibit the multiplication of HIV to multiply. They are Nevirapine, Delavirdine and Efavirenz.

Chemokine co-receptors antagonists: These medications prevent HIV from infiltrating cells. Doctors rarely prescribe them. They are Maraviroc.

Entry-inhibitors: Intracellular entry inhibitors are drugs that block HIV from entering T cells. HIV is unable to multiply if it does not have access to these cells. They include, Maraviroc, Ibalizumab, Fostemsavir.

Complementary and alternative medical treatment

Many people living with HIV test with complementary, alternative, and herbal treatments. But, there is really no evidence to show that they are beneficial. While mineral and vitamin supplements may be beneficial in various ways, it is crucial to discuss these with a healthcare provider before taking them because some natural items may interfere with HIV medications.

Pharmacoenhancers: Cobicistat and ritonavir are two pharmacokinetic enhancers or boosting medications that are commonly used in HIV treatment. Both drugs are CYP3A4 inhibitors, with cobicistat being a more specific CYP inhibitor than ritonavir, according to the manufacturer. Cobicistat, in contrast to ritonavir, does not possess antiretroviral action.

Living with AIDS

Many HIV-positive individuals enjoy long and fulfilling lives. However, because of the possibility of immune system impairment, it is critical to implement the following techniques.

Implementing a medication regimen: It is important to take HIV medication as prescribed; failing to do so even for a few doses could compromise the therapy. A person should devise a daily medication-taking practice that is compatible with their treatment plan and time limits. Side effects might make it difficult for people to adhere to their treatment regimens at times. If any adverse effects that are difficult to manage, contact healthcare practitioner [24]. They are able to offer a medication that is more tolerable as well as provide other recommendations on the treatment strategy.

Boosting overall health: It is essential to take precautions to avoid sickness and other illnesses. People living with HIV should engage in regular physical activity, consume a well-balanced, nutritional diet, and refrain from engaging in harmful activities such as smoking [25]. In particular, it is crucial to avoid exposure to bacteria that cause infection [26]. A person may be required to cease eating unpasteurized foods and undercooked meats as well as prevent contact with animal wastes and cat litter. It is also essential to wash hands thoroughly and on a frequent basis in order to avoid infection. Antiretrovirals, as a whole, lessen the need for the precautions listed above.

Maintaining communication with medical professionals: HIV is a lifetime illness, and checking in with a healthcare team on a regular basis can ensure that a person's therapy is appropriate for their age and any other health conditions they may be experiencing. The treatment plan will be reviewed and adjusted as necessary by the team.

Promoting and assisting with mental health: It is widely believed that HIV and AIDS are highly stigmatized and cloaked in myths and misunderstandings. As a result, the patient may be persecuted, isolated, or excluded from certain social situations. It is typical to have symptoms of worry or despair after receiving an HIV diagnosis [27]. Speaking with a mental health expert, as well as with a trusted doctor, can be quite beneficial. An extensive list of resources is provided by the Centres for Disease Control and Prevention Trusted Source, which can assist people in dealing with stigma and prejudice while also receiving further support.

Prevention of AIDS

Preventing new HIV transmissions is a critical step toward bringing the HIV pandemic to an end in its current form. Researchers financed by the National Institute of Allergy and Infectious Diseases (NIAID) have been working since

the beginning of the AIDS epidemic in the 1980s to identify prevention methods that will keep people healthy. Many HIV prevention approaches are now available for use in combination or on their own, and scientists are continuing to work on the development and improvement of cutting-edge tools and procedures that may be used to prevent HIV in a variety of populations around the world [28]. The following techniques can help to avoid coming into contact with HIV.

Using barrier protection and pre-exposure prophylaxis (PrEP): Using a barrier protection measure, such as a condom, during every sexual act can significantly minimize the likelihood of developing HIV and other sexually transmitted infections (STIs) (Sexually Transmitted Infections). In their 2019 guidelines, the Preventive Services Task Force recommends that doctors only recommend pre-exposure prophylaxis (PrEP) to people who have recently tested negative for HIV. They also approve a PrEP formulation consisting of the antiretroviral drugs tenofovir disoproxil fumarate and emtricitabine. People who take PrEPare advised to do so once a day. The Food and Medicine Administration (FDA) Trusted Source has approved a second combination drug, tenofovir alafenamide and emtricitabine, for use as PrEP (Pre-exposure Prophylaxis) [29].

Adopting safe injection techniques: The use of intravenous drugs is a major source of HIV transmission. Using shared needles and other drug-related equipment might increase risk of contracting HIV and other infections, such as hepatitis C. As a result, anyone who injects any type of medication should do so with a clean, unused needle. Needle exchange programs and addiction recovery programs can both aid in the reduction of HIV prevalence.

Avoiding contact with body fluids: Reducing contact with blood, menstrual fluid, vaginal secretions, and other bodily fluids that can transmit the virus can help minimize the chance of infection with HIV. Skin cleansing on a regular basis and properly after coming into contact with bodily fluids can also help to lower the risk of infection [30]. When exposure to these fluids is likely, healthcare personnel utilize protective equipment such as gloves, masks, protective glasses, face shields, and gowns, and they adhere to established procedures to prevent transmission.

Pregnancy: Certain antiretrovirals can be harmful to the foetus during pregnancy, an effective and well-managed treatment regimen can prevent transfer to the foetus. Additionally, if the person's HIV infection is properly controlled, vaginal deliveries are achievable. It's also possible that the virus could be transmitted through breast milk as well. Although the Centres for Disease Control and Prevention (CDC) Trusted Source does not suggest breastfeeding, it is crucial to examine all of the choices thoroughly with a healthcare professional, regardless of a person's viral load or whether or not they are taking antiretrovirals.

Education: Education is a critical component in the effort to stop the spread of HIV/AIDS. Even if education were to be a total success, it would still be necessary to maintain a continuous process. A new generation of humans becomes an adult with the need to know how to protect oneself from infection with each passing generation.

CONCLUSION

HIV/AIDS continues to be a major public health concern on a global scale. Providing therapy is the most effective means of lowering the mortality rate. Maintaining a clean environment away from AIDS patients can aid in the containment of infection. The use of antiretroviral medication is mandatory when a patient has been infected by a virus. HIV will not always be detected in a test at early stage, and patients will need to be tested again to ensure that the results are accurate. In clinical practise, an HIV test is only administered if the patient requests it. Despite the fact that a person with no symptoms is more likely to seek medical attention, there is still a substantial risk of transmission. As a result, specialists recommend that everyone undergoes frequent testing to ensure that they are aware of their HIV status. The spread of HIV has increased in recent years as a result of a lack of public knowledge among the general public. We must raise awareness among the general public in order to prevent the spread of infection.

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REVIEW ARTICLE

An Experimental Investigation of Performance Parameters of CI Engine using Biofuels (Neem Seed Oil and Rice Bran Oil)

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ABSTRACT

Major portion of energy requirement is fulfilled by fossil fuels around the globe. But the fossil fuels are exhaustible in nature and pollution produces by fossil fuel is a major concern. The trend has taken a paradigm shift as the world is moving towards use of renewable and less polluting sources which results in conserving our fossil fuels and reducing the pollution which creates a win-win situation. The present work focuses on using the bio fuels (Rice bran and Honge) in Diesel engine in duel fuel mode and comparing the performance of the engine in Diesel mode and Dual fuel mode of operation. The results indicated that the performance was improved by using bio fuel when compared to Diesel operation. This encourages to use different biofuels in IC engines to enhance the performance and lessen the pollution which is the order of the day.

Keywords: Biodiesel, engine performance, Diesel, dual fuel, fossil fuels.

INTRODUCTION

The most essential requirement for human survival and activities is energy. The main portion of energy consumed throughout the globe comes from fossil fuels. Nevertheless, these sources are insufficient and are exhaustible in nature. The alternative and renewable sources of energy are the possible answer for several existing social concerns resulted from air pollution, global warming as well as sustainability problems at large. The chief benefits of employing biodiesel are that it is environmental-responsive, can be employed with no major modification in the current engines and generates fewer toxic emissions namely CO, HC and SOx. Biodiesel is principally extracted from vegetable or animal fats; it offers several other benefits namely negligible sulphur and aromatic content, high flash point, Cetane number and non-toxicity. In contrast they have the drawbacks namely higher viscosity, lower calorific

value, lower volatility and oxidation instability. There have been a lot of researches done on different vegetable oils and their biodiesels namely sunflower, peanut, soybean, cottonseed, rapeseed, jatropha etc. But in India, oil plants namely Jatropha, Pongamia, Neem and Mahua are found abundantly. From previous studies, it was reported that the vegetable oils can be employed directly in CI engines effectively with no alterations. But it poses problems owing to high viscosity and low volatility so it is necessary to lessen the viscosity of these by employing the process such as preheating, blending and trans-esterification, to utilize them effectively as an alternative to diesel in the traditional diesel engines with no major modifications. The improvement in volatility of mixture by preheating is important in improving the fuel evaporation resulting smoke reduction. Out of the above mentioned techniques trans-esterification is the best and cheapest method for biodiesel production. A noteworthy progress in the engine performance characteristics was observed while using trans-esterified biodiesel when compared to diesel engine operation. The thermal efficiency of the engine was enhanced while brake specific fuel consumption was reduced. In this present work methyl esters of Neem and Rice Bran oils were produced using trans-esterification process and tests were conducted to determine their performance in diesel engine.

Literature Survey

Researchers have focused on investigating the performance of CI engine using different biofuels and the studies have also put light on different blends that can be used with diesel and are known as biodiesel blends. The viscosity of biodiesel is a major problem in order to reduce this trans-esterification is carried out. The transesterification process is recognized as an efficient technique of decreasing the viscosity of vegetable oil and reducing operational issue following evaluation of the performance and emission characteristics of linseed, mahua, rice bran and linseed methyl ester (LOME) during a CI engine operation [Kumar and Aggarwal, 2008].The esterification process of the vegetable oil reduced its viscosity; cetane number, molecular weight, and fuel spray infiltration distance were improved. [Pandian and Devaradjane, 2007]. Transesterification process was carried out with an aim of yielding good quality biodiesel from the rice bran oil through improvising parameters such as temperature, concentration of catalyst, quantity of methanol as well as reaction duration. The optimal conditions were 55 °C reaction temperature, 60 min reaction period, 9:1 molar ratio and 0.75% catalyst (w/w) to produce utmost biodiesel [Shailendra et.al., 2008]. Experimental investigations was conducted with different biodiesel blends and diesel from 5% - 50% ester on a 4-cylinders, diesel engine to investigate the performance as well as emission characteristics and it was observed that biodiesel considerably lowered the emissions of toxic emissions from CI engines with no effect on the performance of engine[Sinha and Agarwal, 2005]. The performance with emissions of diesel engine using biodiesel blends and diesel at several injection pressures was carried out. Investigations were carried out for Karanja and Neem blends with varying percentages. It was found that Karanja and Neem biodiesels could be used in CI engines with no alterations. The performance was reduced to some extent whereas brake specific fuel consumption was enhanced when biodiesels were used.

The brake thermal efficiency of B10, B20 and B60 are found to be more compared to B100 however lesser compared to Diesel operation. When compared to traditional diesel, CO and HC emissions are decreased whereas NOx emissions were enhanced with biodiesel blends when compared to diesel operation [Ravi et.al, 2012].The performance as well as emission features of traditional diesel, rice bran oil and diesel-biodiesel-ethanol blends on a single cylinder CI were carried out. The results indicated that highest brake thermal efficiency was 28.2% with B10E15. The exhaust gas temperature of the blend B10E15 was a little lesser compared to diesel fuel all over the entire range of the engine load, he CO emissions of the biodiesel and blends were found to be lesser compared to diesel operation. The HC emissions enhanced with an increase in ethanol proportion in the diesel- biodiesel-ethanol blends, however lesser than that of diesel operation at higher loads. The NO_x discharge of the biodiesel and the blends were found to be lesser at lower loads and high at higher load as compared to that of the diesel operation [Syed et.al, 2009]. Performance tests were conducted on 2 cylinders, 4-stroke CI engine employing diesel and different blends of waste cooking oil yielded from waste vegetable oil, in similar operating states which determined the performance of C I engine using diesel fuel and waste cooking biodiesel was found to be comparable [Ghobadian et.al.,2009]. Investigations on the consequences of use of Karanja oil methyl ester on the performance, emission

characteristics, wear behaviour of 6 cylinders, CI engine in comparison with the diesel operation, results confirmed 35% lower wear when engine was run in biodiesel mode when compared diesel operation along with decreasein CO and HC discharges as well [Anand and Nandgaonkar,2011]. Investigations on the performance of CI engine with rice bran oil methyl ester and blends was carried out and it was observed that the CO, HC and soot emissions were enhanced and the NOx emissions were somewhat enhanced with the rise in blends in comparison with diesel mode [Gattamaneni, 2008]. It was found that to the extent of 50% Neem oil could be replaced for diesel for use in a diesel engine with no major operational challenges [Pramanik, 2003]. An experimental investigation was done to assess and compare the use of oil of cottonseed, soybean and sunflower with their related methyl esters the results indicated that tested biodiesel blends, could be employed in a safe manner [Altın et.al, 2001]. Investigations on performance of a CI engine with inner jet piston employing biodiesel were carried out,it was reported that the brake thermal efficiency was enhanced; the CO and Smoke release were reduced at maximum load, NOx emissions were enhanced at maximum load conditions in comparison with diesel fuel with diesel process variables for the manufacture of methyl ester of neem oil and fuel categorization for engine performance was done, the influence of parameters namely molar ratio, preheat temperature, catalyst absorption and reaction duration was investigated to regulate the transesterification process to assess the highest ester revival with least probable viscosity. Neem oil at 6:1 M ratio preheated at 55ºC temperature and retaining 60ºC reaction temperature for 1hr in the existence of 2 % KOH and allowed to settle for 1 day so as to obtain least kinematic viscosity (2.7 cS) with revival of ester (83.36%) [Rajan and Kumar, 2010]. An experimental investigation was performed investigation with Jatropha, Karanja and Polanga methyl ester in a CI engine. It was observed that maximum peak cylinder pressure and shorter ignition delay occurred for biodiesels in comparison with diesel operation [Ragit et.al, 2010].

METHODOLOGY

It is a method of conversion of the long branched triglyceride molecules of straight vegetable oil into tiny straight chain molecules which has a structure compatible to that of diesel. The basic reaction is that the raw vegetable oil reacts with alcohol in the existence of alkali catalyst to generate the methyl ester and glycerol as derivative. The methyl ester formation by transesterification reaction depends on many parameters such as reaction temperature, concentration of reactants, nature of catalysts, reaction time etc. This includes allowing the triglycerides of Neem/Rice bran oil to react with methyl alcohol in the occurrence of catalyst to yield glycerol and fatty acids. Known quantity of Neem/Rice bran oil of methyl alcohol and sodium hydroxide put in a circular bottom flask, these substances were mixed until ester formation was initiated. This content was then heated to 70°C and maintained that temperature by means of steady stirring for 60 min. After that it is allowed to settle under influence of gravity in separation funnel for 24 hours. Two separate filaments are formed in the funnel, top filaments were of methyl ester (Biodiesel) and bottom filaments consisted of glycerol substance. The biodiesel is separated and washed with warm water to remove the catalyst.

Blending

The performance of the biodiesel blends is found to evaluate comparably to petroleum products in all aspects ranging from power output to efficiency. Even though the Biodiesels can be employed in its pure form, widespread application of it is observed as biodiesel blends or blended with petroleum fuels. Biodiesel fuel blends are known as "X20" consisting 20% biodiesel by volume and 80% petroleum content. The larger the proportion of biodiesel in its blend, the more environment-responsive is the fuel

RESULTS AND DISCUSSIONS

Experimental Setup

A 4-Stroke, Diesel engine was employed for the performance evaluation investigation. The specifications of the CI engine aregiven in Table 2;figure of experimental arrangement is indicated in figure 3.The experimental

investigations were performed in the beginning using pure diesel to produce base line information. Once the base line information was recorded, investigations were performed with 10%, 20% and 30% biodiesel blends. Investigations were performed at different loads and the parameters associated with performance namely brake thermal efficiency, mechanical efficiency and brake specific fuel consumption were recorded.

BSFC vs. LOAD for Rice bran

The above graph indicates the brake specific fuel consumption at different load conditions using Rice bran oil. Using Rice bran least brake specific fuel consumption was achieved for increasing load because of lower calorific value of Rice bran at 20% blend when compared to diesel.

Mechanical Efficiency vs Load of Rice bran oil

From above graph, it is obvious that mechanical efficiency enhances with increasing load. The mechanical efficiency of Rice bran biodiesel blends are found to be higher that of l diesel, as the proportion of blends increases the efficiency has a tendency to increase proportionately this is because of complete combustion of fuel.

Brake Thermal Efficiency vs. Load of rice bran oil

From above graph, it indicates that as load on engine increases, brake thermal efficiency increases. The reason is brake thermal efficiency being higher with respect to biodiesel blends because the existence of additional oxygen atoms in the biodiesel.

Bsfc vs. Load for Neem oil

In the above graph indicates that as load goes on increasing, brake specific fuel consumption decreases. One cannot find much difference between the blends of Neem N10, N20, and N30 along with pure biodiesel, the graph won't show much variation between diesel and biodiesel.

BTE vs. Load of Neem oil

The above graph indicates the variation of load vs. brake thermal efficiency. As engine load increases, the brake thermal efficiency increases. The maximum brake thermal efficiency was achieved at 20% because less amount of fuel was consumed while using Neem blends.

Mechanical Efficiency vs. Load of Neem oil

Above graph shows that curve of N20 is nearer to diesel curve. Hence we can conclude that 20% is giving better mechanical efficiency compared with other blends.

CONCLUSIONS

The engine was run without the manufacture's modifications for Rice bran and Neem blends with pure diesel. Brake thermal efficiency of 20% Rice Bran and 20% Neem was high. By using biodiesel as an alternative fuels, it results in reducing the greenhouse gas emissions and global warming and also reduces the dependency on the use of fossil fuel. The performance was found to be efficient when using for 20% Neem. When compared between the Rice bran and Neem, power output of engine was higher for Neem than Rice bran because of higher calorific value resulting in higher heat generation and thus higher output.

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Table 1: Diesel, Neem and Rice bran oil properties

PROPERTIES	DIESEL	NEEM SEED OIL	RICE BRAN OIL
Calorific value(kJ/kg)	42000	39500	38900
Flash point (^{0}C)	51	350	126
Fire point(^{0}C)	57	365	152
Density(kg/m3)	836	875-900	909
Kinematic viscosity at 40^0 C (cSt)	3.72	38	30
Specific gravity	0.81-0.96	0.84	0.835
Cetane number	>51	>49	50

Table 2: Specifications of Engine Test Rig

Category	4-Stroke, Water Cooled , Clengine
Manufacturer	Kirloskar AC-1
Rated power	3.7 KW ,1500rpm
Bore and Stroke	85mm x 110mm
Compression ratio	16.5:1
Cylinder dimensions	624.19cc
Dynamo meter	DC Machine with swinging Field
Cylinder pressure	By Piezo Sensor
Starting	Auto start
Orifice diameter	15mm
Electrical supply	220AC, Single Phase ,15 Amps

Table 3: Engine test observation values at 1000 W load

Sl No.	Fuel	Torque (Nm)	Speed (rpm)	Time for 10 cc of fuel	Air flow
1	Diesel	5.02	1515	58	6.25
2	R10	4.54	1492	61	6.35
3	R20	4.35	1506	57	6.4
4	R30	4.78	1506	63	6.42
5	N10	4.58	1503	61	6.4
6	N20	5.03	1506	61	6.02
7	N30	4.66	1494	62	6.3

Table 4: Comparison of Performance parameters comparison of diesel with Rice bran and Neem oil blends at 1000 W load

Parameters	Diesel	Blend	Rice Bran Oil	NeemSeed Oil
Fuel consumption ($\times 10^{-6}$ Kg/s)	146	10%	139	139
		20%	144	139.3
		30%	134	137.09
Brake power (KW)	0.7963	10%	0.709	0.720
		20%	0.686	0.793
		30%	0.753	0.728
Indicated Power (KW)	1.0071	10%	0.95	0.935
		20%	0.856	1.013
		30%	0.969	0.953
Specific Fuel consumption Kg/KWh	0.66	10%	0.705	0.695
		20%	0.754	0.634
		30%	0.64	0.676
Mechanical efficiency	79.9	10%	74.6	77.7
		20%	80.1	78.28
		30%	82	76.4
Brake Thermal efficiency	12.9	10%	12.4	12.5
		20%	11.7	17.6
		30%	14	12.9
Indicated thermal efficiency	16.42	10%	16.6	16.2
		20%	14.6	13.8
		30%	18	16.9

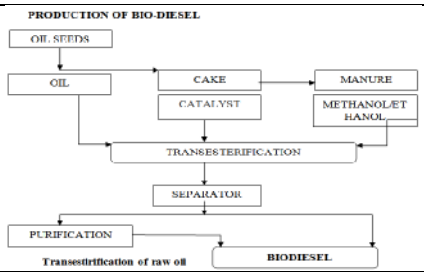

Fig 1: Flow chart of production of biodiesel

Fig2: Transesterification unit

Fig 3: CI engine test rig

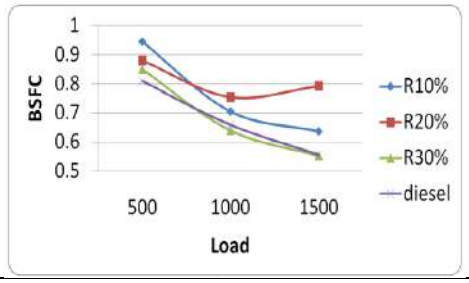

Fig4: BSFC vs. Load

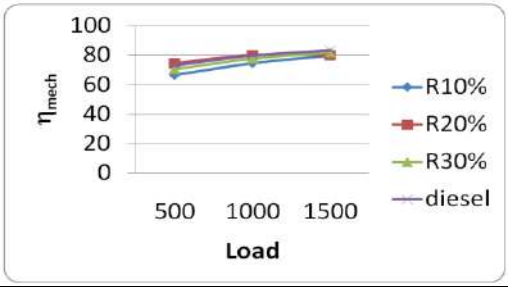

Fig 5: Mechanical efficiency vs. Load

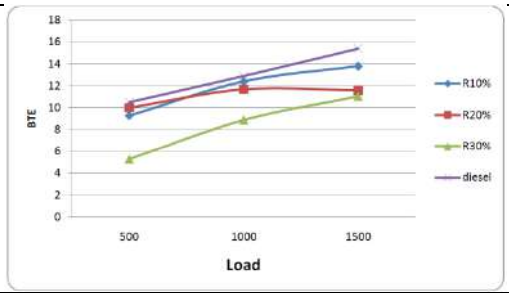

Fig 6: BTE vs. LOAD

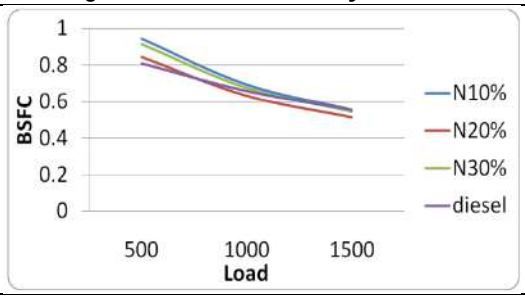

Fig 7: BSFC vs. LOAD

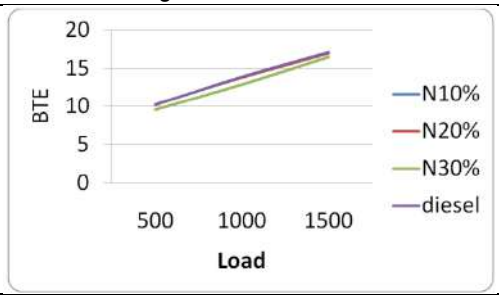

Fig 8: BTE vs. LOAD

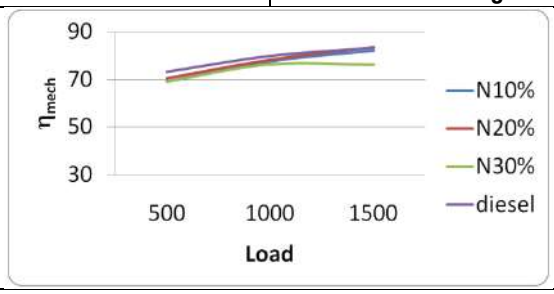

Fig 9: Mechanical efficiency vs. load

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REVIEW ARTICLE

Edible Tropical Wild Blood Fruit (*Haematocarpus validus* (Miers.) Bakh. f. ex Forman): A Review

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ABSTRACT

Blood fruit (*Haematocarpus validus* (Miers.) Bakh. f. ex Forman) is an underutilized potential fruit largely represented by woody climbers. This review aims to summarize the potential value and applications of this lesser-known fruit. This review includes the habitat, fruit content, dominant chemical composition, nutritional aspects and propagation of this plant besides its application as natural colourants, and its usefulness in ethnic medicine, pharmaceuticals and value-added nutraceuticals. Additionally, the review also suggests to have human dietary intervention studies as this fruit is found to be rich in polyphenols.

Keywords: underutilized, composition, pharmaceuticals, nutraceuticals, colourants

INTRODUCTION

The rise in population along with manmade and natural calamities are posing a threat to the natural wealth of the country. Forests are known to be a rich source of natural wealth and a number of edible plant varieties are still not tapped to its full extent which could supplement the requirement of nutritious food varieties for the use of the common man. Divergent varieties of wild edible plants are distributed across the country and are said to be vital, inexpensive and rich source of fibres, vitamins, antioxidants, minerals and nutrients. Traditional knowledge and evidences suggest that a wide variety of edible plant species have played a prominent role in providing health and nutritional security to man and animals [1]. Among the wild varieties, *Haematocarpus validus* is a promising dicotyledonous plant species restricted to the tropics and subtropics [2]. It was first described by John Miers [3]. The present article tries to consolidate the status and importance of this plant based on the investigations of various researchers.

Habitat and Distribution

Blood fruit is native to South East Asia and is mainly distributed in India, Bangladesh, Indonesia, Singapore, Thailand and Sri Lanka. In India, the fruit is found growing wild in Andaman & Nicobar Islands, Arunachal

Pradesh, Mizoram, Tripura, Assam and Meghalaya. The plant is generally found growing wild but not cultivated. *Haematocarpus validus* is an evergreen perennial creeping woody climber capable of growing under extreme conditions, from very dry environments to highly acidic soils and grows up to 1000 m and more in height with dark green smooth profusely branched stems [4]. It creeps and grows on other big trees like banyantree, jackfruit, or other long supporting tree. A recent study by Singh and Bedi (2016) in Meghalaya stated that the forest type of species occurrence was characterized by the presence of subtropical moist evergreen trees, and an enormous number of herbaceous undergrowth on a hilly landscape.

METHODOLOGY

The objective of the present study has been comprehended from the retrospections of the previous publications by various researchers on blood fruit.

Botanical illustration

Haematocarpus species are large woody climbers which spread on tall trees [5]. Leaves are simple, alternate, non-peltate, elliptic, 3 veined, petiolate. Bark is light grayish brown, rough, branches stout, wood consisting of consecutive layers of thin radiating plates. Inflorescence is cauliflorous, axillary, extra-axillary, terminal panicleorraceme. *Haematocarpus* species are dioecious where the male and the female flowers are borne separately. Inmale flowers, sepals 12-15, in 3 series, usually inner series larger, imbricate, petals 6,3 of the inner series auriculate at the base, stamens 6, free, enlarged connective projecting inwards. In female flowers sepals and petals similar as in male flowers, staminodes 6, minute, carpels 6, style reflexed. Fruits are drupes, narrow near the base, stalked, style scar near the base, smooth endocarp. Seeds are curved, non- endospermic, radicle short, cotyledons thick and long. Seeds may be dispersed by barochory *i.e.*, gravitational dispersal, zoochory *i.e.*, dispersal by birds or animals, anthropochory *i.e.*, dispersal by humans as reported by [2]. The species is known to tolerate varied climatic conditions and highly acidic soils [4]. In addition, it thrives well in Andaman Nicobar Islands which receives heavy rainfall with 3-4 months of dry phase [6]. The flowering time varies depending on the place. It has been observed that in Andaman Nicobar Islands, the species flowers more than once in a year. Peak season of harvesting is from Aprilto August [7]. In Bangladesh, the vines produce flower in mid-November-January and the fruiting season is May to August [8]. In Garohills of Meghalaya, the climber flowers from October to December and the fruits are available in the local markets from last week of March till June [9]. A study on the morphological characteristics of this plant conducted by Sangma (2016) in Garo Hills region of Meghalaya reveals that the plants have a climbing growth habit on to tall trees and shape of the fruits were recorded to be ovoid.

Phytochemical and Nutritional composition

Evaluation of phyto-chemicals is important in recognizing the potential of wild edible fruits as reliable supplement towards food and nutrition. The fruit tastes sour but with a pleasant flavor when fully ripe. The fruit contains 90.12% moisture, highly acidic pH (2.77), TSS (12.40%), titratable acidity (5.08%), total sugars (27.232%), reducing sugar (6.90%), non-reducing sugar (26.67%) and phenol of 0.51% [9, 4]. The total polyphenol content of the fruit is (400 -500 GAE mg/100g), flavonoid (542REmg/100g), tannin (275.56TAE mg/100g), anthocyanin (203.77 C3GE mg/ 100g) and beta carotene (Table 1) as reported by Singh *et al.*(2014). Quinic acid (a hepato-protectant) predominates among volatiles in the fruit (29.13%) and was quantified to be 292.95 mg/100 g dry weight along with five macro elements and 17 microelements [10]. The fruit has very high K/Na ratio (280.65) and both the fruit and leaf are rich in Fe content (57.29 and 38.44 mg/100 g dry weight respectively) [10]. High unsaturated fatty acid/ saturated fatty acid ratio and abundance of linoleic acid (55.54% of total fatty acids) in leaf along with relatively high content of conjugated linoleic acid suggest its potential usefulness in pharmaceutical and nutraceutical industry as reported by [10]. Pulp of fully ripe fruits contained 8.76 mg/g of total anthocyanins and Pelargonidin was the dominant anthocyanin, followed by Cyanidin, Peonidin and Petunidin [11].Blood fruit was also found to contain nitrate, phytate, oxalate and saponin (Table2) and the analysis of three different parts of blood fruit showed that the pulp, an edible portion had low nitrate and oxalate content and in a safe health limit for consumption [12].

Propagation

Considering the potential of this species and a viable source of income for the locals, natives and the tribes, efforts have been made for its domestication in the Andaman and Nicobar Islands of India [6].In order to conserve the species, seed pre-treatment for improving seed germination has been studied and was found that pre-treatment of the seeds with 0.1% thiourea gave the best seedlings and its standardized [6]. Seed germination of *H.validus* has been very effective when vermicompost has been used as the substrate for growth [13]. Morphological and physiological studies suggests that there were profound differences among the variants obtained from different geographical locations in terms of their TSS content, fruit size and anthocyanin content[14].

Applications

Traditionally the blood fruit and the plant has been used as a curative for jaundice, anaemia, itching, inflammation, body acheetc [4]. A part from this they are also used in preparing wines, squashes, in pickles and also can be dried and stored for future use [7]. The higher anthocyanin content of this tropical fruit makes it an excellent natural food colouring agent and a natural dye for clothes and handicrafts [12]. The blood fruit juice when extracted contains lots of minerals and nutrients but, being thermosensitive normal processing techniques cannot be employed for preserving the nutritive content of the juice [15]. Thermosonication assisted extraction of blood fruit is a better technique for processing its juice without losing the bio active compounds and nutritive value [16].The processing of blood fruit juice/pulp by the technique of spray-drying to produce powder is said to be the best among various processes resulting in better and superior-quality powders that are easy in packing, transportation, and with increased shelflife [17].

DISCUSSION AND FUTURE PROSPECTS

Many of these edible fruits are plentifully available in the forest and wild areas, and huge quantities of wild fruits are usually not collected and wasted because, their therapeutic properties and potential as subsidiary food sources are practically unknown to the village and rural communities [18].This being a fruit with many saturated and unsaturated fatty acids are to be investigated for their applications in the food and pharmaceutical industry. Blood fruit (*Haematocarpus validus*) has important bioactive compounds, antioxidant properties, and some essential minerals, which play a significant role in human nutrition and traditional medicine for treating arthritis, jaundice, hypertension, cancer, etc. In this contest human dietary intervention studies can be initiated to study the effect of this important species in regulating several life style diseases like diabetes, blood pressure, cholesterol, non-alcoholic fatty liver and cardio vascular diseases (CVD).

Evidences of earlier studies on fruit polyphenols reveal that they are effective on the four risk factors like platelet function, blood pressure, vascular function and blood lipids that lead to CVD [19]. Some evidences suggest that fruits containing relatively high concentrations of flavonols, anthocyanins and procyanindins, such as pomegranate, purple grapes and berries, were effective at reducing CVD risk factors, particularly with respect to anti-hypertensive effects, inhibition of platelet aggregation and increasing endothelial-dependent vasodilation than other fruits investigated [19]. As blood fruits are rich in anthocyanins, its effectiveness in reducing cardiovascular diseases and hypocholesterolaemic effects can be investigated.

As Blood fruit is used traditionally as natural colorant and dye its industrial uses can be explored both in food and textile industry. Use of such natural dyes in these industries either fully or in combination with artificial ones could reduce the extant of pollution in the environment as well as reduction in carcinogenic contents in the environment. Literature also shows a good amount of tannins in blood fruit and therefore can be studied for its uses in the tanning industry, water purification, food industry, packaging industry, pharmaceutical industry, wood industry, paints and as nutraceuticals, antibacterial, anticancerous, antiviral agents, for live stocks and in beverages. New technologies like molecular genetics, GI tagging, GIS mapping and documenting the traditional-ethnic knowledge would help in the conservation, preservation and development of the plant species.

CONCLUSION

The habitat, propagation traditional values and uses of Blood fruit (*Haematocarpus validus*) has been reviewed and documented. Considering the rich nutritive and antinutritive factors studies and research are to be further conducted for exploring its utility extensively among the entire population. In this contest, it is suggested that its propagation and conservation is domesticated such that the use of the plant species is known and valued by everyone invariably benefitting the socio-economic development of the society. More over the under-utilized plant species has to be brought to the fore front in terms of its applications in various fields of use for the benefit of mankind.

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Table 1: The nutritional composition of fruit (per 100 gm)

Parameters	Unit	Contents
Moisture	gm	90.12
Protein	gm	0.6
Carbohydrate	gm	6.99
Fat	gm	1.44
Crude fibre	gm	1.22
Ash	gm	1.23
Energy	Kcal	50
Vitamin C	mg	13.15
Carotenoids	µg	1170
β- carotene	µg	9.0
Iron	mg	0.57
Copper	µg	129.57
Zinc	µg	0.14
Manganese	µg	152.04
Calcium	mg	9.16
Magnesium	mg	6.86
Sodium	mg	0.42
Potassium	mg	255.70
Phosphorus	mg	39.50

(Source: Khatun *et al*., 2014)

Table 2: Anti-nutritional factors (mg/100g) in *H. validus* fruits

Fruit fraction	Anti-nutritional factors (mg/100g)			
	Nitrate	Phylate	Oxalate	Saponin
Pulp	16.25	422.68	34.95	85.56
Pericarp	25.00	506.83	39.82	85.28
Seed	19.58	415.83	33.82	100.06

(Source: Singh *et al*., 2014)

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RESEARCH ARTICLE

Dairy Products Export Marketing Potentials in India

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ABSTRACT

Marketing of dairy products the eatable and non-eatable product contains dairy ingredient. India is exporting large numbers of dairy products to other countries. The risk was faced by the companies while manufacturing, marketing and exporting. The risk was divided in two particular aspects respectively which is internal risk and external risk. The secondary data from APEDA(Agricultural & Processed Food Products Exports Development Authority) the percentage was calculated for three years and calculated compound annual growth rate for five years the data is taken from DGFT (Directorate General of Foreign Trade). The dairy marketing distribution channel divided in two main aspect organized structure and un-organized structure respectively.

Keywords: Dairy products, Marketing of dairy products, Channel of distribution, Risk management, Exporting dairy products.

INTRODUCTION

India is one of the top most countries in the world in exporting the dairy products. The large numbers of dairy products are manufactured and exporting to other country and also the milk has been exporting as dairy products raw material from very long period. Milk marketing was get tremendous growth in decade. The causes of Covid-19 the export marketing was get shutdown. The milk market in India got affected in that particular time. The farmers who are daily staple of milk suffered a lot also the enterprises and company where got affect. The export sales are gone down. People also avoid eating ice creams, milk shakes, curd, and butter milk. So, more number of companies sales got affected the manufactured good the half of goods only sold. But the mean time milk sale was increased

inside of India. The milk has been supplied to Hospitals, Health care centers and Covid-19 centers in pandemic periods. Even though the risk is high the market manipulation leads the market in the right pathway.

Objectives of the study

- To identify export marketing potentials of dairy products
- To find out growth rate of dairy products exports
- To find out standard deviation of dairy products exports
- To find out forecast of dairy products exports

METHODOLOGY

Dairy products exported secondary data has taken for analysis. The following statistical tool has used for analysis.

- Compound Annual Growth Rate (CAGR) / Kegger
- Standard Deviation
- Forecasting
- Diagrams

Milk

Various chattels milk is a daily staple for many people in our country. The Food and Agriculture Organization (FAO) announce 1st June of every year will be celebrating World Milk Day in 2001. The United Nations to recognize the importance of milk gave approval to celebrate the day. Milk contain Iron, Selenium, Vitamin B-6, Vitamin E, Vitamin K, Niacin, Thiamin, Riboflavin are all make the body healthy. India is continually exporting raw milk to various countries.

Milk Marketing

India has the largest cattle and buffalo population in the world. More than 67% of dairy animals are owned by marginal and small farmers which constitute the core milk production sector in the country. Milk contributes more to the national economy than any other farm commodity more than $10.5 billion in 1994-1995. The tremendous growth of India in dairy products it changes the world market. More number of companies where ready to make a contract with India. The dairy co-operative societies play a vital role in rural poverty by augmenting rural milk production and marketing. According to Agricultural & Processed Food Products Exports Development Authority was clearly examining the dairy products.

Areas of Dairy Product Production

The major milk production and exported states are Uttar Pradesh, Maharashtra, Himachal Pradesh, Madhya Pradesh, Punjab, Rajasthan and Tamil Nadu these are major states production of dairy products in India. India was exporting of dairy products more to the Arab, Bangladesh, USA, Bhutan and Singapore in 2020 -2021.

Eatable Dairy products

More number of the companies are using the milk as the raw material to manufacture the dairy products. The products are in two types eatable and non-eatable. Most of the non- eatable products are manufacturing in other countries, so they demand are high to buy the milk in India. The following listed products are using milk as a main raw material to finish the product.

Eatable Dairy – By Products

Milk is main raw material for producing dairy product and remaining wastage are used for producing Chewing gum, Bread, Ready to eat meals, Instant Potatoes, Instant Coffee/Tea and Hot Chocolate, Medicine

Non-eatable Product contains dairy ingredients

Milk is used for produce non-eatable products like Gloves, Nail Polish, Shampoo, Soap, Face Cream, Paper, Plastic, Cosmetics. These products are producing in India but these are not exporting to foreign countries. The foreign countries are importing milk and using milk to producing those non-eatable products.

Marketing Channels Dairy Products in India

The two main marketing strategies are followed in India.

Organized Sector

The organized sector is a sector where the employment terms are fixed and regular and the employees get assured work.

Organized Sector marketing channel

Producer ⇨ Retailer

Producer ⇨ Organized Sector ⇨ Retailer

Producer ⇨ Organized Sector ⇨ Government ⇨ Retailer

Producer ⇨ Organized Sector ⇨ Village Co-operatives ⇨ Government ⇨ Retailer

Producer ⇨ Organized Sector ⇨ Village Co-operatives ⇨ District Milk Producers Co-operative Union ⇨ Retailer

Producer ⇨ Organized Sector ⇨ Village Co-operatives ⇨ District Milk Producers Co-operative Union ⇨ Village Level Milk Producers Co-operative Society ⇨ Retailer

Producer ⇨ Private Retailer

Unorganized Sector

The unorganized sector where direct opposite to organized sector. It unorganized sector the employment terms are not fixed and regular. It is irregular because the enterprises are not registered with the government.

Unorganized sector marketing channel

Producer ⇨ Retailer

Producer ⇨ Unorganized Sector ⇨ Retailer

Producer ⇨ Unorganized Sector ⇨ Milk Vendor ⇨ Retailer

Producer ⇨ Unorganized Sector ⇨ Milk Vendor ⇨ Wholesaler ⇨ Retailer

Risks in Agricultural Enterprises

According to the marketing ethics where there is more risk and there will be more returns. The marketing of dairy products have very high risk. The risk is divided in two aspects namely internal risk and external risk. The internal risk affects the production and distribution. The external risk also known as market risk it affects the market.

Rainfall is very important role play in dairy forming. Where the rainfall is high dairy forming will increase animal foods like grass, grains and all other animal eatable agricultural goods will increase. The health food grains will improve the quantity and quality of the milk naturally. At the same time, the animals may be affected by the various diseases during rainy season.

Agricultural & Processed Food Products Exports Development Authority – APEDA

The APEDA is one of the departments working under the Indian government. The statistical data shows the products selling performance in other countries. The top 50 companies in India were exporting the dairy products to foreign countries. These companies are acting as manufactures, merchants and agents. Tamil Nadu state is one of the exporting large numbers of dairy products to foreign countries. Among these top 50 companies the 10 companies are in Tamil Nadu State.

Comparative Statement of Export for Dairy Products (April-May 2020) and (April-May 2021)

In the comparative statement of export given by - APEDA. The dairy products are coming under the head of processed fruits and vegetable. In the given table we can saw the comparative statement of dairy products quantity and price. In April-May 2020 the total dairy product exported from India is Qty 14356 it cost of 303 Crores in Indian money 40 million in USD. In April-May 2021 the total dairy product exported from India is Qty 21641 it cost of 459 Crores in Indian money 62 million in USD. When compare 2020 dairy products export with 2021 export, it is 51.41 percentage increased in 2021. The huge number of countries importing the dairy products from India. So, here top 10 importing countries were taken to analysis. The U Arab Emtsimporting more dairy products from India. This country has occupied first place in the importing dairy products number in the world. Followed by Bangladesh Pr is occupied second place and followed by USA occupied third place in the world importing. The dairy products percentage of world share increased as 19.54% in April-May 2020-2021.Here, India foreign currency gradually increased and sizable economic growth also increased.

India's Dairy Products Exports From 2015-16 to 2020-21(HS CODE: 0403)

The exporting of dairy products was good start in the year of 2016-2017 and also it was again raised in (2017-18) next year India's export was Rs. 1006.31 lakhs worth of dairy products. It was fall in 2018-2019 due to causes of Covid-19 the export market was temporarily stop for few month. So only the export market unable to reach the target at particular year but in the following year the exporting of dairy products from India is accelerate the sale. India export dairy products Rs.1315.65 lakhs in the year of 2020 -2021.

Standard Deviation for the top 6 countries

Formula: $\sigma=\sqrt{1N\sum Ni=1(Xi-\mu)2}$

σ is Standard Deviation

X is set of numbers

Mean is average of the set of numbers

n is the size of the set

Average = 8.3

Standard Deviation = 5.1

In the top ten countries the seven countries variance is above SD.

Forecasting of Dairy Products for next five years

CONCLUSION

Milk is main raw material for producing dairy products. India is exporting milk and various dairy products. Many states are producing dairy products marketing in India and exporting to foreign countries. This export market affected in COVID-19 pandemic period many milk producers and dairy product producers were got affected. The exporting of dairy product was decreased. The central and state government wants to take necessary step to develop the dairy product marketing in home and foreign countries.

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Table-1 List of Eatable Dairy Products

S.NO	PRODUCT	PRODUCT TYPES
1	Beverages	Coffee,Flavoured Milk,Milk Shake,Tea and Whey Drink
2	Fermented Products	Butter Milk,Curd,Lassi, Panner and Yoghurt
3	Hot Milk	Hot Milk
4	Ice Cream	Ball Ice cream,Candy,Casatta,Cone,Cup Ice cream,Delight,Kulfi,Scoop and Tutty Fruity
5	Mik	Badam Powder
6	Butter	Dry Fruit Mix,Milk Powder,Ghee andGulab Jamun Mix
7	Sweets	Chocolates,Gulab Jamun,Khova,Milk Peda, Mysore Pak and Rasagulla

Source: APEDA (apeda.gov.in)

Table-2. Risks in Agricultural Enterprises

INTERNAL RISKS					**EXTERNAL RISKS**		
Production Risks	**Equipment Risks**	**Financial Risks**	**Personnel Risks**	**Other internal Risks**	**Market Risks**	**Political Risk**	**Other external Risks**
Animal health	Construction risk	Interest rates	Sickness	Liabilities	Price risk	Agricultural Policy	Malicious Damage
Diseases	Machinery breakdown	Basel II	Fluctuation		Supply risk	Environmental Policy	Burglary
GMOs			lack of motivation		Quality problems	Tax Policy	Theft
Pest					quantity problems	Construction Laws	
Fungi							
Weeds							
Weather							

Source: LEHRNER, 2002.

Table-3 Comparative Statement of Export for Dairy Products

PRODUCTS	**April - May 2020**			**Unit Value**	**April - May 2021**			**Unit Value**	**% Changes**		**% Share in APEDA's TOTAL EXPORT**	
	QTY	**VALUE**		**In USD Per Tonnes**	**QTY**	**VALUE**		**In USD Per Tonnes**	**Rs**	**USD**	**Rs**	**USD**
	MTs	**Rs. Crores**	**USD Million**		**MTs**	**Rs. Crores**	**USD Million**					
PROCESSED FRUITS &VEGETABLE:	**14356**	**303**	**40**	**2789**	**21641**	**459**	**62**	**2862**	**51.41**	**55.01**	**1.67**	**1.67**
DAIRY PRODUCTS												

Source: APEDA (apeda.gov.in)

Tabel-4 Export of Dairy products statistical report from 2018-19 to 2020-21 (Rs. In Lakhs)

		2018-19		2019-20		2020-21		%age growth on	%age share
Rank	Country	Qty in MT	Rs. Lacs	Qty in MT	Rs. Lacs	Qty in MT	Rs. Lacs	previous year	In 2020-21
1	U Arab Emts	10,266.95	30,532.33	7,055.07	26,400.92	7,905.70	29,145.88	10.4	19.54
2	Bangladesh Pr	21,745.26	27,003.23	287.1	797.01	8,792.61	17,715.47	2,122.74	11.88
3	U S A	2,614.13	10,453.59	2,320.86	10,237.58	3,805.73	16,964.26	65.71	11.37
4	Bhutan	9,375.86	14,431.99	10,918.18	16,072.48	11,007.48	16,746.39	4.19	11.23
5	Singapore	4,673.88	7,503.47	4,905.18	8,153.32	5,927.75	11,328.08	38.94	7.59
6	Saudi Arab	2,788.37	9,549.45	2,402.69	8,462.38	2,458.86	8,512.28	0.59	5.71
7	Malaysia	7,745.53	9,508.19	251.64	1,026.79	2,966.06	6,342.15	517.67	4.25
8	Qatar	1,618.93	5,317.89	900.6	4,001.67	1,318.41	6,295.70	57.33	4.22
9	Australia	1,093.65	4,818.71	737.38	3,759.95	1,118.01	5,842.37	55.38	3.92
10	Oman	1,257.47	4,602.55	1,061.07	4,351.23	1,449.63	5,514.90	26.74	3.7

Source: APEDA (apeda.gov.in)

Tabel-5 Compound Annual Growth Rate / Kegger (Rs. in Lakhs)

	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	2020-2021	5 YEARS CAGR
SALES (RS IN LAKHS)	361.03	796.84	1006.31	784.94	908.92	1315.65	**30%**
GROWTH		120.71%	26.29%	-22.00%	15.80%	44.75%	

Directorate General of Foreign Trade (DGFT) calculated in MS-Excel

Tabel-6. Standard Deviation for the top 6 countries

S.No	Country	Growth Rate
1	U Arab Emts	19.54
2	Bangladesh Pr	11.88
3	U S A	11.37
4	Bhutan	11.23
5	Singapore	7.59
6	Saudi Arab	5.71
7	Malaysia	4.25
8	Qatar	4.22
9	Australia	3.92
10	Oman	3.7
Total		**83.41**

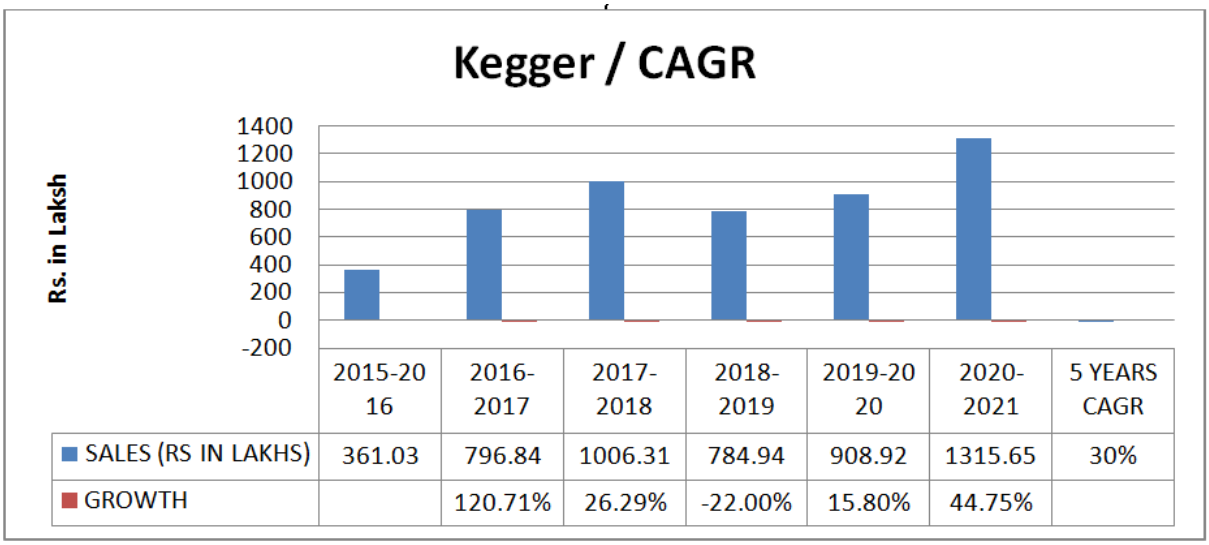
Source: calculated in MS-Excel

Tabel-7 .Forecasting of Dairy Products for next five years

YEAR	EXPORT
2018-2019	123721
2019-2020	83263
2020-2021	124407
2021-2022	111150
2022-2023	357147
2023-2024	111836
2024-2025	112179
2025-2026	112522

Source: calculated in MS-Excel

Fig.1.Compound Annual Growth Rate / Kegger



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RESEARCH ARTICLE

An Experimental Approach for Plant Disease Prediction System using Machine Learning

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ABSTRACT

The Agricultural field plays an important role for the growth any country. Most of Indian populations are depend for their livelihood on the agriculture/crops. Presently, the Indian agriculture is facing a number of hurdles because of the change of climate, water pollution/ shortages, lack of fertilizers, old methods/technologies, different plant's diseases and many more. These factors are not good for better food production to fulfill the public demand on time. The crops may be ruined by the infections in the plants, it may harm to our food security. To detect the diseases within the plants is not easy. DFS (Disease forecasting System) for Potato crops using ML (machine learning) is the best method to predict the plant's diseases for necessary solution to prevent it, timely.

Keywords: ML, AI, Disease forecasting System, Image processing, Internet of things (IoT)..

INTRODUCTION

Quality agriculture relies on gathering comprehensive information from farmland, which includes not only the environmental information but also the plant's information. For example, the environmental condition, such as temperature, soil moisture, humidity, soil composition, solar radiation, wind speed and rainfall, are considered to reveal the weather change and soil pollution, and can help to improve management of fertilizer usage and other inputs. The plant information, such as plant growth, plant disease and insect pest, are useful to predict the production and make decision about pesticide or organic applications. This Disease Prediction System will give a better prediction of disease on Potato and it will recommend suitable pesticide/ organic materials applications using Machine Learning. Python Language is most widely language for Machine Learning, AI, Data Science, Deep Learning and Image Processing because of its simplicity and power full library. Open source python language is used to implement this system with some libraries like OpenCV, pytesseract, NumPy, SciPy, Tensor Flow etc.
In this Disease Prediction System, Machine Learning and Clustering Algorithms can be applicable for plant growth monitoring. In this solution aggregation of data helps to convert raw data coming from plant into exact accurate data

which will be understood by farmers. Finally exact accurate information processed by Disease Prediction system is conveyed to farmers via messaging / App service on their mobiles, which will help to predict the application of pesticide to the plants

Literature Survey on Disease Prediction Techniques

Andreas Kamilaris et. al. [2018], author discussed and perform a survey of 40 researches efforts that employ deep learning techniques, applied to various agricultural and food production challenges. To study the agricultural problems stated under each work, the specific models and frameworks employed the sources, nature and used dataset and overall performance achieved with help of used methods, comparison of deep learning with other techniques also done. It is stated that deep learning provides high accuracy by using image processing techniques [1]. Konstantinos P. Ferentinos et. al.[2018], author discussed about convolutional neural network models were developed to perform plant disease detection and diagnosis through deep learning methodologies using plants leaves images. Training of proposed models was done with the use of an open database of 87,848 images, containing 25 different plants in a set of 58 distinct classes of [plant, disease] combinations. Some models gets success 99.53% in detecting the corresponding combination [plant, disease]. High rate of success model is very usefull to early detection tool and also have possibilities of further expanded by researchers [2].

Shimaet.al. (2018) proposed that plant disease cause decrease in food production. For detection purpose machine learning techniques are used by many researchers like RF processes, SVM processes, K-means processes, CNN processes. The random forest algorithm does the classification. The aim of author is to detect the disease with random forest classifier. We have to convert RGB type images to an HSV type's image [3]. Premet.al. (2018) proposed that some symptoms are visible from the eyes are wilting, spot, powdery mildew, galls, and dryness. Different attributes are taken in dataset, different techniques are used and different plots like box plot, bar plot are performed. With the help of statistical tests the prediction is done on inbuilt dataset. Many techniques are compared and the accuracy is different from each sample dataset [4].

Budiariantoet.al. (2018) proposes Machine Learning techniques for recognition of disease in corn plant which is a main source of carbohydrate. CNN technique is used to improve plant disease. Researcher used different algorithms and use support vector machines (SVM), Decision Tree (DT), Random Forest (RF), and Naive Bayes (NB) to compare the results. By normal seeing of plant we can understand the problem like color difference. Different parameters are used for dataset attribute [5]. Sherlyet.al. (2019) proposed there are different type's bacteria or fungus is responsible for many different plant diseases. It can be predict using algorithm of Machine Learning. Many researchers try many algorithms and get differ results. The classification of diseases is hard to done by algorithms. By CNN technique we can identify the mulberry plant disease [6].

Balwant J Gorad et al.(2019)gives a better disease prediction system for potato plant. K-means clustering used to split the data that is provided by the farmers. Farmers collect images from their phone, tablet, camera and other sources that is forwarded to the system and then system create dataset from that and periodically it is done and hence plants diseases predicted by the system [7]. Monalisa Sahaet al. (2020) takes the tomato and potato plants leaves to predict plant diseases. They collect both plant leaves images from internet sites and some images they collect with their digital camera from farming places. In proposed system after clustering if we give it(cluster) in multiple SVM classes then it gives better results and the performance analysis is 99% and the individual algorithm efficiency like k-means gives 88.6% and SVM gives 91%. Hence the proposed system is better than k-means and SVM [8].

SrideviSakhamuri et al. (2020)describe that there are three types of plant leaves diseases so they collect the leaves and maintain dataset according disease type. They collect different plants leaves like jasmine, grape, apple, beans, rose etc. and used different methods to detect leaves disease and get different accuracy like with k-means algorithm the accuracy was 88.8%, through SVM 95% accuracy was achieved and through ANN it was 70% to 95% for different diseases [9]. Krishnaswamy Rangarajan et al. (2020) proposed an automated disease diagnostic system for ten

diseases of four crops (eggplant, hyacinth beans, lime, lady finger). Author used six pre-trained deep learning models for training and validation of created dataset [10] . Pranesh Kulkarni et al. (2021) had taken public dataset for their research in which healthy and unhealthy images of apple, corn, grapes, potato and tomato plants were included. For the classification they used Random Forest Classifier. They got 93% accuracy through the system they developed [11].

Sahil Thakur et al.(2021) authors develop the model with CNN to identify the plant diseases with image processing. They used plants like potato, grape, corn, apple etc [12]. Gianni Fenu et al.(2021) review the researches from past 10 years in which different plants and crops were used like cherry, coffee, barley, grape etc. and used methods were SVM, SVR, KNN, ANN and many. Author observed that researchers need high quality labeled data for their research work [13]. Kowshik et al. (2021) used Convolutional Neural Network and Deep Neural Network to detect plant diseases. Author detect similar diseases from different plants like banana, beans, jackfruit, lemon, mango etc. The proposed method with CNN and DNN is feasible for early plant disease detection [14].

Jayashri et al. (2021) reviews the existing image processing techniques for disease prediction of pomegranate. They used SVM, ANN, KNN and PNN classifier to detect bacterial, fungal and viral diseases in fruit. K-means clustering for image segmentation, Fuzzy c means gives highest accuracy. According to them very few diseases were covered in the existing system [15]. Punithast al.(2021) reviewed many research papers on detection of plants disease using image processing techniques. For image processing they follow the procedure image acquisition, image pre-processing, image segmentation, feature extraction and disease classification. After comparison of many different models (which used SVM, ANN, KNN and other approaches on different plants) they found that SVM is most accurate method followed by ANN [16].

The challenges in disease forecasting system for Potato Crops

Potato is a popular vegetable and widely used for the various food productions in all around the world. Many species of potato are cultivated with different size and colors/shapes by the Indian farmers for better profit and result. The main factors/issues are necessary to point out and observe for good production of potato crops which are-

- To monitor the overall growth of the Potato plant, time to time.
- To check and forecast the plant's infection through its leaf's conditions.
- To observe and suggest suitable/required resolution for forecasted plant's disease in reliable way.
- To fulfill the public need of good and secure /healthy food items.
- To maintain the overall cost and production of potatoes in suitable manners for the people.

The Research Objectives

With the help of machine learning (ML) and image processing techniques, we can easily solve a number of issues regarding to protect potato crops from the various infections. This research work will be helpful to predict the potato plant's diseases and can suggest the required pesticides/fertilizers for the prevention of infection in potato plants. The objectives of this study/work are-

- To provide a logical forecast for Potato crops through the changing of leaf conditions.
- To recommend the proper pesticides or fertilizers for the predicted diseases of potato crops.
- To design a disease forecasting system, this predicts the growth of plants using ML techniques.
- To publish a new approach/work for agricultural research and various industries.

The Proposed Model

The architecture of DPS is human friendly system which contains separate back-end server for plants image processing and wireless devices for farmers such as mobile, tablet, PDA, laptop etc. The camera of farmer's mobile / PDA / Laptop of DPS is responsible for capturing image of plant leaf / actual Potato to test with earlier created model. Once image captured will be send to back-end for processing with trained model.

K-means Clustering algorithm is used to insert new image into appropriate cluster of disease categories. Using a desktop GUI form / web browser, a user can request and view historical data and along with this the last gathered "almost real-time" data from the back-end server. A Historical data as well as the last gathered "almost real-time" data, plant disease and insect pests of plant is sent to the farmers on their mobile using messaging /App service which can help to improve management of fertilizer usage & other inputs such as pesticide application.

Research Mythologies

The Research will be carried out using following methodologies for development of Intelligent Monitoring Solution for Indian Agriculture.

- Literature study/review is to be carried out on Disease Prediction System for various plants along with strength and drawback in Agricultural Applications.
- Issues and challenges related to DPS for Agricultural Application Systems is to be studied from available literature & Books.
- Periodically Visits are arranged to Potato farm with farmers and agricultural professor / researcher which will help to monitor the growth of plants
- The probabilistic models for DPS are developed to resolve the issues such as prediction of disease on Potato leaf and on actual Potato for Indian Potato crop.
- Mobile Network and traditional network will be interconnected so that IP addressing, Client-Server architecture will be utilized.
- The developed schemes are being tested through application designed / simulation for it.
- Plant growth monitoring system is to be designed which will send information about management inputs such as pesticide application by messaging /App service to farmers.
- Application is to be make available to public on various platforms so that people will get it easily (ex. Google Play Store)
- Performance comparison with standard existing models.
- At every stage, results obtained will be published in suitable conference and journal.

Proposed Methodology

Dataset

In this paper, for further recognition / disease prediction processes an appropriate and perfect dataset (collection of images related to various leaves of potato plants etc.) is required. First step of the proposed system is to train the system. After data is given to the system, evaluate the performance. Machine learning algorithm dataset is required. For this training data we take images and specially captured data images of potato plants. A total of 1000 images are collected from different potato plants for train system And 2000 images are collected for test the system operation.

Augmentation

In this paper, after the collection of dataset, convolutional neural network (CNN) is used for feature extraction which is one of the deep machine learning technique. For feature extraction on the above data set done using a supervised learning technique convolutional neural network (CNN).For this we take fundamental considerable information. Large amount of data can give large and accurate amount of feature attribute in CNN. All the data set are divided into their different data category. Take a data image and rotate the image as 90,180,270 respectively in mirroring each rotated images expanding the dataset using CNN. The process of CNN is different in different images. It is divided into 3 different convocational layer. Each layer have in between a max-building blocks. First take an image as an input. Give the input to the initial convocational block and modifies the entered image with 36 kernels of size 3.5x3.5.And give the result of primary convocation building block to the max block as an result. After first max building block gives the output to the second convocation building block as an input data. In the second convolutional block image will be filter with 64 kernels of size 4x4.Give this output to the second max pooling layer as an input data. After that max building block gives the result to final convolutional

block. It filters images / data as 128 kernels of size 1x1. And give the output as fully connected 512 neurons layers. The result given to soft max function. The soft max function gives a prospected circulation of the four result categories. The last layer is connected to MLP. All convocation layer output have the activated ReLu function. And it fully connected to the layers. The system is trained using Adam. The batch size is size of 100 for 1000 epochs. In that way, we collect the features of the image dataset using the CNN algorithm.

Classification Principle

In the data analysis and database management technique clustering is one of the data structure management technique. Lots of data can be sub divided into subgroup. Same type of data can be placed in same group. Using this method we can define task of identification. Basically find homogeneous subpart inside the data point. Euclidean-based distance or correlation-based distance is use to identify this methods. It is an application-specification. Base on the features sub grouping clustering analysis is used. Clustering is an unsupervised machine learning method. Clustering can be done in different way. Partition the dataset features which are taken from Convolutional neural network (CNN). Each partition of dataset is non-overlapping clusters where every point of features is belongs only one groups. Decide the total number of clusters. First centroid the random data point and iterating data. If centroids are not change then iterating repeatedly. Data points assign in same cluster. After that calculate the sum of the squared distance between data points and all data centroids.

After that call SVM algorithm to evaluate k number of clusters. Sort number is denoted as a T. create a condition where every value can be evaluate as a newly generated solution. Then it will give kSVM solution.
ksvm-model = {(c1,Lsvm1),(c2,Lsvm2)...(ck,Lsvmk)};
Where, k= local model= no of cluster;
y=it is presented as parameter which is hyper of kernel function of RBF;
c= the error rate of SVM.

And in the very last return the global best solution. Repeat till all such cluster is pruned. And it gives final classification. Then, we can classify and identify the images very efficiently. And calculate the accuracy of the prediction system. In the fields of Machine Learning, perhaps K-Means is the most known and studied method for clustering analysis. K-means is a method of clustering which helps to feed up new scanned data or images into required form of blocks for image/picture categories. Using a desktop GUI form / web portal, a client can demand and visualize the historical images with the past gathered data from the server.

THE RESULTS AND DISCUSSIONS

The performance, related to disease prediction / recognition system is calculated, here. The overall performance is calculated as how much time is taken for the recognition process. In this way performance is calculated. For this experiment Anaconda navigator software system is used. Instanced of python programming using jupyter notebook is more efficient. After the proposed system total loss will be 0.02313.Using multilevel k-means and SVM algorithm efficiency of performance will be calculated. After clustering if the data will go through the SVM multi-level classification will be better result. 3500.00 potato data images are used for train the proposed system. Test accuracy with best parameter set is 0.9790. In this fig 4 we derived that individual algorithm like k-means process give efficiency 88.09% and SVM give efficiency 89.99%. But in the proposed methodology it gives better result. The performance analysis is 95.60%. The accuracy is better than individual algorithm performance. Table 1 shows the accuracy rate of different classifiers for image recognition.

Research Outcomes

The proposed method of approaching is a valuable approach, which can be give better performance. K-mean algorithm didn't work well in universal cluster and it does not work well with cluster of different data size and

different data mass. So that after clustering if we give the clusters in the multiple SVM class then it is give better classification .In this method, it is found that big amount of dataset can be easily trained and tested to recognize the different images of potato leaves. This proposed model gives a better result than other classifiers. Now in daily life, this kind of approached is very useful. Future work can be developing the algorithm better segmented techniques. So there is a scope of improvement in the techniques. Following are the research objectives to be carried out in upcoming period for Disease Prediction System. Following are the research objectives to be carried out in upcoming period for topic of development of Intelligent Monitoring Solution for Indian Agriculture

- Design of Disease Prediction System which will predict disease on Potato crop based on symptoms of leaf and Potato in reliable way.
- The probabilistic model is to be developed which will recommend appropriate solution in terms of pesticides / organic material for earlier predicted disease.
- Designing of human friendly system (Computer / Mobile) which monitors plants growth by using machine learning techniques.

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Table 1. Accuracy by Different Classifiers

S No.	Classifier	Average Rate (%)
1	K-Mean	88.09
2	SVM	89.99
3	PM	95.60

Fig. 1"Dataset of disease affected and healthypotato plant images which are used to create trained model"

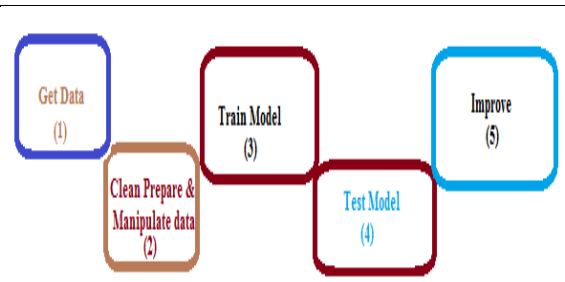

Fig.2"Working Process of Machine Learning Classifiers"

$$J = \sum_{i=1}^{m} \sum_{k=1}^{K} w_{ik} \left\| x^i - \mu_k \right\|^2$$

Fig. 3 " SVM Calculation"

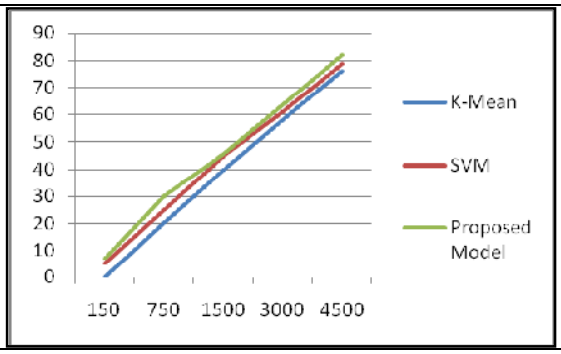

Fig.4 "Experimental Work"

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RESEARCH ARTICLE

Prescribing Pattern of Drugs of Common Psychiatric Illness in the Psychiatric Outpatient Department in a Tertiary Care Hospital, Salem.

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ABSTRACT

To facilitate the rational prescribing and to access the prescribing pattern of drugs as it provides effective feedback to doctors. Antipsychotic medications are utilized for a number of decades. Yet the prescribing pattern has been limited with few studies in Indian population. The American psychiatric Association has provided guidelines for the use of atypical antipsychotic antidepressant drugs rather than typical agents due to risk of adverse drug reactions**.** A prospective observational study was conducted in the Psychiatry outpatient department of a tertiary care center at Salem, for a period of six months. The diagnosis and treatment was made in psychiatric department and prescription analysis was done by the department of pharmacology**.** A total of 100 patients prescription was scrutinized in which 65% were females and 35% were males. Quetiapine is the most commonly prescribed drugs followed by Olanzapine and Risperidone. Polypharmacy was noted in 32%. Majority of drugs were written in generic names.

Keywords: Drug utilization pattern; Psychiatric Outpatients; Psychotropic Drugs

INTRODUCTION

Analysis of prescribing pattern gives an idea about the rational use of drugs among doctors in the community also it provides the information about irrational prescribing pattern and chances of providing feedback to the physicians. The prevalence of mental disorders vary from 9.5 to 370/1000 in Indian population [2,4] which is much similar to prevalence worldwide [3]. The mental diseases are classified into major and minor thereby extending from common

mental diseases to severe psychiatric disorder [4]. It is a challenge for the psychiatrist to treat the major diseases while the minor ill-ness are sparingly present throughout the community being unnoticed. [5].The patient with the mental illness donot attend the hospital or psychiatric clinic, due to the social stigma which prevents their voluntariness [6]. As per the recent study the most common diseases which are more prevalent is childhood and adolescent mental illness followed by mood disorders, alcohol dependence syndrome, geriatric disorder and schizophrenia[7,8]. The common risk factors to develop the mental diseases increases with advanced age, female sex, student population, chronic co-morbid conditions, disabled persons in custody(9). Because this disparity lies on reporting, an attempt was made to find out the prevalence of mental disorders in the outpatient psychiatric department in a tertiary care centre, Salem. In this present study the prescribing pattern of antipsychotic drugs in the Psychiatric outpatient department for Depression, Anxiety, Insomnia, Bipolar disorder, Psychosis, ADHD in a tertiary care hospital, Salem.

METHODS

Study Design

It is a prospective drug utilization study.

Ethical Considerations

The study was conducted after obtaining the institutional ethical committee (IEC) approval and carried out in Psychiatry outpatient department of VMKVMC&H, Salem.

Inclusion Criteria

All patients who attended psychiatry OPD of VMKV Medical College and Hospital, Salem from Dec 2020 to Feb 2021, diagnosed to have mental diseases were included along with drug prescription.

Exclusion Criteria

Patients admitted as inpatients.
Non cooperative patients.
Patients on non psycho-pharmacological therapy was excluded.
Follow-up patients were excluded.

Sample Size

A total of 100 prescriptions was analyzed.

Study Procedure

The data was collected from the patient leaving the OPD and was recorded in a separate structured case report form (CRF).

Data Collection

Demographic details of the patient, prescription details, name of the individual drugs, any fixed combination prescribed was recorded. Then the dose, dosage form, dosing schedule, duration of treatment was collected.

Statistical Analysis

The data's was entered in Excel sheet and analyzed using SPSS software version V27.

RESULTS

Characteristics of study participants

The percentage of female to male patients was about 65% and 35% respectively and the average range was 11-60 years with majority of persons was being in the age group of 31-40 years.

Distribution of psychiatric disorder attending the psychiatric OPD

Around 41% of the prescriptions were of depressive disorder followed by 18% of schizophrenia, 14% of bipolar disorder and 13% of mixed anxiety, depression disorder (Table 2).

Prescribing pattern

This table 3. shows the prescribing pattern of various drugs for mental diseases. Olanzapine was the most commonly prescribed drug for depressive disorder, followed by bipolar illness and Schizophrenia. Quetiapine was the next commonly used drug for Schizophrenia followed by depressive disorder. This list also included the prescribing pattern of Risperidone and Aripiprazole in few cases.

Antidepressive drug usage pattern in percentage

This table showed the drugs used in the management of depression. Fluoxetine which was more commonly prescribed for depressive disorder (37%) followed by 34% in mixed anxiety and depressive disorder. Escitalopram was seen in 20% of the prescription of depression and 21% in mixed anxiety and depression disorder. While prescription of Sertraline constitutes 45% in depression cases and 6% in Mixed anxiety and depression disorder.

Concomitant medication usage pattern

This table explains that the sedative hypnotics were commonly added to the major and minor illness with 64%,while Trihexyphenidyl of 8% followed by 7% propranolol. Carbamazepine was found to be the most common drug prescribed for Bipolar disorder (18%) and Acamprosate in alcohol dependance 34%.

DISCUSSION

Antidepressants were the most commonly prescribed medications followed by antipsychotic drugs (10). We observed the gender prevalence was found to be seen more in females than males. This was very similar to Western studies where the majority of patients affected belong to 30 - 40 age group followed by 21-30 years. The study carried in western world have a preponderance after 40 years of age(11,12). A large number of drugs were prescribed by generic names 84% since good quality drugs are available in our hospital pharmacy, substitution of drugs was foremost avoided. Depression disorder was the most common disease affected which was followed by schizophrenia and bipolar disorder in our population. The most common antidepressant prescribed was fluoxetine, followed by escitalopram and sertraline. Fluoxetine was commonly prescribed drug followed by escitalopram which is quite different from other studies, where escitalopram is the most common SSRI's prescribed for depression(13).The current NICE guidelines recommends SSRI should be the first choice for the treatment of depression with the head o head acceptability as per the finding of Ciprani et al(14,15). Fluoxetine and sertraline was found to be superior than fluoxetine and clomipramine (15).The NICE guidelines emphasized that antidepressants should be continued for 6 months following remission of a major episode of depression. The most common antipsychotic drugs was quetiapine followed by olanzapine and then risperidone .Even the cost of the 2nd generation compounds was generally high, the effectiveness was more with the second generation drugs with minimal adverse effects. Moreover quetiapine usage was seen in our prescription when compared to other studies where olanzapine was commonly prescribed (16).

Sedative hypnotics was the most commonly prescribed drug along with antipsychotics, antidepressant drugs which was followed by anticholinergics and propranolol. Rational prescribing was followed as per the principles of prescription writing. Sodium valproate and carbamazepine were the commonly prescribed drugs in the treatment of Mania. Vasudev et alin his study concluded both carbamazepine and sodium valproate was feasible for the treatment of mania(17).Weisler et al in his study proved carbamazepine is better in the treatment of mania which supported by our study (18). Acamprosate was used in the treatment of alcohol dependence. Katiewit Hiewitz et al in his study found out Acamprosate to work by promoting a balance between excitatory and inhibitory neurotransmitters and currently 3 drugs was found to be effective(19).US FDA approved disulfiram, naltrexone and acamprosate to be the proved medications in the treatment of alcohol dependent patients (20).

CONCLUSION

The findings of the study can lead to rational, evidence based improvement in the prescription pattern of antipsychotic drugs.

LIMITATIONS OF STUDY

The sample size was less in number and lacks facility indicators, patient care indicators. Olanzapine was the commonly prescribed drug followed by risperidone, as antipsychotic drug in the psychiatric OPD. Our study showed a predominance of atypical drug prescription over typical drug which is quite different from other studies.

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Table 1: Age wise distribution of the Patients

Age group	No. of patients
1-10	Nil
11-20	12
21-30	28
31-40	34
41-50	16
51-60	10
Total	100

Table 2: Distribution of psychiatric disorder attending the psychiatric OPD

DISEASE	NO. OF CASES
Depressive disorder	41%
Schizophrenia	18%
Bipolar disorder	14%
Mixed anxiety and depression disorder	13%
Manic disorder	9%
Alcohol dependance	5%

Table 3: Prescribing pattern of Psychiatric Drugs

Disease	Quetiapine	Olanzepine	Risperidone	Aripiprazole
Schizophrenia	8	5	3	2
Depressive disorder	7	15	1	1
Bipolar disorder	1	8	2	-
Mixed anxiety	1	2	1	-

Table 4: Prescribing pattern of Antidepressive drugs

Disease	Fluoxetine	Escitalopram	Sertraline
Depressive disorder (41)	27	10	4
Mixed anxiety and depression disorder	24	11	6

Table 5: Concomitant medication usage pattern

Drug class	Prescribed in no. of cases
Sedative hypnotics	64%
Trihexyphenidyl	8%
Propranolol	7%
Acamprosate	5%

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RESEARCH ARTICLE

A Solar-Powered Water Pumping System using a Cuk Converter-Based Brush Less Direct Current Motor

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ABSTRACT

The Paper proposes the best and Efficient Methodology for Photo Voltaic (PV) water pumping system using the maximum power point tracking technique. To reference optimal power, the optimum is suspended. This technique was created to ensure that the buck–boost converter's chopping ratio is ideal. The suggested MPPT technique is utilised to improve the efficiency of a solar water pumping system.An adaptive controller based on Fuzzy logic controller is utilised to optimise the duty ratio for PV maximum power at each irradiation level.The Cuk converter controls the DC connection voltage between the PV and the VSI. The Continuous Conduction Mode is used to manage the DC bus voltage in the Cuk converter, which helps to reduce DC-DC converter losses.The Brush Less DC motor's speed is controlled by a voltage source inverter with Pulse Width Modulation control. The Hall Effect sensor is used to generate the PWM pulse. The Brush Less Direct Current motor's Pulse Width Modulation switching will reduces switching losses while enhancing efficiency. MATLAB Software is used to simulate the whole system.

Keywords: Cuk Converter, MPPT, solar power, Brush less DC motor.

INTRODUCTION

Solar-powered water pumps are becoming increasingly popular in rural places where electricity transmission is either unfeasible or uneconomical. Solar energy is also non-polluting, abundant in nature, and costless. As a result, solar power can be used to replace the majority of traditional energy sources. For water pumping systems, solar-powered AC and DC machines are proposed [1-6].AC motors have a complicated control system and have a lower efficiency at low speeds. Brushes and commutation issues necessitate routine maintenance on the DC motor. A study

proposes PMBLDC for water pumping systems using multiple MPPT algorithms.[5].The PMSM has also been proposed for a water pumping system. The SRM [7], which has a quick response, strong torque, and a wide operating range, has also been mentioned. However, because of their high efficiency, minimal maintenance, and wide range of speed control, BLDC motors are widely popular and recommended for driving applications [8, 9]. They are used in various residential applications [10], hybrid automobiles [11], robotics [12], and other areas because of these advantages. Brushes and a commutator segment are not present in a BLDC motor. As a result, the brushes' wear and tear properties, as well as the problem of sparking, must be addressed. However, this paper has substantial switching losses and low efficiency. The SEPIC converter-based PFC is presented as well as the circuit's substantial switching losses. When compared to FCM Segmentation- Boosting, FCM Segmentation, the Fuzzy Bee Segmentation Bagging method is used to improve accuracy. In order to track the maximum power, fuzzy logic control with MPPT is used, which employs linguistic variables to alter the inverter's firing angle. In the early stages of lung cancer, a neural network is used to diagnose tumours and develop novel therapeutic strategies.

To compensate for difficulties including power factor, current imbalance, and current harmonics, a four-leg inverter has been developed, as well as to inject energy generated by renewable energy sources. Sustainable energy power sources at the same time a few of ZETA, SEPIC, CUK, and buck-boost papers proposed. The goal of the converter was to reduce switching. Despite the fact that there were losses, the overall cost of the system climbed, as did the cost of the system as a whole implemented the continuous conduction mode (CCM) and discontinuous conduction mode of operation. The current multiplier is employed in continuous in this paper. In this application, the conduction mode (CCM) and voltage follower are used.

PROPOSED CIRCUIT

Figure 1 shows a solar-powered Cuk converter for water pumping systems based on a three-phase voltage source inverter (VSI) supplied BLDC motor (1). To alleviate voltage and current strains on its switching devices, the Cuk converter is designed to run in continuous conduction mode (CCM). In the Cuk and voltage source inverter, the MOSFET (IRFP840) is used as a switch (VSI).The CCM also realised that the DC-DC conversion is unaffected by the load. The discontinuous conduction mode (DCM) increases switching losses and creates electromagnetic interference noise. As a result, by using the CCM mode, these flaws are eliminated. PWM pulses are generated using a Hall Effect sensor positioned on the shaft and adjusted to the BLDC motor's rotor position.

METHODOLOGY AND OPERATION OF PROPOSED METHOD

Fuzzy logic Maximum Power Point Tracking is utilised in this circuit to get the most power from the solar panel, while PWM control is used to regulate the voltage source inverter, which regulates the speed of the BLDC motor that pumps the water.

Maximum Power Point Tracking for Solar Power Systems

Solar energy is inherently intermittent. To get the most electricity out of a solar panel, various strategies are used.In this paper, a fuzzy logic controller is used to extract the greatest amount of power from the solar panel seen in Figure 1. The MPPT controller uses this information to track the maximum power generated by changing sun intensities.

Cuk Converter CCM Operation

1) In this Cuk converter, the input inductor (Li) saves energy when the switch (Sw) is closed, and the intermittent capacitor stores energy when the switch (Sw) is open. The energy is discharged through Cd and stored in the output inductor (Lo).

2) When the switch (Sw) is open, the input inductor (Li) discharges its energy through C1. The energy held in the output inductor (Lo) is discharged to Cd in the meantime.

As a result, substantial values for the input inductor (Li), output inductor (Lo), and intermittent capacitor (C1) ensure that some energy is always available for continued operation throughout switching periods.

Switching Sequence of VSI

The VSI switching pulses are generated by a Hall Effect sensor. The rotor position is used to generate a signal by the Hall Effect sensor, which is positioned on the shaft. With the help of an encoder, pulses are generated. It's the procedure for making something. By transforming three hall signals into six switching pulses (s1-s6),It's worth noting that only two switches are required. Inverter with a voltage source that operates in a 120-degree mode. As a result, Overall efficiency is improved when switching losses are decreased.

SIMULATION RESULTS

A water pumping system using a solar-fed Cuk converter-based BLDC motor simulation circuit. Fuzzy logic MPPT is utilised in this circuit to get the most power out of the solar panel. Solar energy is delivered into the Cuk converter, which then feeds the output to a 3-Φ voltage source inverter. The MOSFETs are employed by VSI to regulate the BLDC motor as a switch. A Hall Effect sensor is added and fed into the comparator to sense the speed of the BLDC motor. To control the BLDC motor's speed, PWM pulses are created and fed to VSI.

CONCLUSION

The performance of a solar-powered water pumping system with a Cuk converter-based BLDC motor has been simulated in a MATLAB/ Simulink environment. By using a Cuk converter, switching losses are decreased. In addition, the VSI decreased by operating in the 120-degree conduction mode, switching losses are reduced. Hall signals effectively regulate the speed of the BLDC motor. By doing so, the cost of the circuits is effectively decreased when using the other form of regulating sensors. The total efficiency has increased.

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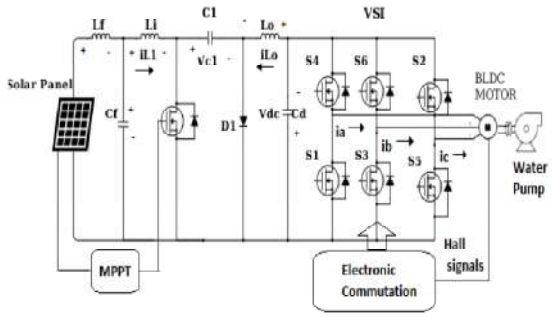

Fig.1: Solar-fed Brush Less DC motor with Cukconverter suggested circuit diagram for water pumping system

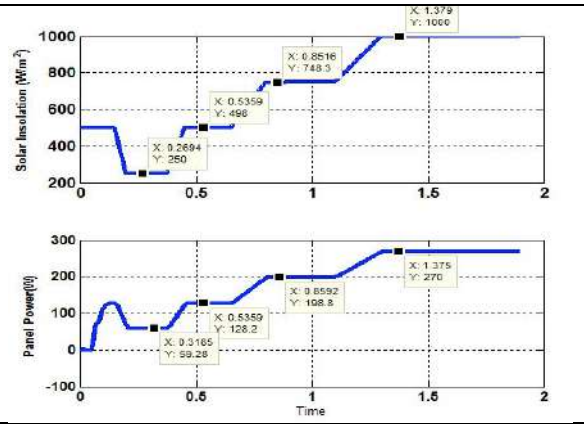

Fig.2: MPPT regulation of solar energy extraction and insolation

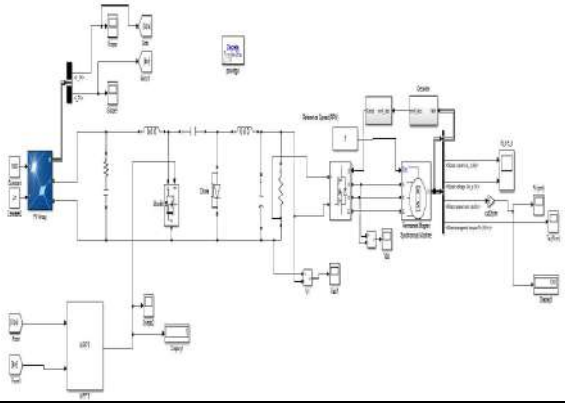

Fig.3: simulation circuit of Solar-powered Brush Less DC motor driving system

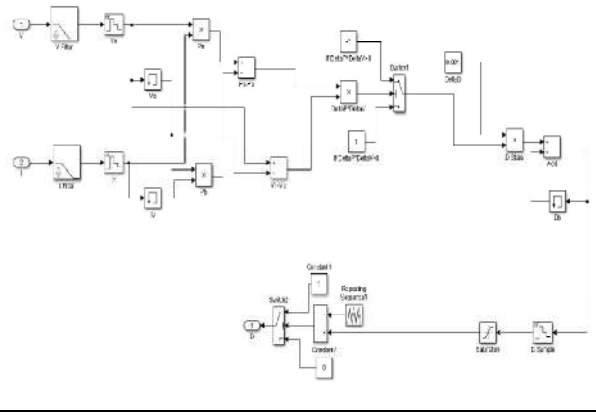

Fig.4: MPPT Model

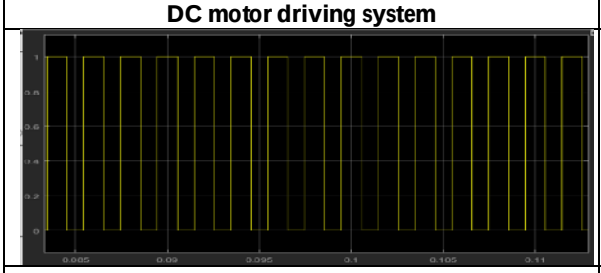

Fig.5.1: MPPT Voltage

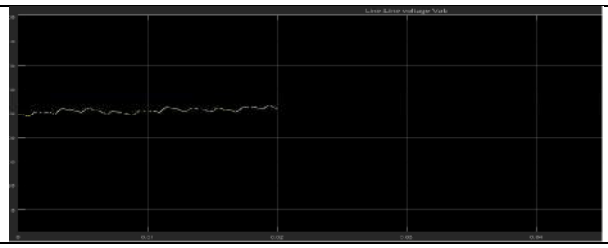

Fig.5.2: Converter output Voltage

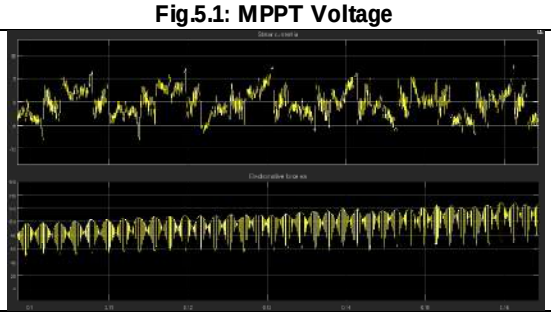

Fig.5.3: Stator Current and Voltage

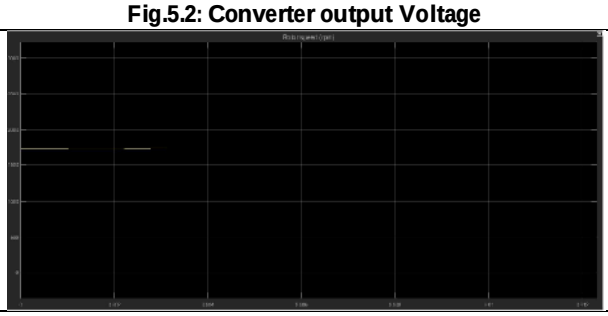

Fig.5.4: Rotor Speed

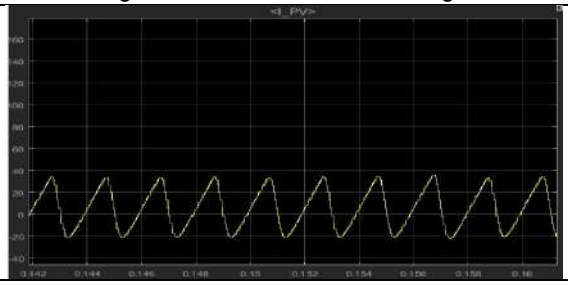

Fig.5.5: Solar output Voltage

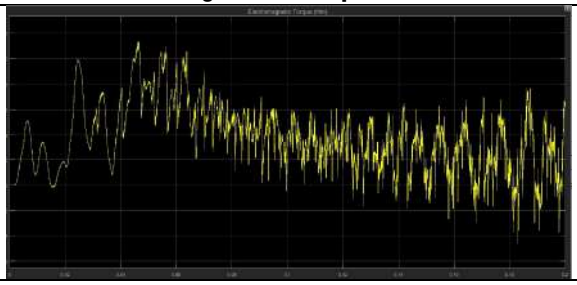

Fig.5.6: Electromagnetic Torque

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RESEARCH ARTICLE

Effects of Urea Coated Hydroxyapatite Nanoparticles on Various Biochemical Parameters in Two Varieties of *Brassica juncea* L

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ABSTRACT

In the present investigation, urea coated hydroxyapatite nanoparticles (UHANPs) were synthesized, and characterized by using X-ray diffraction, FESEM, and FTIR techniques. Then, their effects at different concentrations (250 ppm, 500 ppm, 750 ppm, 1000 ppm) were observed for biochemical attributes of two varieties of *Brassica juncea* L. i.e. GIRIRAJ, and NRC-BH 101. Those were also compared with control plants and plants grown in treatments with the same concentrations of urea. Results revealed that synthesized nanoparticles were 23-32 nm in size, and for all the parameters, biochemical attributes were the maximum at treatment with 500 ppm of urea coated hydroxyapatite nanoparticles when compared to others while higher concentration of these nanoparticles (1000 ppm) lower seed germination attributes. However, lipid per oxidation decreased with increasing concentration of UHANPs. All the results were statistically compared.

Keywords: Urea coated hydroxyapatite nanoparticles, biochemical attributes, *Brassica juncea* L., lipid peroxidation etc.

INTRODUCTION

For an edible crop, its quality is determined by its biochemical constituents. Biochemical contents like carbohydrates, proteins, lipids, and antioxidants are important for various nutritious and therapeutic purposes. Amount of these biochemical compounds in a crop varies according to availability of nutrients in soil. Presence of various macronutrients and micronutrients in optimum concentration is essential for a better crop production. Nitrogen is important macronutrient for growing plants as constituent of proteins, nucleic acid, and chlorophylls (Barita, Y. *et al.,* 2018). Plants uptake nitrogen from soil as organic matter, N fixation, irrigation water, atmospheric dumping, and fertilizer application. Applying N fertilizers in overflow is a common implementation to secure plants. Excessive Nitrogen cannot be utilized by plants (Hopkins, B.G., 2020). Application of fertilizers is the main issue for sustainable

agriculture to lower the negative effects of farming on the surrounding environment (Zebarth, B.J.*et al*., 2009). Green leafy vegetables carry high nitrate levels (Prasad, S. *et al*., 2008) which may cause severe malfunctioning in Human beings (Mensinga *et al*., 2003). The use of large number of chemical fertilizers has resulted in the development of environment pollution. Recent studies have shown that slow-release fertilizers are effective of delivering their nutrients moderately over a certain period of time. These slow- release fertilizers increase nutrient uptake proficiency of plants, lower continual application, decrease in money and labour, and upgrade storage and managing properties (Liu *et al*., 2011). Manufacturing of novel and innovative slow releasing fertilizers using nanotechnology is required to resolve the issues of nutrient losses because of their nanoscale size and high surface to volume ratio (DeRosa *et al*., 2010; Kottegoda *et al.,* 2011; Gunaratne *et al*., 2016; Pulimi and Subramanian, 2016; Dimkpa and Bindraban, 2018; De Silva *et al*., 2020). Urea coated hydroxyapatite nanoparticles are one of those slow releasing fertilizers. It has been studied that these nanoparticles release N slowly than conventional urea. These nanoparticles are widely renowned for their intrinsic biocompatibility and biodegradability, being the main component of human bones and teeth. So, use of these nanoparticles as fertilizers should not raise any concern on human and environmental health (Gomez-Morales, J., *et al*., 2013; Tampieri, A., *et al.,* 2016; Sprio, S., *et al.,* 2017.). In the present investigation, urea coated hydroxyapatite nanoparticles were synthesized, and characterized. Effects of those synthesized nanoparticles were observed on different parameter of biochemical yields in two varieties of *Brassica juncea* L. (family Cruciferae) i.e. GIRIRAJ, and NRC-BH 101 which are edible oil crops.

MATERIALS AND METHODS

Synthesis and characterization of Urea coated hydroxyapatite nanoparticle

Hydroxyapatite nanoparticle (HANPs) were synthesized using the method of Sandhofer *et al*., 2015. Urea coated Hydroxyapatite nanoparticles (UHANPs) were synthesized by using method of Kottegoda *et al*., 2013. For this, saturated urea solution, HANPs, and double distilled water were used. All the reagents were used of analytical grade. All solutions were prepared in double distilled water. These synthesised nanoparticles were characterised by using X-Ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM), and Fourier Transform Infra-Red (FTIR) techniques.

Plant source and surface sterilization

Seeds of two verities (Giriraj and NRC-BH 101) of *Brassica juncea* L. were obtained from Rajasthan Agricultural Research Institute (RARI), Durgapua, Jaipur, Rajasthan, India. Variety Giriraj was given name S1 while variety NRC-BH 101 was given name S2. Seeds of both the varieties were washed with running tap water. Those were surface sterilized with 5% NaOCl for 5 minutes and then washed repeatedly for two to three times with distilled water to prevent fungal/bacterial contamination. Filter papers were also sterilized in autoclave to reduce any chances of microbial growth. Then seeds were surface sterilized in 0.01% mercuric chloride ($HgCl_2$) solution for 2 min followed by washing with autoclave water and dried on sterilized filter paper.

Seed germination protocol

Seeds were germinated in sterile glass Petri dishes of 15 cm diameter lined with filter paper circles moistened with control and four different concentrations (250 ppm, 500 ppm, 750 ppm, and 1000 ppm) of each of urea and U-HANPs. Nearly 10 seeds were sown in each Petri dish and incubated in growth chamber set at 25 ± 2°C for 7 days and each treatment was replicated thrice. Occurrence of Germination was considered when roots were 2mm long. Germination percentage was recorded in every 24h, till the end of experiment. Distilled water was used as control. According to treatments, for both the species, Petri plates were named as mentioned in Table 1.

Biochemical profiling

Estimation of total carbohydrates: Total carbohydrates were estimated according to the method of Yemn and Willis (1954). To 4 ml of chilled Anthrone reagent, 50 µl of ethanol extract and 950 µl of 20% ethanol was added. Tubes were shaken gently to mix the solution. These were then covered with glass marbles and immediately placed

in boiling water bath for 10 min. and cooled in ice bath. The absorbance of blue green coloured solution was read at 625 nm in spectrophotometer against blank containing 4 ml Anthrone reagent and 1 ml of 20% ethanol. The concentration of total sugars (mg g^{-1} DM) was calculated from the standard curve plotted with known concentrations of glucose.

Estimation of total reducing sugars: The reducing sugars were estimated according to the method given by Miller (1959). To 1 ml of DNSA reagent, ethanol extracts (250 µl) and 20% ethanol (750 µl) was added. The tubes of reaction mixture were kept at 100° C for 12 minutes in boiling water bath. 2 ml of distilled water was subsequently added and absorbance was recorded at 560 nm against blank containing 1 ml of DNSA reagent and 1 ml of 20% ethanol. The concentration of reducing sugars (mg g^{-1} DW) was calculated from the standard curve plotted with known concentrations of glucose.

Estimation of total proteins: Total proteins were estimated according to the method of Bradford (1976). For the quantification of total soluble proteins, the fresh plant material (0.1 g) was homogenised in 1.5 ml of 0.1 M phosphate buffer (pH 7.5) and transferred to Eppendorf tubes. The homogenate was centrifuged at 8000 rpm for 10 min. and the supernatant was collected and used as protein extract. Reaction mixture was prepared by taking 0.1 ml of supernatant and diluted to 1 ml by 0.1 M phosphate buffer (pH 7.5). then 5 ml of Bradford reagent was added and mixed thoroughly absorbance was recorded at 595 nm against the reagent blank. Protein content of the sample was determined from the standard curve.

Estimation of free amino acids: The estimation of free amino acid content was determined according to the method of Lee and Takahashi (1966). Ninhydrin reagent (3.8 ml) was added to 1 ml of ethanol extract and the contents were shaken vigorously. The mixture was heated in boiling water bath for 12 min. and cooled to room temperature in running tap water. The absorbance of the coloured solution was read at 570 nm against a blank containing 20% ethanol. The concentration of free amino acids (µg/ ml DM) was calculated from standard curve plotted with known concentration of glycine.

Estimation of chlorophyll content: chlorophyll content was estimated according to the method given by Coombs *et al.,* (1985). For chlorophyll extraction, fresh leaves were washed, blotted dry and then homogenised in 80% acetone followed by centrifugation at 10000 rpm for 15 min. the absorbance of the supernatant was read at 647 nm and 664 nm against blank containing 80% acetone. Total chlorophyll was calculated according to following formula:
The amount of chlorophyll a, chlorophyll b and total chlorophyll (mg/g FW) were calculated according to following formula:
Chlorophyll a = (13.19 x A664 – 2.57 x A647) V/ (1000 x W)
Chlorophyll b = (22.10 x A647 – 5.26 x A664) V/(1000 x W)
Total chlorophyll = (7.93 x A664 + 19.53 x A647) V/ (1000 x W)
Where,
A = absorbance,
V = total final volume of the extract,
W = Fresh weight in gram of the tissue

Estimation of Lipid peroxidation: The lipid peroxidation was measured by determining the level of malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) content as indicators of lipid peroxidation.

MDA: Malondialdehyde was assayed by Thiobarbituric acid reactive substances (TBARS) content, a method given by Heath and Packer (1968). 0.1 g of fresh plant tissues were homogenised in 5 ml of (w/v) 0.1% TCA. The homogenate was centrifuged at 10,000 rpm for 10 min. TCA containing 1 ml aliquot of the supernatant was treated as extract. 1 ml of supernatant was mixed with 4 ml of 0.5% TBA (Thiobarbituric acid) in 20% TCA. The mixture was

heated at 95° C for 30 min and then quickly cooled in ice bath. The absorbance of the supernatant was measured at 532 nm. The level of lipid peroxidation is expressed as mole of MDA formed using an extinction coefficient of 155 nM^{-1} cm^{-1}.

Estimation of hydrogen peroxide: H_2O_2 was estimated as described by Mukherji and Chaudhari (1983). 0.5 g of fresh plant material was homogenised in 5 ml cold acetone (80%) and filtered through Whatman No. 1 filter paper. To 5 ml of extract, 4 ml of titanium reagent was added and then 5 ml of ammonia solution was added, the mixture was centrifuged at 10000 rpm for 10 minutes and supernatant was discarded. The residue was dissolved with 1 N H_2SO_4 and absorbance recorded at 410 nm against blank containing 5 ml of 1 N H_2SO_4. Calculations were made with standard curve plotted with pure H_2O_2. Concentration of H_2O_2 was determined using standard curve plotted with known concentrations of H_2O_2.

Data analysis

All the experiments were carried out with 3 triplicates. The results for each parameter were calculated as mean value with standard deviations of each triplicates. The obtained data were analyzed by descriptive analysis. The statistical significance of the treatments was evaluated by one-way analysis of Variance (ANOVA). Means were compared, according to Fisher's statistical test by least significant difference.

RESULTS AND DISCUSSION

Characterisation of nanoparticles: XRD pattern of the synthesised nanoparticles indicated the presence of peaks due to UHANPs. The results of FESEM studies revealed that the average particle size of UHANPs ranges from 23-32 nm. It was also observed that FESEM results are in good agreement with size distribution of UHANPs measured by XRD by the FTIR studies, structure of UHANPs were confirmed. Results of characteristic analysis of these nanoparticles are shown in Table 2 and Graph 1, and Graph 2.

Estimation of total carbohydrates and total reducing sugars: Effect of UHANPs at different concentrations with comparison of urea treated plants and control are shown in Table3and Figure3 while total reducing sugars content in all treated plants of both varieties are shown in Table 3 and Figure 4. maximum carbohydrate content and maximum total reducing sugars were recorded in P_7($UHANPs_{500}$) in both GIRIRAJ (136.53 mg/g DM &13.87 mg/g DM) and NRCBH 101 (137.33 mg/g DM& 16 mg/g DM) varieties respectively while with increasing concentration of nanoparticles, total carbohydrates and total reducing sugars decreased. These values were significantly higher than control and other treated plants.

Estimation of total proteins, and free amino acids: Total protein content and total free amino acid content were shown in Table 3 and Graph 4. It was observed that in P_7 ($UHANPs_{500}$) protein and free amino acids were found to be maximum in both GIRIRAJ (31.45 mg/g FW& 17.28 mg/g DM respectively) and NRCBH 101 (29.89 mg/g FW& 17.21 mg/g DM respectively) varieties while with increasing the concentration of UHANPs, these contents were decreased.

Estimation of chlorophyll content: Effect of UHANPs on chlorophyll a, chlorophyll b, and total chlorophyll in two varieties of the plants are shown in Table 3 and Figure 5. It was observed that in P_7 plants chlorophyll a, chlorophyll b, and total chlorophyll content were found maximum in both GIRIRAJ (0.89 mg/g FW, 0.29 FW, and 1.18 FW respectively) and NRCBH 101 (0.87mg/g FW, 0.23mg/g FW, and 1.1 mg/g FW respectively) varieties of the plant. Statistical analysis showed that variation in these parameters in all treated plants were not significant.

Estimation of lipid peroxidation: Results of MDA and H_2O_2 analysis are shown in Table 3 and Figure 6 and 7 respectively. It was observed that the highest MDA content was recorded in P_1 (control) in both varieties GIRIRAJ and NRCBH 101 (0.83 nM^{-1} cm^{-1} and 0.90 nM^{-1} cm^{-1}) respectively which show highest peroxidation in these plants

while minimum in P9 (0.12 nM^{-1} $cm^{-1}$for both varieties). H_2O_2 content was also found maximum in P_1 (control) while minimum in P_9 for both GIRIRAJ (16.73 µg/g FW) and NRCBH 101 (17.05 µg/g FW) varieties of the plant. Higher MDA and H_2O_2 contents showed higher level of lipid peroxidation. So, in the present investigation, it was revealed that with increasing the concentration of UHANPs, lipid peroxidation decreased. MDA amount varied significantly in treated plants while significant variation was not found in H_2O_2 content among treatments.

CONCLUSION

Results of the present study concluded that Urea coated hydroxyapatite nanoparticles play significant role in production of various biochemical compounds in *Brassica juncea* L. However, 500 ppm concentration of U-HANPs was found to be optimum for production of the biochemical compounds. Higher concentration of these UHANPs caused reduction in their production.

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Table 1: Name of different treatments given for the experiment.

Treatment	Distilled water	Urea 250 ppm	Urea 500ppm	Urea 750ppm	Urea 1000ppm	UHANP 250ppm	UHANP 500ppm	UHANP 750ppm	UHANP 1000ppm
Name to Petri-dishes	P_1 (Control)	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9

Table 2: The main compound diffraction peaks corresponding with d spacing by XRD method.

Compound Name	Obs. Max	d (Obs. Max)	Net Height	FWHM	Intensity
	2-Theta°	Angstrom	cts	2-Theta°	%
Urea	22.4899	3.95344	2224.09	0.1476	100.00
Calcium phosphate complex	24.8876	3.57773	263.77	0.1476	11.86
Calcium phosphate complex	26.1309	3.41026	116.77	0.2952	5.25
Urea	29.5101	3.02699	335.07	0.0886	15.07
Calcium phosphate	31.8967	2.80575	448.80	0.1181	20.18
Urea	35.7779	2.50978	176.94	0.1771	7.96

Table 3: Effect of Urea coated hydroxyapatite nanoparticles on biochemical parameters in *Brassica juncea* L. (Variety GIRIRAJ)

Plant	Total Carbohydrates (mg/g DM)	Total reducing sugars (mg/g DM)	Free amino acids (mg/g DM)	Total protein (mg/g FW)	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chlorophyll (mg/g FW)	MDA (nM^{-1} cm^{-1})	H_2O_2 (µg/g FW)
P_1	94.67	9.60	7.94	19.52	0.75	0.22	0.97	0.83	18.78
P_2	98.13	11.56	28.80	25.82	0.78	0.21	0.99	0.70	18.47
P_3	111.73	12.62	14.36	29.56	0.79	0.24	1.03	0.32	18.00
P_4	93.87	6.44	8.60	18.24	0.71	0.17	0.88	0.25	17.52
P_5	61.87	5.69	6.09	11.27	0.72	0.13	0.85	0.19	17.21
P_6	97.33	11.37	12.44	24.49	0.78	0.23	1.01	0.45	18.47
P_7	136.53	13.87	17.28	31.45	0.89	0.29	1.18	0.32	18.15
P_8	89.86	6.7	12.04	15.06	0.72	0.18	0.9	0.19	15.94
P_9	73.33	4.62	7.05	10.18	0.74	0.16	0.9	0.12	16.73

Table 4: Effect of Urea coated hydroxyapatite nanoparticles on biochemical parameters in *Brassica juncea* L. (Var. NRCBH 101)

Plant	Total Carbohydrates (mg/g DM)	Total reducing sugars (mg/g DM)	Free amino acids (mg/g DM)	Total proteins (mg/g FW)	Chl a(mg/g FW)	Chl b (mg/g FW)	Total Chlorophyll (mg/g FW)	MDA (nM^{-1} cm^{-1})	H_2O_2 (µg/g FW)
P_1	95.47	10.13	7.79	19.66	0.74	0.21	0.95	0.90	18.94
P_2	90.40	13.33	12.41	24.25	0.77	0.22	0.99	0.77	18.63
P_3	112.53	14.04	14.25	28.47	0.78	0.25	1.03	0.38	18.15
P_4	85.60	6.93	8.71	14.64	0.77	0.17	0.94	0.25	17.52
P_5	63.73	5.69	5.58	10.04	0.75	0.12	0.87	0.19	16.89
P_6	96.53	11.73	12.30	24.16	0.77	0.22	0.99	0.51	18.47
P_7	137.33	16	17.21	29.89	0.87	0.23	1.1	0.38	18.31
P_8	83.73	8.53	9.27	15.25	0.71	0.16	0.87	0.25	17.68
P_9	56.26	5.86	7.64	11.46	0.72	0.12	0.84	0.12	17.05

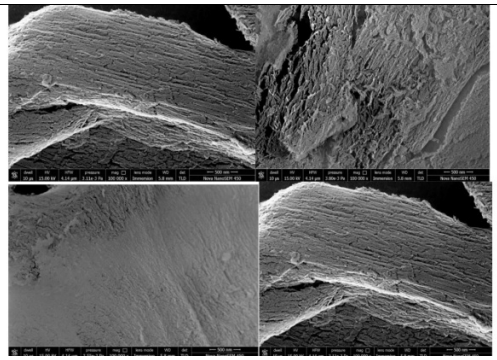

Figure 1: FESEM images of synthesized Urea coated hydroxyapatite nanoparticles.

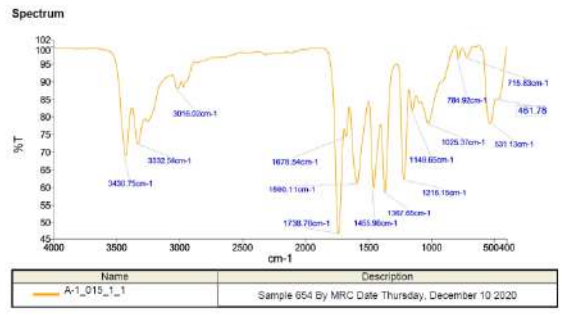

Figure 2: FTIR Spectrum of the synthesized urea coated hydroxyapatite nanoparticles.

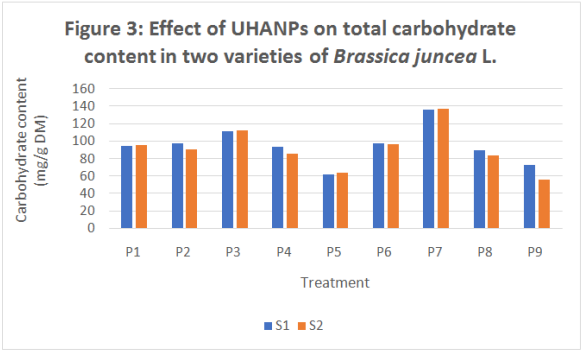
Figure 3: Effect of UHANPs on total carbohydrate content in two varieties of Brassica juncea L.
Carbohydrate content (mg/g DM)
160
140
120
100
80
60
40
20
0
P1 P2 P3 P4 P5 P6 P7 P8 P9
Treatment
S1 S2

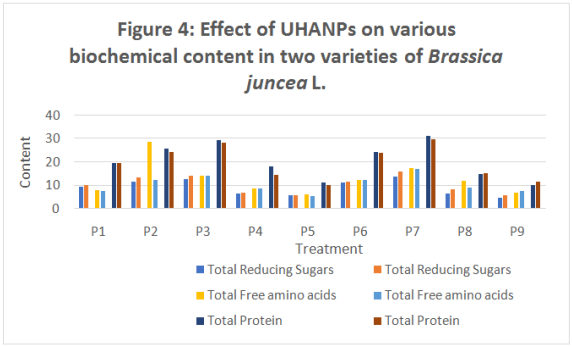
Figure 4: Effect of UHANPs on various biochemical content in two varieties of Brassica juncea L.
Content
40
30
20
10
0
P1 P2 P3 P4 P5 P6 P7 P8 P9
Treatment
Total Reducing Sugars
Total Reducing Sugars
Total Free amino acids
Total Free amino acids
Total Protein
Total Protein

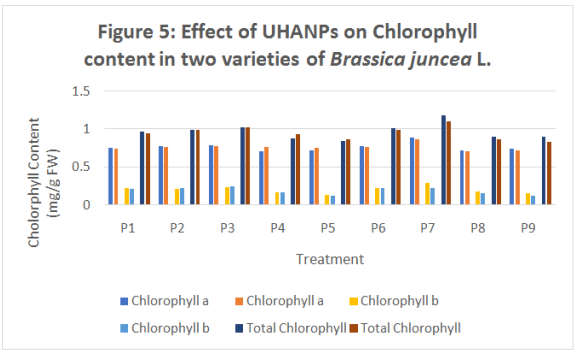
Figure 5: Effect of UHANPs on Chlorophyll content in two varieties of Brassica juncea L.
Cholorphyll Content (mg/g FW)
1.5
1
0.5
0
P1 P2 P3 P4 P5 P6 P7 P8 P9
Treatment
Chlorophyll a
Chlorophyll a
Chlorophyll b
Chlorophyll b
Total Chlorophyll
Total Chlorophyll

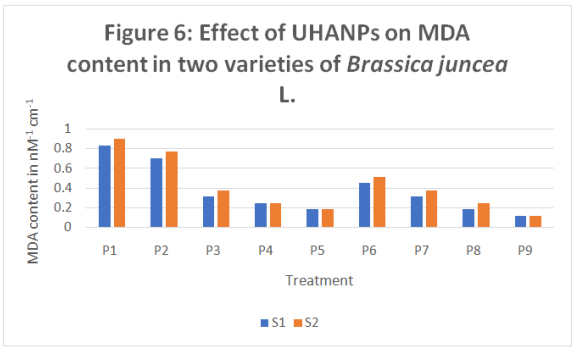
Figure 6: Effect of UHANPs on MDA content in two varieties of Brassica juncea L.
MDA content in nM-1 cm-1
1
0.8
0.6
0.4
0.2
0
P1 P2 P3 P4 P5 P6 P7 P8 P9
Treatment
S1 S2

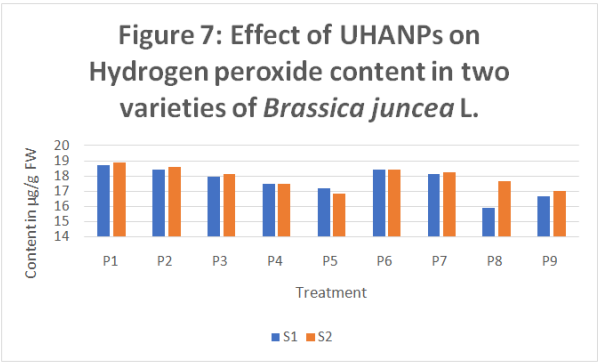
Figure 7: Effect of UHANPs on Hydrogen peroxide content in two varieties of Brassica juncea L.
Content in µg/g FW
20
19
18
17
16
15
14
P1 P2 P3 P4 P5 P6 P7 P8 P9
Treatment
S1 S2

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RESEARCH ARTICLE

Effect of Acid Rain on Nickel based Abradable Coating used in Aero Engines

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ABSTRACT

The corrosion potential of abradable nickel based coating used for aero engine components (for sealing and clearance control in compressor of aero engines) was explored and the effect of acid rain was observed for varied length of time. Coating plays an important role in aero engine manufacturing industry as it provides surface protection against corrosion, erosion, friction and wear, when subjected to harsh conditions *viz*., high temperature and corrosive environment during service period. The coating deposition was done by using thermal plasma spray method. Porosity was observed to be high at critical plasma spray parameter (CPSP). Phase transformation and interlayer oxide formation was also observed during plasma spray coating. A comparative study was established between acid rain treated and untreated samples by evaluating the corrosion resistance potential, microstructural characterization, composition, and phase analysis of coatings of treated samples using scanning electron microscopy, X-ray diffraction, and EDS. The weight gain of coating was also evident after acid rain treatment of up to ten weeks of immersion.

Keywords: Critical plasma spray parameter (CPSP), plasma spraying, acid rain corrosion.

INTRODUCTION

A series of sprayed abradable coating has been developed for improvement in achieving aero engine efficiency. The term sacrificial coating is used synonymously as rubbing of the engine part that leads to its worn-out while protecting the required engine part [1,2]. Abradable or sacrificial materials have been successfully employed to reduce the rotor to shroud clearance for aircraft engine compressor application [8]. Ceramic materials like aluminium

or nickel-based powders are coated by the plasma spraying method to produce abradable coating on aero engine casings [4,5]. Abradable nickel based coating produced by plasma spraying method is significant enough to sustain the working temperature of the aero engine up to 650 ^{0}C, thereby improving the engine efficiency. The coating characteristics depend on various factors like substrate preparation, spraying parameters, etc. which are responsible for determining the sustainability of the coating on different environmental conditions [9,10]. However, the perusal of literature has shown scanty of information on evaluation and characterization of acid rain corrosion effect on abradable plasma spray coating used for aero engines as no concrete studies have been taken up in this regard. Pertinent to this fact, the present investigation was undertaken with an objective to study the acid rain corrosion behaviour of plasma sprayed nickel based abradable coating.

MATERIALS AND METHODS

Preparation of Substrate

Nimonic, a nickel based high temperature low creep super alloy, was selected as a substrate for Nickel based coating. Substrate roughing of nimonic plates was done at VTC Surface Technologies (P) Limited, Vishakhapatnam during 2018-19 by subjecting the surface to blasting with Al_2O_3 powder of grit size 60 micron. Utmost care was taken to clean/ sterilize the substrate in order to remove all possible contaminants prior to exposing it to coating [5, 6]. For achieving the desired results of effective coating, the surface of prepared substrate was subjected to coating within 2 hours of blasting.

Coating powder preparation

Coating powder with varied composition was prepared by amalgamating nickel powder, cadmium oxide, hexagonal boron nitride, copper oxide and graphite.

Spraying parameters

The coating characteristics as well as its sustainability were known to have been resolved by the spraying parameters of plasma spray [11]. Hardness of coating was kept in optimum level due to its abradable property. The key parameters were decided on the basis of required obtainable hardness[3]. After preparation of the coating specimen, the same was subjected to heating up to 650 ^{0}C for a period of 1 hour in order to release the stress. The critical plasma spraying parameters to obtain the required hardness are listed in table 1.

Preparation of acid rain and specimen for corrosion test

Acid rain preparation

Acid rain solution was prepared in Chemistry Laboratory, Centurion University of Technology and Management, Odisha during 2018-19 by using distilled water, bromo methylene blue solution and solid sulphur as shown in reaction 1.

$S(s) + O_2(g) \rightarrow SO_2(g)$

$O_2 + SO_2 + H_2O \rightarrow H_2SO_4(aq)$

$$SO_2(g) \xrightarrow{O_2} SO_3(g) \xrightarrow{H_2O} H_2SO_4$$

Method of preparation

The solution was prepared by adding bromo methylene into distilled water taken in a glass jar on drop basis till the distilled water showed a trace of light blue colour. 10 g of solid sulphur was then taken and burnt using gas lighter. The white fumes from burnt sulphur were allowed to blend completely with the prepared solution of distilled water and bromo methylene in a jar which was then covered with a lid properly. For better blending and settlement of the fumes, the jar containing the solution was shaken well and due to specific reaction resulting from the presence of

acid in bromo methylene solution, change in colour from blue to yellow was observed in the solution. pH of the prepared acid rain solution was measured in μ pH System 361 pH meter in Chemistry laboratory, Centurion University of Technology and Management (CUTM) during 2018-19 and was observed to be 3.0.

Specimen Preparation

The nickel based abradable coated specimen was cut with wire cut Electrical Discharge Machining (EDM) after coating seven specimens with required thickness of 3-4 mm and weight of 3-6 g. The prepared nickel based coated samples were then subjected to acid rain corrosion test by dipping the samples into prepared solution of acid rain having a pH of 3.0 for varying length of time periods.

RESULTS AND DISCUSSION

Corrosion Test

Prior to corrosion test, cleaning of the specimens was done by distilled water followed by drying at a temperature of 110-120 ^{0}C for a period of 1 hour. Specimens were weighed before putting them into prepared acid rain solution by using electronic balance with least count of 0.01 g. Observations were made on the specimens which were dipped into prepared acid rain solution at different time interval. After ten weeks of immersion, samples were taken out followed by drying at 110-120 ^{0}C for 1 hour. Weighing of the samples was also done (Table 2).

Data pertaining to 10 weeks of acid rain treatment revealed the susceptibility of nickel based coating to the attack of acid rain (Figure 1). However, the severity was observed to be low as compared to sea water treatment. The weight gain behaviour was evident in all five specimens under treatment at different time intervals and an increasing trend was observed in weight gain of treated samples. Consequently, it may be assumed that the coating sample during immersion reacts/ interact with foreign elements, which leads to deposition of corrosion product on the coating surface [7].

Characterization of coated and acid rain treated sample

Micro structural observation

Scanning Electron Microscope (SEM) micrography and Energy Dispersive Scanning (EDS) of coating sample with critical plasma spray parameter (CPSP) and acid rain corroded sample were carried out to observe the effect of acid rain on abradable nickel base coating (Figure 2-5). Results pertaining to characterization of coated and acid rain treated sample revealed that the coating composition thus developed by using CPSP was of superior quality and had the potential to cause porosity despite of developing a good quality coating with required hardness (Figure 2 and 3). As plasma sprayed ceramic coating mostly contain porosity, it may affect the corrosion resistance property of the abradable coating [3]. The observations made during SEM and EDS studies revealed that the grain structure was not prominent in untreated sample (Figure 2 and 4) and prominent in acid rain treated sample (Figure 3 and 5).

Data pertaining to EDS analysis presented in table 3 and 4 revealed that there was an increase in sodium and a decrease in the amount of nickel with a drastic increase in the level of oxygen which may be contributed by the fact that some level of oxidation had taken place during the course of contact with acid rain which resulted in deposition, leading to weight gain of sample during immersion. Presence of sodium in the EDS analysis exhibited that oxidation may have taken place up to the substrate level as the coating contained sodium silicate. A perusal of literature has shown the occurrence of corrosion only at pores, however, the results of the present investigation contradicted from the published literature and revealed that apart from pores, corrosion was also observed in the sodium silicate coating.

X-ray diffraction study

Nickel based abradable coating samples were examined for identification of phases, by using Phillips Diffractometer after acid rain treatment (Figure 6). The x-ray diffraction was taken using copper target.

Different phases of oxide were observed in the graph. The formation of boron oxide was observed which may be due to oxidation of boron nitride present in the composition. Further study for the confirmation in this regard is under process.

CONCLUSION

The present investigation was undertaken with an objective to evaluate the characterization of nickel based abradable coating used in aero engines, after acid rain treatment. Preparation of coating was done by using plasma spray method with specific critical plasma spray parameters in order to obtain the required hardness. The formation of pores in the coating with required good quality coating deposition was observed. Continuous gain in weight was also observed in the samples when they were treated in acid rain for 10 weeks implying to the fact that coating may not be immune to oxidation or corrosion. Nickel based abradable coating is susceptible to acid rain corrosion. Therefore, proper care is needed to be taken during exploitation and storage of aero engines/ aircrafts to avoid acid rain corrosion of coatings, which may lead to premature failure/ withdrawal of aero engines from service.

ACKNOWLEDGEMENT

The authors are indebted to the authority of Centurion University of Technology and Management, Paralakhemundi, Odisha for providing necessary financial assistance during the period of study.

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Table 1. Spraying parameters for plasma deposition

Sl. No.	Parameter	Value
1	Primary plasma Gas	Argon (47 lpm)
2	Nozzle Dia	6 mm
3	Secondary Plasma-gas	Hydrogen (6 lpm)
4	Current	470 Amp
5	Powder feed rate	88% on machine scale
6	Powder carrier gas	Argon (3 lpm)
7	Spray distance	118 mm

Table 2. Per cent change in weight with treatment time

Sl. No.	Weight (in g)		No. of days	Difference	No. of weeks	% Change
	Before	After				
1	3.61	3.68	13.00	0.07	1.86	1.94
2	4.67	4.79	39.00	0.12	5.57	2.57
3	4.96	5.13	52.00	0.17	7.43	3.43
4	4.23	4.38	61.00	0.15	8.71	3.55
5	5.15	5,37	70.00	0.22	10.00	4.27

Table 3. EDS of untreated sample (Composition)

Element	Weight %	Atomic %
N K	16.71	27.55
O K	31.49	45.45
Na K	4.79	4.81
Al K	5.82	4.98
Si K	3.26	2.68
Ti K	0.69	0.33
Ni K	30.11	11.84
Cu K	5.63	2.05
Cd L	1.50	0.31
Total	**100.00**	

Table 4 . EDS of acid rain treated sample (composition)

Element	Weight %	Atomic %
O K	29.39	52.92
Al K	10.27	10.96
Si K	14.69	15.06
Ni K	26.22	12.87
Cu K	16.24	7.37
Cd L	3.19	0.82
Totals	100.00	

Table 5 .Phase analysis of acid rain treated sample

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	00-004-0850	55	Nickel, syn	0.001	1.007	Ni
*	01-088-2485	43	Boron Oxide	0.352	0.125	B_2O
*	01-073-2095	22	Boron Nitride	0.035	0.196	BN
*	00-026-1083	24	Carbon	-0.161	0.043	C

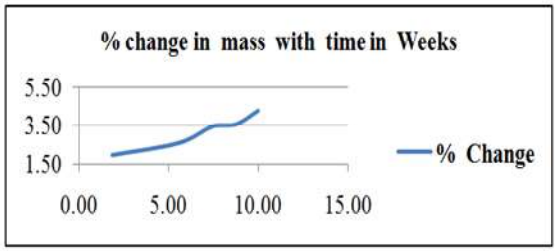

Figure 1: Per cent change in weight Vs time curve (weeks) in acid rain corrosion

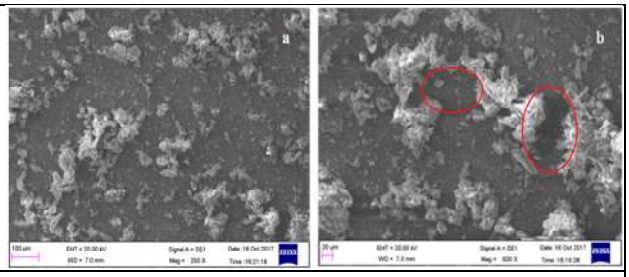

Figure 2: SEM of untreated nickel based coating at a) 250X and b) 500X

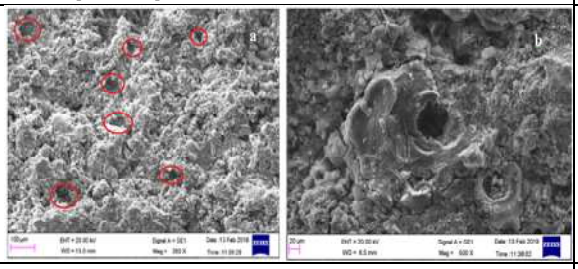

Figure 3: SEM of acid rain treated nickel based coating at a) 250X and b) 500X

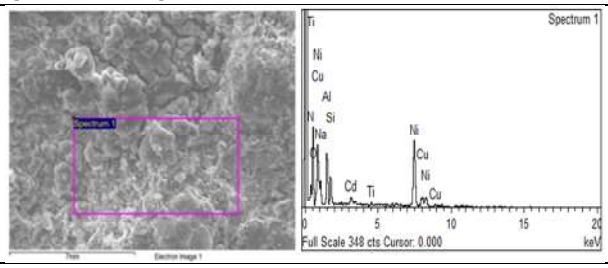

Figure 4: EDS of untreated nickel based coating

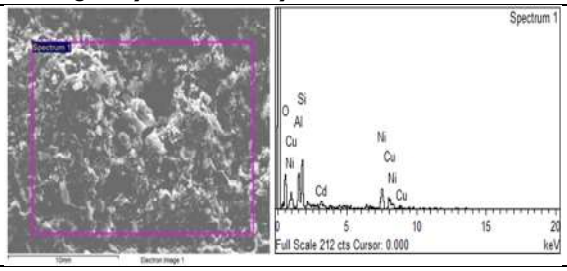

Figure 5: EDS of acid rain treated nickel based coating

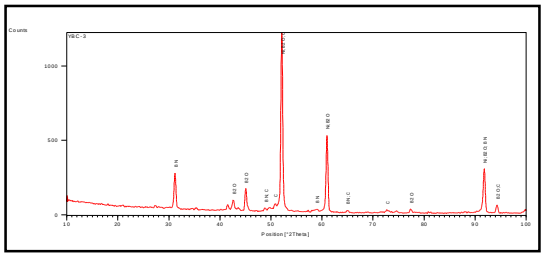

Figure 6: XRD pattern of acid rain treated nickel based abradable coating

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RESEARCH ARTICLE

Functional Requirement (FR) Classification using Convolutional Neural Network (CNN)

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ABSTRACT

Text classification is a prediction of classes based on the available labeled data. Automation using different approved its effectiveness in text classification. Software Requirement (SR) is text based and the task of classifying it is highly needed. The main classes of SR are: FR (Functional Requirement) and NFR (Non Functional Requirement). Multi classification of FR and NFR is exist as well. The automation is applied and recently using ML (Machine Learning) and DL (Deep Learning) models is the most successful approach. Different models have been used and achieved better results than other automation techniques. CNN (Convolution Neural Network) is one of the frequently used models for SR classification. Results are promising but there is still a demand to improve the accuracy and focus on different missing classifications related to SR. This study uses CNN model to classify FR to multi classes: solution, enablement, policy, definition, action constraint, and attribute constraint. The model has been trained and tested on FR dataset consists of 600 statements of structured text. The model achieves 98.3% accuracy.

Keywords: Functional Requirement (FR), Classification, Deep Learning, Convolution Neural Network (CNN), Natural Language (NL), Text Classification.

INTRODUCTION

Text classification is defined as assigning classes or labels to textual documents. Text classification is considered as supervised learning as the prediction of new labels or classes is based on the already existing labeled text documents that is passed in earlier phase (training dataset) [1]. Different techniques have been deployed to conduct text classification of the increasing number of textual documents such as, vectorization, supervised, and unsupervised learning [2]. Software Requirement (SR) classification refers to determine the main class which is FR (Functional Requirement) or NFR (Non-Functional Requirement), and further classification of the basic classes may be included. FR refers to services should be found in a system while NFR refers to the constraints on these service [3]. Even if the classes are clear, but the classification process is time consuming and expensive as it requires number of experts. Thus, automation is used to reduce time and cost but there is still a problem of performance and availability. As a solution ML (Machine Learning) and DL (Deep Learning) models have been used. The results are promising but there is a limitation and ignorance in multi classification of FR [4]. In this work, we will investigate improving FR classification to multiclass: Solution, Enablement, Definition, Policy, Action Constraint, and Attribute Constraint using CNN (Convolution Neural Network) model. It has been chosen because it achieved in many studies high accuracy and considered one of the best DL models. There are few numbers of studies used ML or DL models to classify FR to multi classes and the dataset is used only once with ML models. The main contributions of this work were: investigating CNN performance in classifying FR using structured text and balanced dataset and comparison between proposed work and other state-of-art that used CNN model to classify SR. The rest of the paper is organized as follows: summary of related work, methodology that has been used, results and discussion, and conclusion.

Related Work

This section highlights some studies where CNN model and other DL models have been used to classify SR in different ways achieving promising results. In [5] CNN have been used by authors to determine wheither the input text is a SR or a piece of information. The used dataset to train and test the model called DOORS document dataset. The model achieved 81% accuracy, 0.89 recall, and 0.73 precision. Another successful deploy of CNN in SR classification has been done by the authors in [6]using PROMISE corpus dataset. SR classified to FR or eleven classes of NFR. The mode recorded 0.80 precision, 0.785 recall, and 0.77 f-score. In [7] more clssifications have been experiemented by classifying SR to FR or NFR, NFR to multiple eleven classes, and another classification of NFR to security or not security requirment. The best f-score found in binary classification of SR to FR or NFR and classifying NFR to eleven classes achieved 0.91. However, the binary classification of NFR to security or not security related recorded the worst results with 0.77 f-score. A recent study used fine tuned BERT model to classify SR to FR or NFR with 0.93 and 0.90 f-score for NFR and FR respectively. The second classification is NFR to 11 classes with 0.76 f-score, and FR to function or data behaviour with 0.92 f-score[8]. It can be noticed from the reviewed studies as examples of previous efforts in using CNN or other DL models to classify SR that there is more focus on binary classification and multi classification of NFR . Moreover, the performance and accuracy need to be enhanced.

METHODOLOGY

The used machine is ASUS laptop that has x64 Inter(R) Core (TM) i7-9750H processor, RAM with 17.0 GB. The operating system is 64-bit Windows. The implementation tool is Spyder using Python 3.6. The scikit learn has been chosen because it includes useful libraries for DL models and evaluation. The dataset is a new dataset that has been used only once before with ML models in [4]. The dataset includes structured text with 600 FR as each 100 FR belong to one of the classes: solution, enablement, definition, policy, action constraint, and attribute constraint. The phases of classification is displayed in Figure 01. It consists of the following phases:

- Normalization.
- Classification.
- Performance Measure.

The normalization and also known as preprocessing phase is a very important phase before classification and it means cleaning and preparing the input text for classification. The first step is converting all words to lowercase letters. Then, remove stopwords such as, "the", "a" and so on. The next step is to return words to its roots such as, "players" converted to "player" and "specified" became "specify"[2]. The normalized input used for training and testing CNN model. In CNN model as displayed in figure 2., local features are produced by applying convolutional concept [9]. A^(T×D) is the presentation of input as the sentence length is T and the embedding dimentionality is D. Next,w_1,w_2,….w_k. are produced by applying convolution operation by filters that have a specified width based on the word embedding vector size. Thus, different filter sizes L= 2,3, 4,. allowed as long as there is different vertical local regions. Feature maps are generated using active convoluted resuts with different dimensions: H_i={h_1+h_2+⋯+h_(T+L-1) } and the following formula is used for calculation:

$$\boldsymbol{h_t} = \boldsymbol{f}(\boldsymbol{w}.\boldsymbol{x_{t:t+L-1}} + \boldsymbol{b_t}) \quad (1)$$

- x_(t:t+L-1) represents the concatenation of input vectors L.
- $b_t \in R$ is the bias vector.
- *f* is an activation function such as Sigmoid or TanH but it should be nonlinear.

The feature map size is determined by the length of both the input sentence and the filter size. Thus, a pooling(1 max) is applied to find the largest[10]

$$m = \left[max_p(H_1), max_p(H_2), \dots\dots, max_p(H_N)\right] \textit{(2)}$$

- Max-pooling function is $max_p(H_1)$
- Feature from each feature map H_i is represented as *p*.

Performance is measured using confusion matrix to calculate multiple parameters: accuracy, precision, recall, f-score. Confusion matrix is matrix with a size of NxN and it is used mainly to evaluate classifiers. This matrix allows a comparison between actual labels or classes and the predicted ones. Confusion matrix is used to detict errors of the classifiers that are used. In case of the predicted value and the actual vlaue are the same and both are negative, it is represented by TN. In case of the actual value is negaticve but predicted falsely positive by the classifier, it is represented by FP. However, if the actual value is positive and falsely predicted as negative, it is represented as FN. TP represents the case of predicting positively an actual positive value. Accuracy, precision, recall, and f-score are calculated based on the confusion matrix according to the following formulas[11]:

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (3)$$

$$Precision = \frac{TP}{TP+FP} \quad (4)$$

$$Recall = \frac{TP}{TP+FN} \quad (5)$$

$$F1-Score = 2 * \frac{Precision*Recall}{Precision+Recall} \quad (6)$$

RESULTS AND DISCUSSION

Training Results

In training phase 35% is used for training and 35% is used for validation to ensure that there is no overfitting and the hyperparameters are suitable as can be seen in Figure 3. Number of epochs assigned to 60 epochs but it has been noticed that the accuracy is stable and remained the same starting from 10 epochs. The used optimizer is "adam" with its default learning rate (0.001). Dropout is assigned to 0.2 and batch size to 50.

Testing Results

Figure 4 displays the confusion matrix of CNN model that is the base of calculating other evaluation parameters. The model predicted all classes perfectly as four are predicted 100% true and the other three are very close from 100%.

Table **2** displays the evaluatiion parameters of CNN model in classifying FR to multi classes. The accuracy is 98.3% and the other parameters are 0.99. Comparing the performance of CNN model performance in this study with other state-of-art that have used CNN for the same purpose as displayed in . It can be noticed that there is no previous study that clasified FR to multiple classes using CNN model. Moreover, the results of the proposed model are the best and the highest among other studies.

CONCLUSION

CNN model has been selected from DL models to enhance the classification accuracy of SR especially FR multi classification. It has been selected because it has promising previous results in the field. It has been trained and tested on a FR dataset that contains 600 statements. FR has been classified to: solution, enablement, policy, definition, action constraint, and attribute cosntraint. The model achived 98.3%accuracy. Comparing the results with other studies that used CNN model to classify SR, it can be concluded that the proposed model is one of the rare models that classify FR to multi classes and the results considered to be promising and leading. The performance is the best and never been reached before. The model can be utilized in future to include more classifications of SR.

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Table 1: CNN Architecture.

Layer (Type)	Output Shape	Param#
embedding_90 (Embedding)	(None, None, 46)	71438
dropout_93 (Dropout)	(None, None, 46)	0
conv1d_23 (Conv1D)	(None, None, 46)	6394
global_max_pooling1d_23	(None, 46)	0
dense_160 (Dense)	(None, 128)	6016
dropout_94 (Dropout)	(None, 128)	0
dense_161 (Dense)	(None, 6)	774

Table 2: Performance Results for CNN Model.

Reference # Year	Classification	Dataset	Methodology	Results
[5] 2017	Input: Requirement Information	DOORS document	CNN	**Accuracy:** 81% **Recall:** 0.89 **Precision:** 0.73
[6] 2017	SR : FR or 11 NFR classes	PROMISE	CNN	**Precision:** 0.80 **Recall:** 0.785 **F-score:** 0.77
[7] 2018	SR: FR-NFR NFR: 11 classes NFR: security – not security	PROMISE	CNN	**F-score:** 0.945 **F-score:**0.91 **F-score:** 0.772
[8] 2020	SR: FR-NFR NFR: 11 classes FR: function – data behaviour	PROMISE	CNN	**F-score:** 0.90 FR 0.93 NFR **F-score:** 0.76 **F-score:** 0.92
Proposed Model 2021	FR: 6 classes	FR dataset	CNN	**Accuracy:** 98.3% **Precision:** 0.99 **Recall:** 0.99 **F-score:** 0.99

Table 3: Comparison of proposed model with state-of-art models.

Accuracy	Precision	Recall	F-score
98.3%	0.99	0.99	0.99

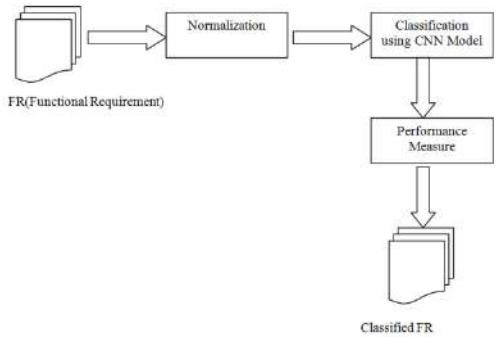

Figure 1:Classification Phases

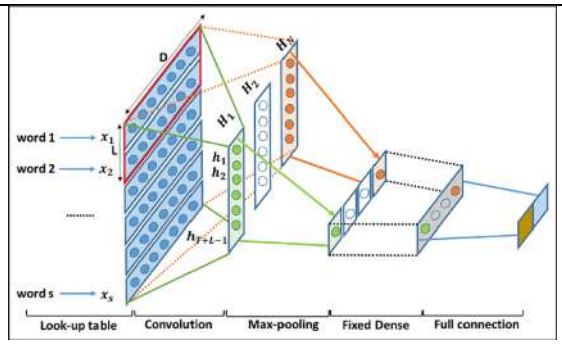

Figure 2: CNN DL model for FR classification [10]

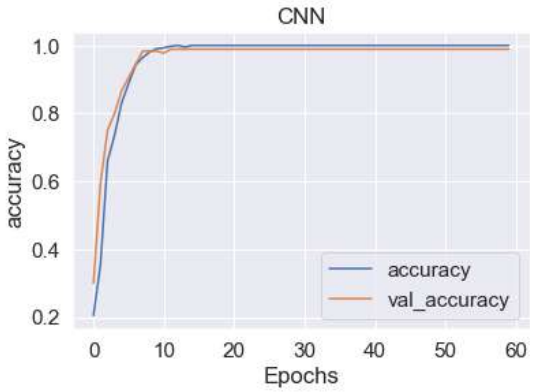

Figure 3: Training and validation results

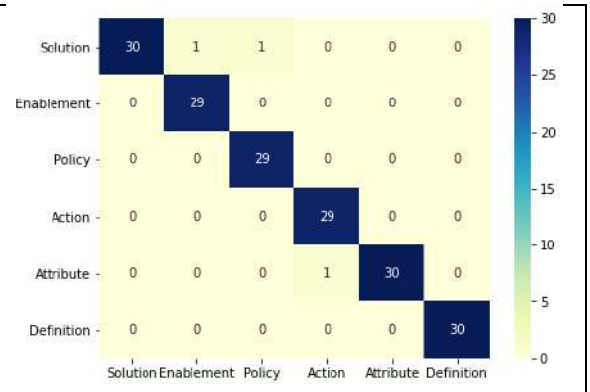

Figure 4: CNN Confusion Matrix

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RESEARCH ARTICLE

In Silico Docking Studies of Aavarai Kudineer Targeting Peroxisome Proliferator Activated Receptor (PPAR – γ)on Non - Alcoholic Fatty Liver Disease. (NAFLD)

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease with the prevalence rate of 25% in many nations. NAFLD persist as Non-alcoholic fatty liver (NAFL) and may upsurge to harmful conditions ranging from to nonalcoholic steatohepatitis (NASH) to liver fibrosis and cirrhosis later it may lead to hepatocellular carcinoma. Till date there is no established drug of choice for the treatment of NAFLD beyond clinical trial. This necessitate to explore a drug for the effective treatment of NAFLD. Aavarai Kudineer (AK), which is an official Siddha poly herbal formulation is selected to explore its effectiveness against the treatment of NAFLD. *In-Silico* molecular docking study, which is a virtual, rational and more effective screening tool, was conducted to estimate the effect of AK against NAFLD. Among the different phytoconstituents present in AK, 21 significant components were taken as the ligand and docking was done against Peroxisome proliferator-activated receptor- γ (PPAR-γ). Pioglitazone was considered as the standard drug. The study revealed that, among 21 components Iguesterin, Mangiferin, Oleanolic acid, Catechin, Luteolin and Gallo catechin had a good binding score (-7.93, -7.57, -7.42, -7.26, -7.12, and -6.93 kcal/mol respectively) compared to standard drug Pioglitazone (-5.99 kcal/mol). Moreover, Mangiferin, Catechin, Luteolin and Gallo catechin showed more than two hydrogen boding. This results clearly depicts that Aavarai Kudineer may be a good drug of choice for the management of NAFLD, which should be ensured with the appropriate*in-vitro* and preclinical study.

Keywords: Non-alcoholic fatty liver disease (NAFLD), Non-alcoholic fatty liver (NAFL), Nonalcoholic steatohepatitis (NASH), In-silico, Molecular Docking, Aavarai Kudineer.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) denotes the buildup of fatty liver (hepatic steatosis) without or less alcohol consumption. NAFLD is a comprehensive term and covers the simple accumulation of adipose tissue in the liver to more progressive steatosis along with hepatitis and further leads to fibrosis, cirrhosis and in some cases finally leads to hepatocellular carcinoma (HCC) [1]. Thus, NAFLD is categorized into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) [2]. NAFL is characterized by steatosis of the liver, involving greater than 5% of fat deposition, with no evidence of hepatocyte injury [3]. Whereas, NASH is serious form of fatty liver disease which involves a necro inflammatory process whereby the hepatic cells become injured in a background of steatosis [3]. NAFLD has become the growing epidemic worldwide with prevalence of 30% in developed nations and nearly 10% in developing countries [4]. Incidence of NAFLD escalates with increase in prevalence of obesity and insulin resistance which leads to liver accumulation of triglycerides and free fatty acids [5]. Treatment for NAFLD in more challenging and Pharmacotherapy is the most important treatment tool. There are neither established therapies nor any evidence-based guidelines for the treatment of NAFLD. The present treatment and management modalities revolve around correcting the underlying metabolic abnormalities associated with NAFLD and terminating hepatotoxic drugs[6]. There is no currently approved pharmacotherapy. Whereas Vitamin E and pioglitazone are available medications with the most evidence of efficacy in the treatment of patients with NAFLD but have side effects and limitations [7].

In this scenario, it is the need of the hour to explore a promising drug formation for the treatment of NAFLD effectively with minimal or/no side effects. In this context herbal drugs will be the ideal choice for this as it is reported to be less toxic. WHO states that 80% of the Asia's population relies for their primary health care on traditional medicine, Traditional medicinal plants scores over the modern medicine owing to lesser side effects and lesser drug reactions [8,9]. During the search of plant drugs, for the treatment of NAFLD, Siddha system of medicine came to the limelight as it is a big source of herbal medicine. Moreover, siddha is a pioneer and successful system in treatment of various hepatic disorders. Aavarai Kudineer (AK) is a Siddha poly herbal formulation containing seven herbal ingredients as given in Table-1[10, 11]. Kudineer formulation is one among the 32 types of internal medicines mentioned in Siddha. Kudineeris also called as Kashaya, Kiyazham, Marundhu neer and Unneer. Herbs are dried and coarsely powdered for Kudineer preparation is called as Kudineer churanam. Kudineer is the extract prepared by means of decoction and it is referred to as khashayas in Ayurveda. AK is rich in chemical constituents with high pharmacological value and it is used in the management of Diabetes mellitus [12, 13]. The formulation has been taken from the classical Siddha Literature "Theraiyar Kudineer" [14] and it is also found in the Siddha literature "Gunapadam mooligai vaguppu" (Siddha Materia medica)[15]. Detailed literature review revealed that most of the ingredients in the AK polyherbal formulation possess/have a chance of possessing hepatoprotective activity[16-22]. With this perspective it was planned to study the hepatoprotective activity of Aavarai Kudineer as a move to repurpose the AK for the treatment of NAFLD by using *in silco* screening method.

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity [24, 25]. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. Docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand. The molecular docking method determines interaction between ligand and target molecule. It predicts binding affinity of ligand to form a stable complex with protein by finding preferred orientation of minimum free binding energy [26].

In this present study *in-silico* docking study was conducted to predict the binding interaction between the important (21 selected) phytoconstituents of AK against the Peroxisome proliferator-activated receptor- γ (PPAR-γ) to estimate the management effect of AK in NAFLD.

MATERIALS AND METHODS

Preparation of Receptor Structure: [27-30]
The protein data bank (PDB) is a crystallographic database for three-dimensional (3D) structure data of large molecules proteins and nucleic acids. Crystal structure of peroxisome proliferator activated receptor -gamma agonist (PPAR – γ) was obtained from PDB (http://www.rcsb.org) with PDB ID :4CI5. The protein consists of two chains that is A chain and B chain with resolution 1.77 Å, expression system *Escherichia coli* and organism:Homosapiens.

Structure Of Ligands From Pubchem:[31]
It is product of the NCBI database. It is most useful for collecting the information about the specified chemicals. It is an online archive containing the information of all known chemicals their properties and biological importance. The structures of the major constituents of Aavarai Kudineer vizLuteolin, Kaempferol, Arjunone, Catechin, Chrysophanol, Gallic acid, Gallo catechin, Glycine, Mangiferin, Myricetin, Oleanolic acid,Physcion, Resorcinol, Rhein,Rotundine, Valeric acid, Cyperene, Diosgenin, Phytol, Rutin andstandard drug pioglitazone was obtained from the pubchem database.

Ligand Structure Preparation:[32]
In this study , for the ligands Luteolin , Kaempferol, Arjunone , Catechin , Chrysophanol , Gallic acid , Gallo catechin , Glycine , Mangiferin , Myricetin , Oleanolic acid , Physcion , Resorcinol , Rhein , Rotundine , Valeric acid , Cyperene , Diosgenin , Phytol , Rutin and slandered drug pioglitazone, the 2D structure , chemical formula and molecular weight were obtained from pubchem (Table-2). The structures of all this ligand was computed by drawing using Marvin sketch software (Marvin NET version 5.4.1.062) and saved in pdb file format. The protein and ligands file which prepared were then taken for docking.

Docking Methodology:[33]
The ligands were docked to the target protein PPAR – γ agonist (PDB ID: 4CI5) using Autodock program version 4.2. Autodock is a suite of automated docking tools. The software is used for modeling flexible small molecules such as drug molecules and its binding receptor protein of known 3D structures. It uses genetic algorithm for the conformational search and is suitable method for docking studies. This technique is based up on grid-based method of energy evaluation. Autodock tool are used to prepare run and analyze the docking simulations and modeling studies. After the grid generation, the prepared ligands were docked with the protein to evaluate the interaction between each target protein and ligands.

RESULT AND DISCUSSION

On the basis of molecular docking studies, we docked natural compounds from Aavarai Kudineer (AK) with protein PPAR- γ agonist (PDB ID :4CI5) using Autodock software (version 4.2). Autodock results were analyzed the study the interactions, bindingenergy and the hydrogen bonding of the docked structures. The best ligand receptor structurer from the docked structures was chosen based on the lowest energy. The grid box size was set at 60×60×60 Å (X, Y and Z). The spacing between grid point was 0.375 Å. TheLamarckian genetic algorithm (25) was chosen to search for the best conformers. Total 21 bioactive ligands from Aavarai Kudineer (AK) was docked into the binding pockets of the protein .on the basis of their docking score Iguesterin (-7.93 kcal/mol, Mangiferin (7.57 kcal/mol),Oleanolic acid (-7.42 kcal/mol , Catechin (-7.26 kcal/mol), Luteolin (-7.12) , Gallo catechin (-6.93 kcal / mol)

and Pioglitazone (slandered drug : -5.99 kcal/mol) was analyzed and compared test with slandered drug (Table-5).Binding energy and hydrogen bond interaction were measured for the best conformers. From the binding energy value and hydrogen bond interaction the Non-Alcoholic Fatty Liver Disease (NAFLD) activity of Ak compounds to corresponding receptor was predicted. Docking of Gallo catechin, Luteolin, Catechin, Mangiferin and Pioglitazone with PPAR γ is given in Fig 1 to 5. Visualisation of the interaction of Mangiferin and Pioglitazone against PPAR - γ performed using chimera-1.15 software is depicted in Fig. 6 & 7.

CONCLUSION

From the *Insilco* docking study,it was revealed that among the selected 21 compounds of AK, Mangiferin, Luteolin, Iguesterin, Catechin, Oleanolic acid and Gallo catechinhad a highest binding score compared to standard drug Pioglitazone against the targetprotein PPAR-γ. Among this Mangiferin showed least binding energy of -7.57kcal/mol and 3 hydrogen bonding (4ci5A:GLN444: HE22,4ci5B:HIS323: HD1,4ci5B:GLU324:HN) whereasstandard drug Pioglitazone have the docking score of -5.99 kcal/mol and 2 hydrogen bond (4CI5A:GLN444:HE22 and 4CI5B:ARG397:HH11),as given in Table-3. This interaction was visualize using chimera 1.15 software.This evidently disclose that components of Aavarai Kudineerhas thebest binding efficacy than that of standarddrug Pioglitazone and so Aavarai Kudineer may be a fruitful drug in the management of NAFLD.This also further encourage us to examine *in-vitro* and *in-vivo* studies on Aavarai Kudineer.

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There is no acknowledgment

Conflict of Interest

There is no conflict of interest.

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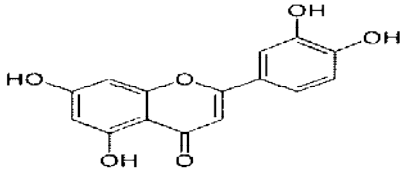
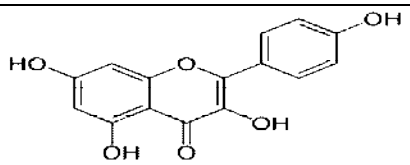
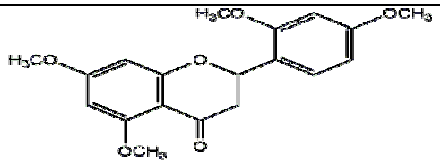
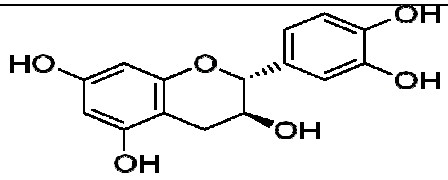
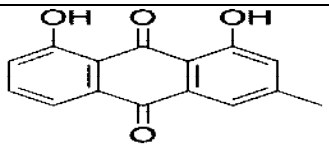
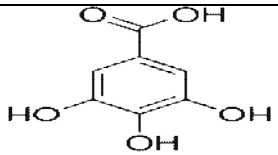
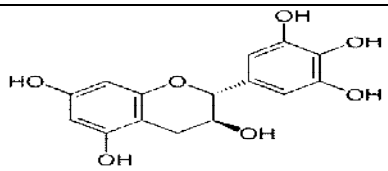
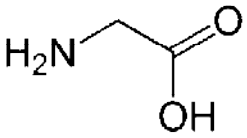
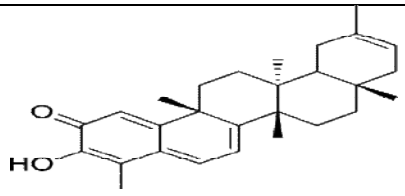
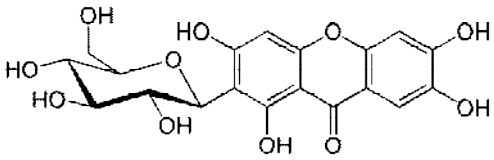
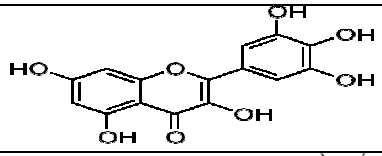
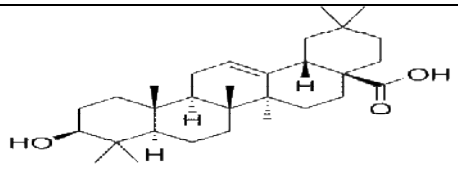
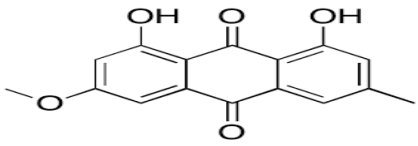
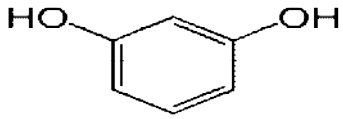
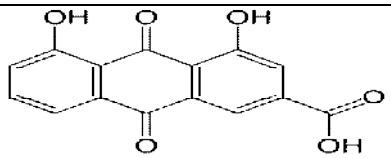
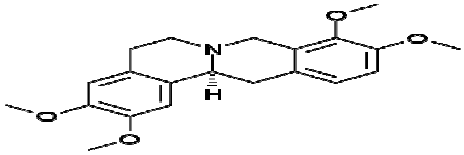
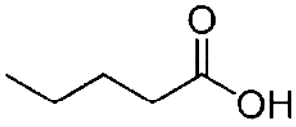
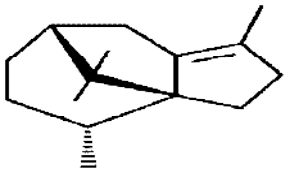
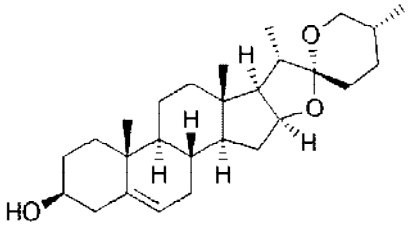
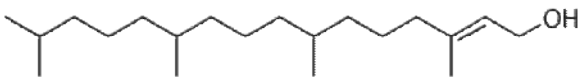
Table 1: Ingredients of Aavarai Kudineer Formulation (AKF) [23]

S. No.	Siddha Ingredients	Vernacular/Tamil name	Botanical Name & Family	Parts used
1	Aavaraiilai	*Tanner's cassia* (Aavaram)	*Cassia auriculata* (Leguminosae)	Leaves
2	Kondraiilai	Golden shower, Purging cassia, Indian laburnum (Kondrai/ Sarakondrai)	*Cassia fistula* (Caesalpinaceae)	Leaves
3	Naval kottai	Malabar plum, Java plum, Black plum (Naval)	*Syzygiumcumini* (Myrtaceae)	Seeds
4	Kadalazhingil	Chinese salacia, Lolly berry (Karukkuvai,Cuntan)	*Salacia chinensis* (Celastraceae)	Roots
5	Korai kizhangu	Coco-grass, Java grass, Nut grass (Korai kilangu, Muthakasu)	*Cyperusrotundus* (Cyperaceae)	Rhizomes
6	Kosthum	Canereed, Wild Ginger (Krrauvam, MalaiVasambu)	*Costus specious* (Coataceae)	Rhizomes
7	Maruthampattai	Arjuna, Arjun tree (Marudhamaram)	*Terminaliaarjuna* (Combretaceae)	Bark

Table 2: Aavarai Kudineer phytochemicals used for Insilco docking study along with its 2D structure

S. No	Compound Name (Pubchem ID)	Molecular Formula	2D structure
1.	Luteolin (5280445)	C15H10O6	
2.	Kaempferol (5280863)	C15H10O6	
3.	Arjunone (14034821)	C19H20O6	

4.	Catechin (1203)	C15H14O6	
5.	Chrysophanol (10208)	C15H10O4	
6.	Gallic acid (370)	C7H6O5	
7.	Gallo catechin (65084)	C15H14O7	
8.	Glycine (750)	C2H5NO2	
9.	Iguesterin (46881919)	C28H36O2	
10.	Mangiferin (5281647)	C19H18O11	
11.	Myricetin (5281672)	C15H10O8	
12.	Oleanolic acid (10494)	C30H48O3	

13.	Physcion (10639)	C16H12O5	
14.	Resorcinol (5054)	C6H6O2	
15.	Rhein (10168)	C15H8O6	
16.	Rotundine (5417)	C21H25NO4	
17.	Valeric acid (7991)	C5H10O2	
18.	Cyperene (99856)	C15H24	
19.	Diosgenin (99474)	C27H42O3	
20.	Phytol (5280435)	C20H40O	

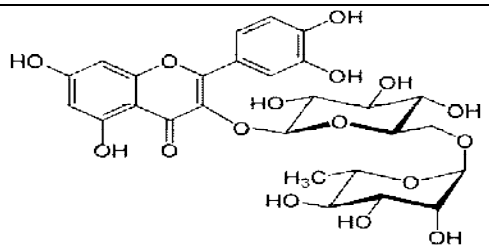
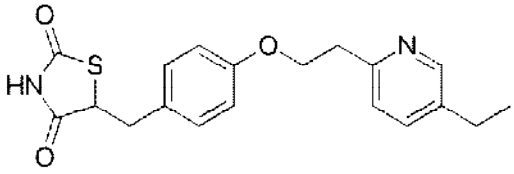
21.	Rutin (5280805)	C27H30O16	
22.	Pioglitazone (4829)	C19H20N2O3S	

Table 3: Overall docking result of ligands of Aavarai Kudineer against PPAR – γ agonist (PDB ID: 4CI5)

Sl. No	Protein PDB ID	Ligand name	Inhibition constant (µM)	Ligand efficiency	Binding energy (Kcal / mol)	Distance A°	No. of bonds	Hydrogen bond
1	PPAR γ AGONIST (4CI5)	Luteolin	6.06	0.34	-7.12	1.687 1.85	2	4ci5B:HIS323: HD1 4ci5B:GLU324:HN
2		Kaempferol	11.21	0.32	-6.75	1.549	1	4ci5B:HIS323: HD1
3		Arjunone	67.85	0.23	-5.69	2.884 2.92	2	4ci5: GLU448: UNKO: O1 4ci5:ASP396: UNKO:O1
4		Catechin	4.79	0.33	-7.26	2.003 1.677 1.879 2.144	4	4ci5B:HIS323:HN1 4ci5B:HIS323HD1 4ci5B:GLU324:HN 4ci5: GLN451:HE21
5		Chrysophanol	34.12	0.32	-6.09	2.07	1	4ci5B:ARG397: HH11
6		Gallic acid	480	0.38	-4.53	2.77 2.186 2.245	3	4ci5A:GLN410: HE22 4ci5A:ARG443: HE 4ci5B:GLN437: HE21
7		Gallo catechin	8.28	0.32	-6.93	1.722 2.179 2.222	3	4ci5B:GLU324:HN 4ci5B:ARG397: HH11 4ci5B:ARG443: HH21

8		Glycine	5.94	0.61	-3.04	1.982	1	4ci5A:LYS438: HZ3
9		Iguesterine	1.53	0.26	-7.93	NIL		NHB
10		Mangiferin	2.8	0.25	-7.57	2.002 2.168 2.191	3	4ci5A:GLN444: HE22 4ci5B:HIS323: HD1 4ci5B:GLU324:HN
11		Myricetin	9.43	0.3	-6.86	1.888 2.036	2	4ci5B:ARG397: HH11 4ci5B:ARG443: HH22
12		Oleanolic acid	3.62	0.23	-7.42	NIL	NIL	NHB
13		Physcion	30.79	0.29	-6.15	2.061	1	4ci5B:ARG397: HH1
14		Resorcinol	420.46	0.58	-4.61	2.0 2.216 2.156 1.909	4	4ci5A:SER394: HG 4ci5A:GLN410: HE22 4ci5A:ARG443: HH21 4ci5B:GLN437: HE21
15		Rhein	84.36	0.26	-5.56	1.973 2.037	2	4ci5B:HIS323: HD1 4ci5B:GLU324:HN
16		Rotundine	10.54	0.26	-6.79	2.752	1	4ci5B: THR447:O
17		Veleric acid	6.28mm	0.43	-3.0	1.898 1.677 1.947	3	4ci5B: HIS323:HN 4ci5B: HIS323:HD1 4ci5B: GLU324:HN
18		Cyperene	1.23mm	0.33	-3.97	2.197 1.839 1.957 1.86 2.092	5	4ci5: MET439:O:H 4ci5:ASP441:O:H 4ci5A:GLN410: HE22 4ci5B:GLN437: HE21 4ci5B:THR440: HG1
19		Diosgenin	28.79	0.41	-6.19	NIL	NIL	NHB
20		Phytol	344.23	0.22	-4.72	1.88	1	4ci5: GLU324:O:H
21		Rutin	59.49	0.13	-5.76	1.769 2.107 2.224 2.132	2	4ci5: GLU324:O:H1 4ci5: ARG443:O:H1 4ci5: ASP441:O:H1 4ci5: ASP44:O:H1
22		Pioglitazone	40.37	0.24	-5.99	2.075 1.92	2	4ci5A:GLN444:HE22 4ci5B:ARG397:HH11

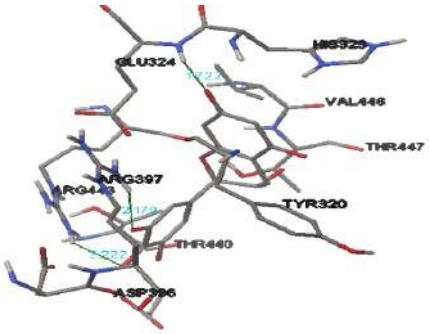


Figure 1: Gallo catechin docked with PPAR-γ

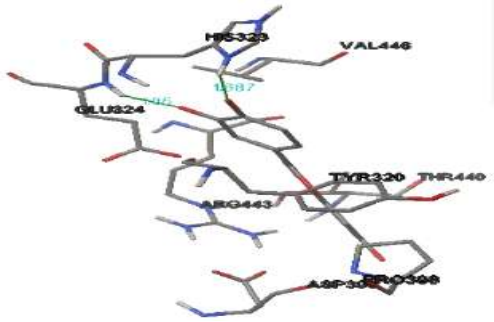


Figure 2: Luteolin docked with PPAR-γ

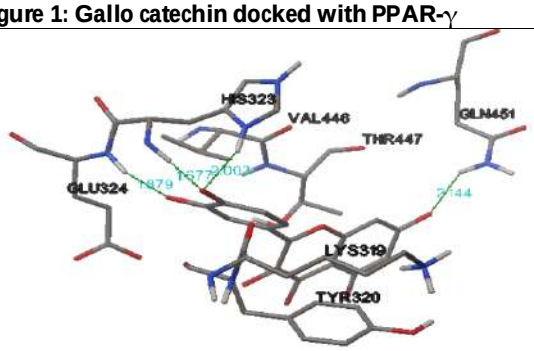


Figure 3: Catechin docked with PPAR-γ

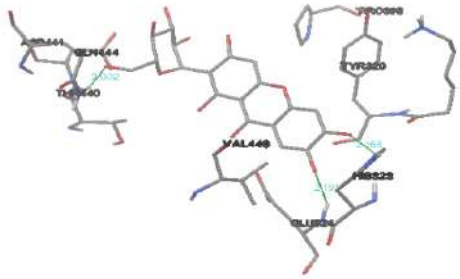


Figure 4: Mangiferin docked with PPAR-γ

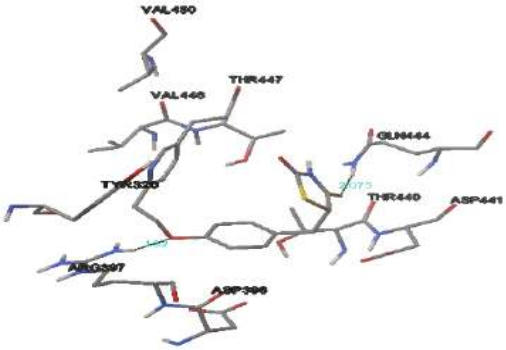


Figure 5: Pioglitazone docked with PPAR-γ

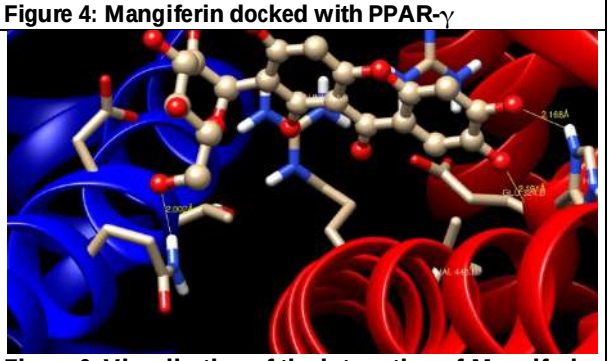


Figure 6: Visualisation of the interaction of Mangiferin against PPAR - γ performed using chimera-1.15 software.

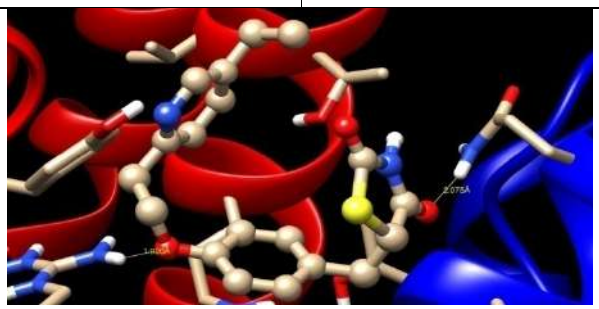


Figure 7: Visualisation of the interaction of pioglitazone against PPAR - γ performed using chimera-1.15 software.

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RESEARCH ARTICLE

Development and Standardization of *Moringa oleifera* Leaf Fortified Soup Mix and Herbal Mix

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ABSTRACT

Moringa oleifera is well-known medicinal herbal plant found in India. Whole tree like pods, leaves, seeds and flowers are highly rich in nutrients. Leaves can be directly consumed fresh or sometimes cooked. Leaves of the moringa can be stored for longer period of time without losing any nutrient by drying them and convert into powdered form. The methodology of the study is to develop the product (moringa soup mix, moringa herbal mix) by incorporating the moringa leaves powder, in different ratio and sensory evaluation was done by semi trained panel members. The study result showed that moringa mix soup (M_3), with incorporation of 30% Moringa powder and 70% Oats is more acceptable. Moringa herbal mix (T_1) made up of 80% Moringa powder and 20% Turmic powder is more acceptable as compare to control and other variants.

Keywords: *Moringa oleifera,* Moringa powder, Fortified food, Product development.

INTRODUCTION

Moringa oleifera is easily available and low cost food item in local area. Moringa called *Shanjana* and botanical name *Moringa oleifera*. *Moringa oleifera* belonging to the family of Moringaceae is an effective remedy for menopause woman, malnutrition, anemia, diabetic, cancer, and hypertension, mainly joint pain. Moringa is rich in nutrition owing to the presence of a variety of essential phytochemical present in its leaves, pods and seeds. *Moringa oleifera* in

rich nutrients vitamin C (ascorbic acid), vitamin A(carrot), calcium (milk), iron (green leaf vegetable like spinach), protein(pulse), potassium(banana). *Moringa oleifera* Lam. (Moringaceae) is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands (Iqbal et al, 2006). *Moringa oleifera* is the most promising tree which has used for nutritional benefits, medicinal properties, environmental conservation, and consumption and is the perennial, multipurpose. *Moringa oleifera* is mainly name such as "cabbage tree", ''drumstick tree" or "horseradish tree", 'beanoil tree' or 'benzoil tree', 'miracle tree' and 'mother's best friend tree , super food'. (Koul and Chase, 2015).

All parts of *Moringa oleifera* plant are used culturally for its nutritional value, medicinal properties and taste, flavor, as a vegetable and seed. *Moringa oleifera* can be eaten fresh, cooked, and stored as a dried powder use for many months without any major loss of its nutritional value (Arabshahi-D et al, 2007; Fahey, 2005). According to Fahey (2005), the content of vitamin C in moringa leaves is seven times higher than that of oranges, quantity of vitamin A is four times to carrots, calcium is four times and protein content is two times to milk. There is also the presence of antioxidant compounds in moringa leaves. Due to these several health benefits and nutrients, the leaves, seeds, pods and flowers are widely used in the preparation of various kind of food (Juice, herbal mix, soup, cake, biscuit).

MATERIALS AND METHODS

The research study was conducted in the laboratory of Food Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan.

Procurement of raw material

The raw materials were procured from the local market (Jaipur, Rajasthan, India) for processing and developing of product. This include Moringa leaves powder, rice , oats ,Turmic powder, Cinnamon powder, black paper, ginger powder, cloves powder, cardamom powder.

Processing for moringa leaves

- Good quality of moringa leaves collect JVWU campus
- Clean properly
- Selected for drying
- Cleaned parts shade drying for 7 days at room temperature
- Weight
- Grinder
- Sieving
- Collect moringa powder

Formulation of Moringa soup mix powder

To make the moringa soup mix, raw materials were weighed (moringa powder ,rice powder, oats powder, cardamom powder, black paper powder, salt as shown in Table no. 1) Take 250 ml water and add moringa soup mix. Heat the content till half of the total volume (125ml) and strainer the soup. Then add salt, Turmic powder, black pepper, cinnamon powder, to the soup.

Formulation of moringa herbal mix

To make the moringa herbal mix , raw materials were weighed (dry moringa leaves powder ,Turmic powder, Black pepper, Cloves powder, Cinamum powder , Cardamom powder, Honey as shown in Table no. 2). Take 250 ml water and add moringa herbal mix. Heat the content till half of its initial volume (125ml) and strainer the soup. Then add salt, turmic powder, black pepper, cinnamon powder, to the herbal mix.

Sensory Evaluation

The moringa powder based product i.e. moringa mix soup and moringa herbal mix were evaluated for their sensory characteristic using 9- point hedonic scale.(Rangana 1986).

RESULTS AND DISCUSSION

Moringa soup mix

The product is prepared by the incorporation of *Moringa* leaves powder in three different concentrations i.e. 10%, 20% and 30% which is compared with the control (Table no.3). The mean scores secured for the colour attribute were ranging from 5.8±0.874 to 7.44±0.06.The mean score obtained for the taste varied from 6.00±0.942 to 7.11±0.99. Moringa soup mix prepared with 20% (M_2) of incorporation obtained the maximum score7.11±0.99. However, the third variant (M_3) with 30% incorporation obtained the minimum score 6.00±0.942 for taste. The data indicated that the mean score secured for the texture were between 5.8±0.94 to 7.22±1.03. The first variant with 10% of incorporation showed maximum score (7.22±1.03). However, the third variant (M_3) with 30% level of incorporation showed score is minimum (5.8±0.94).The mean score for the overall acceptability varied from5.8±0.99 to 6.88±0.99. Moringa soup mix (M_2), with incorporation of 20% moringa powder and 80% oats is most acceptable product as compare to the other variants and control.

Moringa herbal mix

The product is prepared by the incorporation of moringa leaves powder (*moringa Leaves powder)* in three different concentrations i.e. 20%, 30% and 40% which is compared with the control (Table no.4.1.4). The mean score obtained for the colour were ranging from 6.71±1.27to7.85±0.63. The mean score secured for the *taste* of moringa herbal mix were ranging from6.28±0.88 to 7.85±0.63. Moringa herbal mix prepared with 40% incorporation (T2) showed minimum score (6.28±0.88).The data indicated that the mean score obtained for the *texture* were between6.57±0.72 to 6.714±0.90. The third variant (T_3) with 40% incorporation has minimum score (6.57±0.72). The mean score secured for the appearance of Moringa herbal mix were ranging from 6.42±0.90to7.42±0.49. The data indicated that the mean score registered for the overall acceptability varied from6.57±0.72 to 7.57±0.49. The first variant with 30% of incorporation showed minimum score of control (6.57±0.72). Moringa herbal mix (T_1) made up of 80% moringa leaves powder and 20% Turmic is more acceptable as compare to other variants and control.

CONCLUSION

Moringa oleifera is nutritious food that provide sufficient amount of nutrients needed for, cancer, anemia, diabetes, hypertension, malnutrition and menopause woman. The outcome of the study demonstrated that the moringa leaves powder can serve as a good nutritional supplement for combating malnutrition as it is rich source of protein, carbohydrate, vitamin A, vitamin C , Iron and calcium.

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Table .No.1. Moringa soup Mix

Ingredients	Control	10% Incorporation	20% Incorporation	30% Incorporation
Moringa powder	__	10	20	30
Oats	50	90	80	70
Rice	50	50	50	50
Black pepper powder	5	5	5	5
Cinamum powder	3	3	3	3

Table No. 2: Moringa Herbal Mix

Ingredients	Control	10% Incorporation	20% Incorporation	30% Incorporation
Moringa powder	__	10	20	30
Turmeric powder	1	2	3	4
Black pepper powder	3	3	3	3
Cinamum powder	2	2	2	2
Cloves	2	2	2	2
Cardamom powder	3	3	3	3
Ginger powder	2	2	2	2
Honey	5	5	5	5

Table No.3:- Acceptability Evaluation of food product (Moringa soup mix) in the term of sensory attributes.

Attributes	**Control**	**M1**	**M2**	**M3**
Colour	5.8±0.874	6.5±0.95	7.44±0.68	7.44±0.06
Taste	6.00±0.942	6.8±1.09	7.11±0.99	6.88±1.28
Texture	5.8±0.94	6.8±1.22	7.22±1.03	7.11±1.09
Appearance	5.83±1.2	6.7±0.30	7.33±0.81	6.94±1.13
Odor	5.5±0.83	6.5±1.06	6.88±0.99	6.33±0.94
Over all acceptability	5.8±0.99	6.3±1.24	6.88±0.99	6.55±1.25

Table No.4:-Acceptability Evaluation of food product (moringa herbal mix) in the term of sensory attributes

Attributes	**Control**	**T1**	**T2**	**T3**
Colour	6.71±1.27	7.85±0.63	7.00±0.75	6.85±0.83
Taste	6.71±1.16	7.85±0.63	6.85±0.63	6.28±0.88
Texture	6.714±0.90	7.71±0.69	6.85±0.63	6.57±0.72
Appearance	6.42±0.90	7.42±0.49	6.85±0.63	6.58±1.16
Odor	6.71±0.45	7.71±0.69	7.00±0.75	6.57±1.04
Over all acceptability	6.57±1.04	7.57±0.49	6.71±0.45	6.57±0.72

Fig 1. Preparation of moringa soup mix

Fig 2. Preparation of moringa herbal mix

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RESEARCH ARTICLE

Effect of Russian Current Versus Plyometric Exercises on Skill—Related Fitness Variables in Young Female Athletes

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ABSTRACT

Skill-related physical activity is vital for all sports players, and fitness variables are much needed in performing high-intensity workouts. Running is one of the activities which require utmost fitness. Many exercise programs help to improvise fitness. The study compares the effect of Russian current with plyometric exercises on skill-related fitness variables in young female athletes. The study was conducted as an experimental study with 45 female runners selected from the university campus. Those who fall into the selection criteria were given a clear explanation about the study, and those who volunteer themselves were included in this study. All participants were randomly divided into three groups, Group A, who underwent Russian currents. Group B underwent plyometric exercises, and group C underwent basic strengthening exercises. All the group participants receive the treatment three times a week for six weeks. No dropout was noted in this study. All the participants in the groups completed six-week protocols. All the participants were measured with agility, power, and speed. Agility was measured using the shuttle run test, power was measured using a standing broad jump, and speed was measured using a 40-yard run. Data was collected on the first and the last session, and it was evaluated using SPSS 20.0. ANOVA is used to identify the significance between the groups, and the Post Hoc test (Tukey HSD) is calculated to determine which group is significant. This study concluded that the six-week training program on the Russian currents, plyometrics, shows a difference in the groups' skill-related fitness variables. There was also a significant difference in comparing the Russian current with the basic training group and the plyometric with the basic training group. On comparing the Russian current and plyometric, there are no significant differences obtained.

Keywords: Russian Current, Plyometric exercises, Skill related fitness, Agility, Speed, Power, Female Runners.

INTRODUCTION

Russian Currents or Russian electrical stimulation are alternating currents modulated with a frequency of 50Hz with 50% of duty cycle at 2.5 kHz [1]. Russian currents have been beneficial for improving the muscle force-generating capacities and work well as a part of physiotherapy regimen [2]. It also helps to enhance the contractions in the muscles [3]. Russian currents use electrical stimulation primarily for muscle performance gains, and it acts as an adjunct to the strengthening regimens in musculoskeletal conditions [4]. Selkowitz, 1989, [1] has reviewed the experiments of the Russian currents and concluded that there is an increase in muscle force. This study also concluded that there is a significant improvement when compared with the voluntary exercises. Plyometrics is a well-known technique for improving athletic performance. This type of explosive resistance training focuses on increasing speed and power by utilizing the enhanced force output of a muscle's stretch reflex [5]. Plyometrics is the contraction with eccentric loading followed by concentric contractions. It improves dynamic restraints by facilitating sensorimotor system adaptation [6]. Eccentric loading followed by a concentric contraction is known as plyometric exercises. It stimulates the adaptation in the sensorimotor system, which enhances dynamic restrains [6]. Fitness is the core preconditions of health, and it differs from person to person, different from young to age, or sportsperson to average individual [7]. Physical fitness variables comprise of the skill and health-related components. Skill-related physical fitness variables are the ability of the individual to excel in particular sports to improvise the dynamic, balance, power, speed, and agility [8].

Speed, Agility, and power were taken as a measurement in this study. Shuttle run test to measure Agility, here the participants should run 30 feet back and forth between two parallel lines as fast as possible for 120 feet. Two trials may be performed, and the best is taken for measurement. A 40-yard sprint test measures speed, in this, participants need to run for 40 yards, and the timings were recorded. Two trials were conducted after 5 mins of break, and the best timings were recorded. Standing broad jump is the standard measure for power. The participants were asked to stand behind a marked line on the ground and instructed to jump as far as possible and land on both feet without falling backward. Three attempts are allowed; the best length is taken [9]. Skill-related fitness is developed by the standard exercise protocols, which are designed individually. Russian currents are not well used in the skill-related fitness components. Russian currents are most often used to strengthen muscles and are commonly used to re-educate the muscle groups, but it is less considered in sports of skill-specific measures [10]. Plyometrics training improves performances and the ability to do the skill tasks at different levels [11]. Since there was no head-to-head comparison between the Russian currents with Plyometric exercises on various physical fitness, this is the first kind of study as far as our knowledge. The purpose of the study is to identify the effect of Russian currents versus Plyometric training and the basic training program on various skill-related fitness variables for young female athletes.

METHODOLOGY

This experimental study involved female runners who participated in the 100 meters, 200 meters, and 4x 100 relay. The institutional ethical committee, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, approved this study. A notice was displayed in the university notice boards and the university girls' hostel to recruit the participants. Those who volunteered themselves were asked to register in the OPD of the College of Physiotherapy. All the participants were called in a group to recruit in the study. The supervisor described a brief introduction to the research and its effects. A blinded researcher who is not part of the study has assessed the volunteers and selected the participants for the study. Out of 120 athletes who volunteered, only 70 participants were chosen for the study. The seventy athletes screened for their history, pain, functional activity, and fitness. They are

also assessed for the selection criteria, and those who suit this criterion were included in this study. Selection criteria include the age group of 18—25 years, athletes with three years of training, runners who participate at least in university-level competitions, runners without any recent injuries in the lower limbs, without any cardiac or neurological involvements, without any history of pre mensural syndromes, and without any congenital deformity in the lower limbs. Forty-five participants were included in the study by the blinded assessor. All were randomly assigned in three equal groups using computer. An explicit instruction was given to the participants, and written consent was obtained from all the participants. Group A, 15 participants underwent Russian currents for six weeks, Group B, 15 participants underwent Plyometric exercises for six weeks, Group C, 15 participants underwent basic strengthening exercises for six weeks.

This study happened on the outdoor athletic track with a tartan surface. The blinded assessor took the first and the last measurement. Athletes were instructed to have their usual intake of foods and fluids before testing; they were advised not to take caffeine-rich beverages 4 hours before the assessment. All the participants were measured with agility, power, and speed. Agility was measured using the shuttle run test, power was measured using a standing broad jump, and speed was measured using a 40-yard run. Russian current was applied to the quadriceps muscles at 2500 Hz with a sinusoidal pulse with a burst frequency of 50 Hz, with a pulse duration of 200—300μs. It is applied for 10 minutes with a 50% duty cycle and 2 seconds ramp down for comfort and 2—3 seconds ramp-up [3]. All the participants have stimulation three times a week for six weeks. Following this, warming up exercises was given to all the participants for 10 mins. The exercise program was started with warming up the lower limb muscles for 10 mins. Plyometric training for the lower limbs starts with Jumping rope, Skip, Medicine ball tosses, 20-40 cm double leg hurdle jump for the first three weeks, and Single leg hurdle hops, alternate successive short distances 5—30 m, and 30—76 cms double leg hurdle jump in the last three weeks [12]. The basic strengthening program includes Jogging, Running, Leg press, Squats, Half squats, and Hopping. All the exercises were done with resistance at 50% to the 1 RM [13]. The exercises were instructed and supervised by the experience's therapist in the respective fields, and each group was asked to visit the department at different times. All the group participants receive the treatment three times a week for six weeks. There was no dropout noted in this study. All the participants in the groups completed six-week protocols. Data was collected on the first and the last session, and it was evaluated using SPSS 20.0. Normality was assessed using Shapiro-Wilk tests. $\alpha < 0.05$ is the level of significance used in this study.

RESULTS

The Outcome measures used in the study are Agility, Speed, and Power. A blinded assessor collected all the data on the first visit and at the last visit. All the collected data were analyzed with SPSS 20.0 and MS Excel. ANOVA is used to assess the difference between the post-test values of the groups. Once the difference is obtained, the Post Hoc test is used to identify which group is significant. All the test p values were set as p<0.05%. This study identifies that the p-value > α, H0 is accepted. The experiential effects in all the parameters are detailed in Table I, II, and Table III. The table II shows there is marked differences obtained within the groups. There is an improvement on the pre-test and post-test values. All the groups show marked differences in the groups. The inference in Table III shows a marked significant difference obtained when compared between Group A vs. Group C and Group B vs. Group C. In contrast, there is no considerable difference obtained when compared between Group A vs. Group B. This shows no difference between the outcomes when trained with Russian currents or with plyometrics. So, this study's findings show that the magnitude of the difference between the average and is μ0 large, which means there is a tremendous significant difference between the interventions.

DISCUSSION

The study is to determine the effect of Russian current versus Plyometric exercise on various skill-related fitness variables on young female athletes. Runners require good muscle strength and flexibility14. Runners need low impact cross-training, which produces similar running-like movement, preventing decreased fitness and

performances [15]. Russian currents cause depolarization of the sensory and motor nerve fibers. It aids in activating the fast type II motor unit and evokes muscle contractions, leading to the strengthening of the muscles3. Studies suggest that the Russian currents would produce improvement in muscle activation and also improves the fitness variables [16]. Studies also suggest that the current Russian system could cause an increase in muscle strength along with an alteration in the muscle fibre with the capillary system [17]. It is also claimed to relieve pain, increase local blood flow, strengthen the muscles around the joint, Hypertrophy of the muscles, and facilitate muscle contractions [18]. Plyometric exercises have a rapid deceleration – acceleration process, which produces an explosive reaction that increases both speed and power during the lower limb athletic activities [19]. Plyometrics promotes neural adaptations by stimulation of the recruitment of the more significant number of motor units 20. The short stretch drills combined with the rapid voluntary contractions applied on the acceleration phase enhance the sprint performances [21]. Numerous authors have identified an increase in jump height, sprint times, improvement of the running economy with improved joint position senses, and postural control on application of plyometric training on the lower extremities [22, 23, 24].

Basic strength training program may promote the increase of force production in the slow-twitch fibres and the fatigue resistance of the muscle fibres. There is a voluntary contraction of the limited recruitment of the fast-twitch fibres in all but the fastest and most forceful voluntary contractions [25]. Training with higher speed and power would produce muscle adaptation [26]. Related studies showed there is a limited benefit to applying the basic training, whereas combination with resistance training produces significant improvement in the fitness variables [25]. Studies also showed that general strength training would not improve sprint or agility performances [27]. Studies also showed that a combination of plyometric with strength training would produce more significant gains in the physical performances of soccer players [28]. This study's results are interesting since there are no significant differences between the Russian current or plyometric training on physical fitness levels. There is a considerable difference obtained within the groups, whereas there is no significance seen between the groups. The researcher hypothesizes that the reasons for the non-significance of the results may be due to smaller treatment periods (6 weeks and 18 sessions). Another reason would be electrical stimulation causing a series of rapid twitch in the type of fibres, which may reduce the responses. The longer outcome measure is not there, which would be a factor in the non-significance. The present study's findings show that there are no significant differences between the groups of Russian currents and plyometrics. In contrast, there is a difference obtained when compared with the basic training group.

CONCLUSION

This study concluded that the six-week training program on the Russian currents, plyometrics shows a difference in the skill-related fitness variables within the groups. There was also a significant difference in comparing the Russian current with the basic training group and the plyometric with the basic training group. On comparing the Russian current and plyometric, there are no significant differences obtained.

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Conflict of Interest

No potential conflict of interest to this article was reported.

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Table 1: Analysis of the Treatments of the Groups

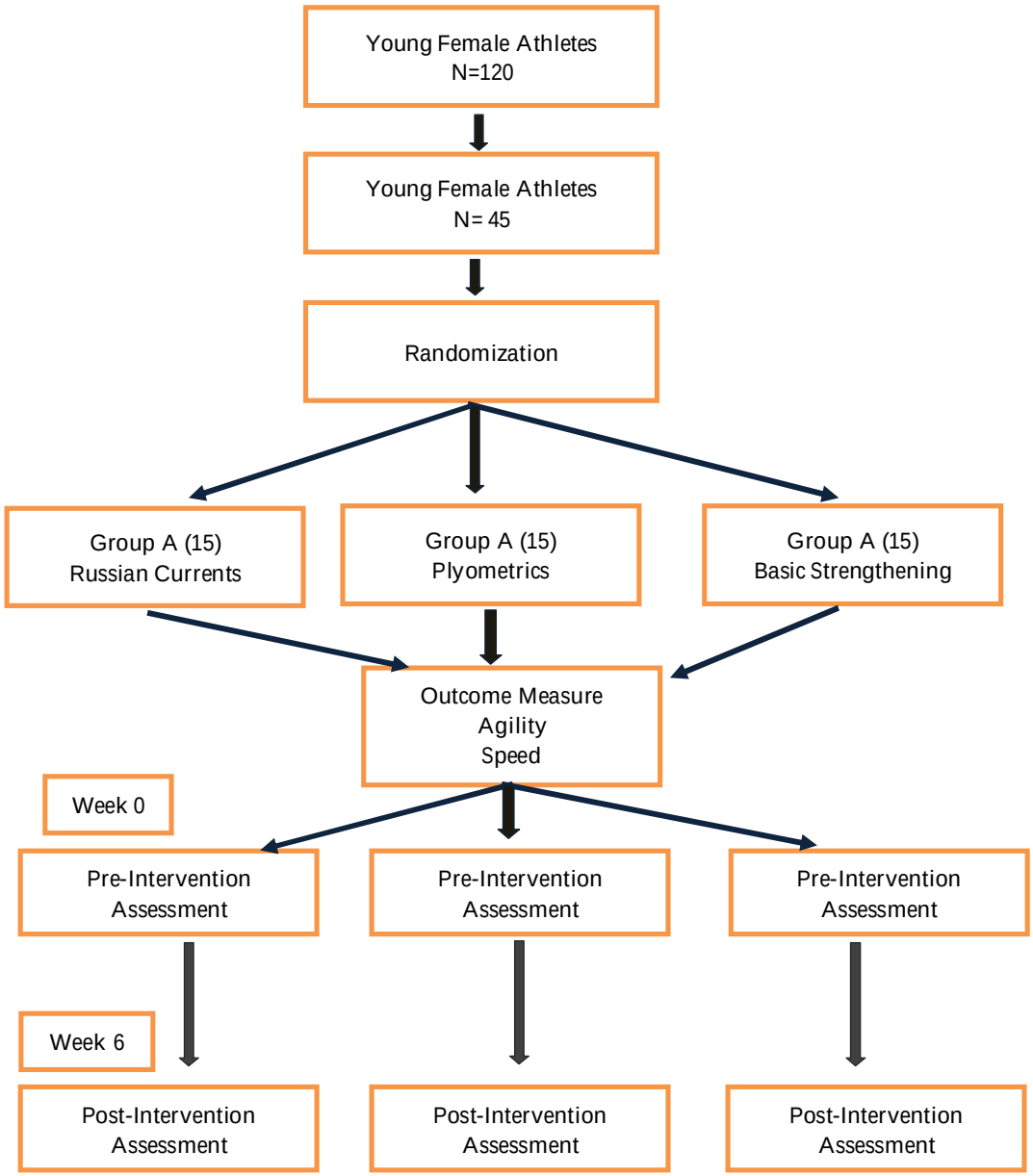
Procedures	Pre-treatment (Mean ± SD)	Post-treatment (Mean ± SD)	Percentage of change	t value	Effect size	P value
Agility Group A	5.31 ±0.122	4.38 ± 0.152	17.51%	18.4	6.74	0.05
Agility GroupB	5.29 ±0.144	4.26 ± 0.188	19.47%	16.9	6.15	0.05
Agility Group C	5.32 ± 0.157	4.91 ±0.128	7.71%	7.91	2.86	0.05
Speed Group A	6.33 ± 0.144	5.26 ± 0.14	16.9%	20.6	7.53	0.05
Speed Group B	6.31 ± 0.155	5.15 ± 0.21	18.48%	17.4	6.28	0.05
Speed Group C	6.38 ± 0.182	5.93 ± 0.19	7.05%	6.65	2.41	0.05
Power Group A	182.93± 1.62	190.47± 1.60	4.12%	12.8	4.68	0.05
Power Group B	182.27± 1.58	191.80 ± 1.82	5.23%	15.3	5.59	0.05
Power Group C	182.47± 1.46	185.80± 1.82	1.82%	5.54	2.01	0.05

Table 2: Anova for the variables between the Groups

Procedures	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	F value	P value
Agility	4.38 ± 0.152	4.26 ± 0.188	4.91 ±0.128	71.04	0.001
Speed	5.26 ± 0.14	5.15 ± 0.21	5.93 ± 0.19	110.81	0.001
Power	190.47± 1.60	191.80 ± 1.82	185.80± 1.82	48.65	0.001

Table 3: Tukey HSD Analysis for the variables

Procedures	Tukey HSD Q Statistics for Group A vs Group B	Tukey HSD Q Statistics for Group A vs Group C	Tukey HSD Q Statistics for Group B vs Group C	Tukey HSD Q Inference
Agility	2.94	12.91	15.85	Group A vs B is insignificant
Speed	2.98	16.56	19.54	Group A vs B is insignificant
Power	2.96	10.33	13.29	Group A vs B is insignificant


Young Female Athletes
N=120
Young Female Athletes
N= 45
Randomization
Group A (15)
Russian Currents
Group A (15)
Plyometrics
Group A (15)
Basic Strengthening
Outcome Measure
Agility
Speed
Week 0
Pre-Intervention
Assessment
Pre-Intervention
Assessment
Pre-Intervention
Assessment
Week 6
Post-Intervention
Assessment
Post-Intervention
Assessment
Post-Intervention
Assessment

Flow Chart

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RESEARCH ARTICLE

A Note on $TSBF_1$ – Algebras

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ABSTRACT

In this paper, we discuss the concept of open sets and subalgebras on $TSBF_1$ – algebras with respect to F(a), which is a special case of TSBF-algebras with respect to F(a) with the property that, $x = (x * y) * (0 * y)$, for all $x, y \in X$. In a $TSBF_1$-algebras with respect to F(a), if we consider {0} as a open set then it implies every subset of F(a) is open, since F(a) is open and F(a) is a subalgebra of X. It produces trivial results. So, we avoid this case in this paper. Therefore, {0} is not open in the chapter.

2010 AMS Classification: 06F35, 54A10, 54A05
Keywords and phrases: TSBF-algebra, $TSBF_1$-algebras, Minimal subalgebra,

INTRODUCTION

Algebra and topology are the two fundamental domains of mathematics. Many of the most important objects of mathematics represent a blend of algebraic and topological structures. In [1], Molodtsov introduced the concepts of soft set as a new mathematical tool for dealing with uncertainties that is free from the difficulties that have troubled the usual theoretical approaches. The aim of this article is to lay a foundation for providing a soft algebraic tool in considering many problems that contain uncertainties. In [2], Walendziak introduced the notion of BF and BF_1 algebras, which is a generalization of B–algebras. In [3], Min Su Kang and Hee Sik Kim introduced the notion of soft BF-algebras. In [4], we introduced the notion of topological BCH–algebras and in [5], we introduced the notion of topological soft BF–algebras. In this paper, we study the connection between soft BF_1-algebras and topology and discuss some of properties.

Preliminaries

Definition 2.1 [2] A BF-algebra is an algebra (X, ∗, 0) of type (2, 0) (that is, a non-empty set X with a binary operation * and a constant 0) satisfying the following conditions

1. x ∗x = 0,
2. x ∗0 = x,
3. 0 ∗(x ∗y) = y ∗x, ∀ x, y ∈ X.

Definition 2.2 [2] A BF-algebra (X, ∗, 0) is called a BF_1-algebra if it satisfies the following identity, x = (x ∗y) ∗(0 ∗y), ∀x, y ∈ X.

Definition 2.3 [2] Let (X, ∗, 0) be a BF-algebra. A nonempty subset N of X is called a subalgebra of X if x ∗y ∈ N, for all x, y ∈ N.

Definition 2.4 [3] A pair (F, E) is called a soft set over U if and only if F is a mapping of E into the set of all subsets of the set U. where, U is an initial universal set and E is a set of parameters.

Definition 2.5 [3] Let (F, A) be a soft set over a BF-algebra X. Then (F, A) is called a soft BF-algebra over X if F(x) is a subalgebra of X, ∀ x ∈ A.

Definition 2.6 [5] Let (F, A) be a soft BF-algebra over a BF-algebra X and τ be a topology on X. Let x ∈ X. Then (F, A, τ) is said to be a topological soft BF-algebra (TSBF-algebra) over X with respect to F(x), if for every a, b ∈ F(x) and any open set W of a ∗b, there exist open sets U and V of a and b respectively such that U ∗V ⊆ W.

Definition 2.7 [5] Let (F, A, τ) be a TSBF-algebra with respect to F(a) over finite X, and A be any subset of X. Then

i. The cardinality of A is defined as number of elements in A. It is written as, |A|.
ii. A is called the least open set of x ∈ X if every open set of x contains A.

Theorem 2.8 [5] Let (F, A, τ) be a TSBF-algebra with respect to F(a) and B is the least open set containing 0. If x ∈ F(a) ∩ B, then B is the least open set containing x.

Theorem 2.9 [6] Let X be a BF-algebra, then 0 ∗x = 0 ∗y implied x = y for any x, y ∈ X.

RESULTS

Results on $TSBF_1$–Algebras

Definition 3.1 Let (F, A, τ) be a topological soft BF-algebra (TSBF-algebra) over a BF-algebra X with respect to F(x), Then (F, A, τ) is said to be a topological soft BF_1-algebra ($TSBF_1$algebra) over X with respect to F(x), if x = (x ∗y) ∗(0 ∗y), for all x, y ∈ X.

Example 3.2 Consider the TSBF–algebra with respect to F(2) over X = {0,1,2,3} with the cayley table,

∗	0	1	2	3
0	0	1	2	3
1	1	0	2	3
2	2	2	0	1
3	3	3	1	0

Let A = X and define a function F : A → P(X) by,
F(0) = {0}, F(1) = {0,1}, F(2) = {0,2}, F(3) = X with the topology τ = {X,ϕ,{0,2}} on X. Here, for every x, y ∈ X satisfies the condition x = (x ∗y) ∗(0 ∗y). Therefore, (F, A, τ) is a TSBF$_1$- algebra with respect to F(2) over X.

Example 3.3 Consider the TSBF–algebra with respect to F(1) over X = {0,1,2,3,4} with the cayley table,

*	0	1	2	3	4
0	0	1	2	3	4
1	1	0	1	0	1
2	2	1	0	1	0
3	3	0	1	0	1
4	4	1	0	1	0

Let A = X and define a function F : A → P(X) by,
F(0) = X, F(1) = {0,1}, F(2) = {0,1,3}, F(3) = {0,1,2,4}, F(4) = {0}, with the topology τ = {X, ϕ, {1}, {0,4}, {0,1,4}} on X.
Here, 2 ≠ (2∗1)∗(0∗1). Implies, (F, A, τ) is not a TSBF$_1$- algebra with respect to F(1) over X.

Theorem 3.4 Let (F, A, τ) be a TSBF$_1$- algebra with respect to F(a) over X, then it satisfies the property (#), x ∗y ≠ z ∗y and y ∗x ≠ y ∗z, ∀ x, y, z ∈ X.

Proof: Let X be a BF-algebra with the property that, x = (x∗y) ∗(0∗y),for all x, y ∈ X. Fix y ∈ X Let x,z ∈ X and $x \neq z$.
Now, x = (x ∗y) ∗(0 ∗y) and z = (z ∗y) ∗(0 ∗y).
If x ∗y = z ∗y then (x ∗y) ∗(0 ∗y) = (z ∗y) ∗(0 ∗y). Therefore x = z. which is a contradiction. Therefore x∗y ≠ z ∗y, ∀ y ∈ X. From theorem 2.4, 0∗y ≠ 0∗z, for any x ≠y. ⟹ 0∗(x∗y) ≠ 0 ∗(z ∗y), ⟹ y ∗x ≠ y ∗z.

Lemma 3.5 Let (F, A ,τ) be a TSBF$_1$- algebra with respect to F(a) over X. A set {x}, x ∈ F(a) is not open.

Proof: From our assumption, the set {0} is not open. Every open set of 0 contains at least one element y (say) from X other than 0.
Let x ∈ F(a) and x ≠ y. Since x ∗0 = x, every open set W of x, there exists open sets U and V of x and 0 respectively such that, U ∗V ⊆ W.
Now, x ∈ U and 0, y ∈ V. ⟹ x ∗0 = x, x ∗y ∈ W. From (#), x ∗0 ≠x ∗y. ⟹ x ∗y ≠ x.
Therefore, W has atleast one element other than x. This is true for all open sets of x. Therefore, every open set of X has atleast two elements. Therefore, the set {x} is not open, x ∈ F(a).

Remark 3.6 In general, if {0} is not open then the set {x} may be open. The above Lemma 3.5 is true only for the case X with the property (#).

Proposition 3.7 Let (F, A, τ) be a TSBF$_1$-algebra with respect to F(a) over X. If 0 ∗y ≠ y, ∀ y ∈ X, then every open set of 0 contains atleast two elements other than 0.

Proof: From our assumption, the set {0} is not open. From Lemma 3.5, {x}, x ∈ F(a) is not open. Every open set of 0 contains more than one element other than 0.
Claim: Every open set of 0 contains atleast two elements other than 0.
Suppose there exist an open set W$_1$ of 0 contains exactly one element (say) y from X other than 0. That is, W$_1$ = {0, y}. Since 0 ∗0 = 0, every open set W$_1$ of 0, there exist open sets U and V of 0 such that, U ∗V ⊆ W. ⟹ U ∗V ⊆ {0, y}.

$\Rightarrow$ U and V has an element x_1 and x_2 respectively.- If $x_1 \neq y$, $\Rightarrow x_1 * 0 = x_1 \notin \{0, y\}$. Which is contradiction . If $x_2 \neq y$, $\Rightarrow x_2 * 0 = x_2 \notin \{0, y\}$. Which is contradiction. Suppose, $x_1 = x_2 = y$. Since $0 * y \neq y$, $\forall\, y \in X$, then $0 * y \notin \{0, y\}$. Which is contradiction Therefore, Our assumption is wrong. Therefore, every open set of 0 contains atleast two elements other than 0.

Definition 3.8 Let (F, A, τ) be a $TSBF_1$-algebra with respect to F(a) over a BF-algebra X and S be any subalgebra of X. Then S is called a minimal subalgebra of X, if there is no subalgebra of X contained in S.

Proposition 3.9 Let (F, A, τ) be a $TSBF_1$-algebra with respect to F(a) over X, where F(a) is a minimal subalgebra of X. then there exist atmost one element y form $(F(a))^c$ such that {0, y} is open.

Proof: For Existence: Let $y \in X$. Assume, {0, y} is open.

Case (i): Suppose $y \in F(a)$. Since $0 \in F(a)$ and $0 * 0 = 0$, every open set W of 0, there exists open sets U and V of 0 such that,

$U * V \subseteq W. \Rightarrow U * V \subseteq \{0, y\}$ (3.1)

From our assumption, {0} is not open. $\Rightarrow$ U and V has atleast one element z_1, $z_2 (\neq 0)$ respectively. Therefore, $z_1 * 0 = z_1$, $0 * z_2$, $z_1 * z_2 \in U * V$. (3.1) $\Rightarrow z_1$, $0 * z_2$, $z_1 * z_2 \in \{0,y\}$.From (#), this is possible only if $z_1 = z_2 = y$. Now, $\{0,y\} * \{0,y\} \subseteq \{0, y\}$. Therefore, {0, y} is a subalgebra of X and contained in F(a). Which is contradicts to F(a) is a minimal subalgebra of X. Therefore, {0, y} is not open. In this case, there exists no y form F(a) such that, {0, y} is open.

Case (ii): Suppose $y \notin F(a)$

Let $x \in F(a)$. From the first condition of BF-algebra definition, $x * x = 0$. $\Rightarrow$ Every open set W of 0, there exist open sets U and V of x such that $U * V \subseteq W$. Therefore,

$U * V \subseteq \{0, y\}$ (3.2)

From Lemma 3.5, {0} is not open. $\Rightarrow$ U and V has atleast one element other than x say z_1 and z_2 respectively. Therefore $x * z_2$, $z_1 * z_2$,$z_1 * x \in U * V \subseteq \{0, y\}$. From (#), $z_1 * z_2 \neq z_1 * x$, $x * z_2 \neq 0$. (3.2) $\Rightarrow z_1 * z_2, z_1 * x, x * z_2 \in \{0,y\}$. This is possible only if $z_1 = z_2$. Therefore, U and V contains x and z_1. Suppose U and V has any other element it contradicts to (3.2). Therefore, $U = V = \{x, z_1\}$.

Claim: $\{x, z_1\}$ is the minimal open set for x. Suppose there exist an open set G of x such that, $z_1 \notin G$. $\Rightarrow G \cap \{x,z_1\} = \{x\}$. G is not open. Every open set of x contains z_1. Since $\{x, z_1\}$ is open, $\{x, z_1\}$ is the minimal open set for x. From the second condition of BF- algebra definition, $x * 0 = x$. Every open set W of x, there exist open sets U and V of x and 0 respectively such that

$U * V \subseteq W. \Rightarrow U * V \subseteq \{x, z_1\}$ (3.3)

Now, $x, z \in U$. We can take,

$V = \{0, y\}. \Rightarrow x * 0 = x$, $z_1 * 0 = z_1$, $x * y = z_1$, $z_1 * y = x$ (3.4)

(3.4) satisfies (3.3). Let $x_1, x_2 \in F(a)$. From the third condition of BF-algebra definition,

$0 * (x_1 * x_2) = x_2 * x_1$ (3.5)

Let $x_1 * x_2 = h \in F(a)$, $x_2 * x_1 = k \in F(a)$.

$\Rightarrow$ {h, h_1} is the minimal open set for h, {k,k_1} is the minimal open set for k, where h_1,$k_1 \in X$. (3.5) implies, every open set W of $x_2 * x_1$, there exist open sets U and V of 0 and h respectively such that,

$U * V \subseteq W. \Rightarrow U * V \subseteq \{k, k_1\}$ (3.6)

We can take, V = {0, y}. (3.6) implies, $0 * h = k$, $0 * h_1$, $y * h$, $y * h_1 \in \{k, k_1\}$. This is possible only if $0 * h_1 = k_1$, $y * h = k_1$, $y * h_1 = k$ (3.7)

(3.7) satisfies (3.6). Therefore, we conclude that, for every x in F(a) if there exist z_1 such that, {x, z_1} is open and satisfies (3.3) , (3.4) and (3.7) then {0, y} is open. If any one them fails then {0, y} is not open. Suppose {0, z}, $z \neq y$ is open, satisfying (3.3) , (3.4) and (3.7) . $\Rightarrow$ {0} is open. Which is a contradiction. Therefore, there exist atmost one y from $(F(a))^c$ such that, {0, y} is open.

Lemma 3.10 Let (F, A, τ) be a $TSBF_1$-algebra with respect to F(a) over a finite BF-algebra X. Then the cardinal number of the least open set for 0 is equal to the cardinal number of the least open set for every $x \in F(a)$.

Proof: Let W_1 be the least open set for 0. Assume, $|W_1| = n$.
Now choose, $x \in F(a)$. Suppose if $x \in W_1$, then from theorem 2.9, W_1 is the least open set for x. Suppose, if $x \notin W_1$. Let U_1 be the least open set for x.

Claim: $|U_1| = n$.
Since $x*0 = x$ for every open set W for x, there exist open sets U and V of x and 0 respectively, such that $U * V \subseteq W$. $\Rightarrow U * V \subseteq U_1$. Now, $U_1 \subseteq U$ and $W_1 \subseteq V$. From (#), $|U_1 * W_1| = n$.
But, $U_1 * W_1 \subseteq U * V \subseteq U_1$. Implies, $|U_1 * W_1| \leq |U_1| < n$. Which is contradiction.

Case (ii): Suppose, $|U_1| > n$. Since $x * x = 0$, for every open set W of 0, there exist open sets U and V of x such that, $U * V \subseteq W$ (3.8)

$\Rightarrow U * V \subseteq W_1$. Now, $U_1 \subseteq U$ and $U_1 \subseteq V$. $\Rightarrow |U_1| > n$ and $|V_1| > n$. From the property (#), $|U_1 * V_1| > n$. Since $U_1 * U_1 \subseteq U * V$, $\Rightarrow |U_1 * U_1| \leq |U * V| \Rightarrow |U * V| > n$. Which is contradiction to (3.8). Therefore, $|U_1| = n$. Therefore the cardinality of the least open set for x in X equals the cardinality of the least open set for 0.

Theorem 3.11 Let (F, A, τ) be a $TSBF_1$-algebra with respect to F(a) over finite X. If W_1 is the least open set for 0, then for every open set U of x in F(a), there is a one - one correspondence with W_1 into U.

Proof: let $x \in F(a)$. Since $x * 0 = x$, for every open set W of 0, there exist open sets U and V of x and 0 respectively such that, $U * V \subseteq W$. Now, $W_1 \subseteq V. \Rightarrow U * W_1 \subseteq U * V \subseteq W$ _____ (1)
From (#), the cardinality of U is atleast $|W_1|$. Suppose there exist an open set U_1 of x such that, $|U_1| > |W_1|$, then (1) fails. Therefore, for every open set U of x in F(a), there is a one - one correspondence with W_1 into U.

CONCLUSION

As a result of this study, we got an idea of building topologies, in $TSBF_1$-algebras with respect to F(a). Based on this study, we can identify some basic sets such as open set, closed set and ideals, which plays main role in the construction of $TSBF_1$-algebras with respect to F(a) and give some results.

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RESEARCH ARTICLE

Transmutation of the Two Parameters-Rayleigh Distribution for Acute Exercise on Serum GH Response in Elite Male Water Polo Players

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ABSTRACT

Statistical analysis is used to analyse lifetime data which is based on some statistical distribution. In this paper, mathematical elucidation for the consequence of severe exercise on serum growth hormone (SGH) levels in elite water polo players using Transmuted Two Parameters Rayleigh Distribution is employed. The result clearly indicates that acute exercise significantly increases the serum GH levels in elite water polo players. Further, it is found that water polo players showed increased levels of probability functions and significantly increased levels of reliability function as compared with sedentary subjects, suggesting that the regular exercise welfares the life span.

Keywords: Water polo players, GH, Rayleigh Distribution, hazard function, survival function

INTRODUCTION

The most important continuous pdf for positive valued rvs is Rayleigh distribution (RD). AS a special case of Weibull distribution, RD is of much importance for survival analysis [1]. It was introduced by Lord Rayleigh (1880) as a single scale parameter [2]. Applications of RD appear in engineering sciences. Longuet-Higgins first demonstrated that in linear waves which have frequency spectrum that is of bandwidth, height of waves attained follow RD. distribution. Uses of RD are broadened to medical and social sciences, marketing etc., [3-6]. Two parameters RD have location and scale parameters. Here, RD is used to analyse the life data for acute exercise effects on SGH levels in elite water polo players. Growth hormone is an area of major research interest analytically in endocrinology [6-8]. Reports show GH level variations on different physical activities. Substantial exercise is a forceful biological stimulant for secretion of GH. Aerobic and insistent exercise showed crucial increase in GH secretion [8]. In the present study, Djelic M. *et al.,* stated data using Transmutation of the Rayleigh Distribution using two parameter is analysed. Hence, we have designated to study the consequences of exercise on SGH levels in elite water polo players in comparison with sedentary participants. Subsequently, regular physical exercise is a path to lead healthy life.

Physical exercise is termed as the movement or activity of the body that maintains the physical fitness which enhances the whole health system. The numerous health benefits are mainly accompanied with the habit of regular physical exercise [9]. Regular exercise gives substantial benefit and effects of numerous stability mechanisms directly involved with endocrine system is in [10-13]. To our knowledge, the life time data was not yet analysed using two parameter Rayleigh Distribution, so far. Moreover, we have analysed the Djelic M. *et al.,* stated data using two parameter Rayleigh Distribution with some modifications to acquire the clear interpretations on probability and reliability functions to express the data in well accepted as well as in understandable manner.

METHODOLOGY

Mathematical Model

Transmuted 2 Parameters RD

The df of a RD is

$f(x,\sigma) = \frac{x}{\sigma^2} exp\left(-\frac{x^2}{2\sigma^2}\right), x > 0, \sigma > 0.$

And the respective cumulative

$F(x,\sigma) = 1 - exp\left(-\frac{x^2}{2\sigma^2}\right), x > 0, \sigma > 0.$

And the transmuted cdf is

$G(x,\sigma,\lambda) = \left(1 - exp\left(-\frac{x^2}{2\sigma^2}\right)\right)\left(1 + exp\left(-\frac{x^2}{2\sigma^2}\right)\right), x > 0, \sigma > 0.$

With the transmuted pdf $g(x,\sigma,\lambda) = \left(\frac{x}{\sigma^2} exp\left(-\frac{x^2}{2\sigma^2}\right)\right)\left(1 - \lambda + 2\lambda\, exp\left(-\frac{x^2}{2\sigma^2}\right)\right).$

A r.v. X possess transmuted distribution if interrelation in Shaw and Buckley [1-6] known as quadratic rank transmutation map is satisfied.

$F(x) = G(x)[(1 + \lambda) - \lambda G(x)], |\lambda| \leq 1$

Differentiating, $f(x) = g(x)[1 + \lambda - 2\lambda G(x)]$

Λ - transmuted parameter,

$g(x)$, $G(x)$ - pdf, cdf of base distribution.

The pdf of 2 parameters RD is

$g(x;\alpha,\beta) = 2\beta(x-\alpha)e^{-\beta(x-\alpha)^2} \qquad x > \alpha, \beta > 0$

β, α – scale, location parameters. The cdf is,

$G(x;\alpha,\beta) = 1 - e^{-\beta(x-\alpha)^2}, x > \alpha$

Cdf of transmuted 2 parameters RD is obtained from the above equation

$F_{TR}(x;\alpha,\beta,\lambda) = \left[1 - e^{-\beta(x-\alpha)^2}\right]\left[1 + \lambda e^{-\beta(x-\alpha)^2}\right]$

Pdf of transmuted 2 parameters RD is,

$f_{TR}(x;\alpha,\beta,\lambda) = 2\beta(x-\alpha)e^{-\beta(x-\alpha)^2}\left[1 - \lambda + 2\lambda e^{-\beta(x-\alpha)^2}\right]$

Theorem

Let X be a r.v. that has $T_R(x;\alpha,\beta,\lambda)$ with $|\lambda| \leq 1$. The rth moment $E(x^r)$ of transmuted 2 parameters RD is

$$\mu_r^{'} = \sum_{k=0}^{r} \binom{r}{k} \alpha^{r-k} \left[(1-\lambda)\left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{\beta^{\frac{k}{2}}}\right\} + \lambda\left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{2\beta^{\frac{k}{2}}}\right\}\right]$$

Proof:

The rth moment is,

$\mu_r^{'} = E(X^r) = \int_{\alpha}^{\infty} x^r 2\beta(x-\alpha)e^{-\beta(x-\alpha)^2}\left[1 - \lambda + 2\lambda e^{-\beta(x-\alpha)^2}\right]dx$

Let $x = \alpha + z$, then

$\mu_r^{'} = 2\beta \int_0^{\infty} (\alpha + z)^r z e^{-\beta z^2}\left[(1-\lambda) + 2\lambda e^{-\beta z^2}\right]dz$

$$\mu_r^{'} = 2\beta \int_0^{\infty} \sum_{k=0}^{r} \binom{r}{k} z^k \alpha^{r-k} z e^{-\beta z^2} \left[(1-\lambda) + 2\lambda e^{-\beta z^2}\right] dz$$

$$\mu_r^{'} = 2\beta \sum_{k=0}^{r} \binom{r}{k} \alpha^{r-k} \int_0^{\infty} z^{k+1} e^{-\beta z^2} \left[(1-\lambda) + 2\lambda e^{-\beta z^2}\right] dz$$

Now, substituting $y = z^2$,

$$\mu_r^{'} = 2\beta \sum_{k=0}^{r} \binom{r}{k} \alpha^{r-k} \left[(1-\lambda) \int_0^{\infty} \frac{y^{\left(\frac{k+1}{2}\right)} e^{-\beta y}}{2\sqrt{y}} dy + 2\lambda \int_0^{\infty} \frac{y^{\left(\frac{k+1}{2}\right)} e^{-2\beta y}}{2\sqrt{y}} dy\right]$$

$$\mu_r^{'} = \sum_{k=0}^{r} \binom{r}{k} \alpha^{r-k} \left[(1-\lambda) \left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{\beta^{\frac{k}{2}}}\right\} + \lambda \left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{2\beta^{\frac{k}{2}}}\right\}\right]$$

The mean of the transmuted 2 parameters RD is,

$$Mean = E(X) = \alpha + \frac{\sqrt{\pi}}{2\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right]$$

$r^{th} Moment\ expression\ of\ the\ transmuted\ 2\ parameters\ RD$

$$\mu_r^{'} = \alpha + \frac{\sqrt{\pi}}{2\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right]$$

$$\mu_1^{'} = \alpha + \frac{\sqrt{\pi}}{2\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{2}\right]$$

$$\mu_2^{'} = \alpha^2 + \frac{\alpha\sqrt{\pi}}{\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{2}\right] + \frac{1}{\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right]$$

Thus, $\mu_3^{'}, \mu_4^{'}, \ldots\ldots$ can be found.

Theorem

Let the r.v. X follow transmuted 2 parameters RD. Variance,

$$\sigma^2 = Var(X) = \frac{1}{\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right] - \frac{\pi}{4\beta}\left[(1-\lambda + \frac{\lambda}{2})\right]^2$$

Proof.

Variance of transmuted parameters RD is,

$$Var(X) = E(X^2) - [E(X)]^2$$

Then

$$Var\,(X) = a^2 + \frac{a\sqrt{\pi}}{\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{\sqrt{2}}\right] + \frac{1}{\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right] - \left[a + \frac{\sqrt{\pi}}{2\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{\sqrt{2}}\right]\right]^2$$

$$Var\,(X) = a^2 + \frac{a\sqrt{\pi}}{\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{\sqrt{2}}\right] + \frac{1}{\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right] - a^2 - \frac{\pi}{4\beta}[(1-\lambda) + \frac{\lambda}{2}]^2 - \frac{a\sqrt{\pi}}{\sqrt{\beta}}\left[(1-\lambda) + \frac{\lambda}{\sqrt{2}}\right]$$

$$Var\,(X) = \frac{1}{\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right] - \frac{\pi}{4\beta}\left[(1-\lambda) + \frac{\lambda}{2}\right]^2$$

Theorem:

Let X possess transmuted 2 parameters RD. Mgf of transmuted 2 parameters RD is

$$M_X(t) = \sum_{r=a}^{\infty} \sum_{k-0}^{r} \frac{t^r}{r!}\left(\frac{r}{k}\right) a^{r-k} \left[(1-\lambda)\left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{\beta^{\left(\frac{k}{2}\right)}}\right\} + \lambda\left\{\frac{\Gamma\left(\frac{k}{2}+1\right)}{(2\beta)^{\left(\frac{k}{2}\right)}}\right\}\right]$$

Proof: The mgf for X is

$$M_X(t) = E(e^{tX}) = \int_a^{\infty} e^{tx} f_{TR}(x, a, \beta, \lambda) dy$$

$$e^{tx} = 1 + tx + \frac{t^2x^2}{2!} + \frac{t^3x^3}{3!} + \cdots \frac{t^r x^r}{r!} + \cdots$$

Then

$$M_X(t) = \int_a^{\infty} \left(1 + tx + \frac{t^2x^2}{2!} + \frac{t^3x^3}{3!} + \cdots \frac{t^nx^n}{n!} + \cdots\right) f_{TR}(x, a, \beta, \lambda) dy$$

$$M_X(t) = \sum_{r=a}^{\infty} \frac{t^r E(X)^r}{r!}$$

$$M_X(t) = \sum_{r=a}^{\infty} \frac{t^r}{r!} \left(\sum_{k=0}^{r} \binom{r}{k} a^{r-k} \left[(1-\lambda) \left\{ \frac{\Gamma\left(\frac{k}{2}+1\right)}{\beta^{\left(\frac{k}{2}\right)}} \right\} + \lambda \left\{ \frac{\Gamma\left(\frac{k}{2}+1\right)}{(2\beta)^{\left(\frac{k}{2}\right)}} \right\} \right] \right)$$

$$M_X(t) = \sum_{r=a}^{\infty} \sum_{k-0}^{r} \frac{t^r}{r!} \binom{r}{k} a^{r-k} \left[(1-\lambda) \left\{ \frac{\Gamma\left(\frac{k}{2}+1\right)}{\beta^{\left(\frac{k}{2}\right)}} \right\} + \lambda \left\{ \frac{\Gamma\left(\frac{k}{2}+1\right)}{(2\beta)^{\left(\frac{k}{2}\right)}} \right\} \right]$$

Reliability Analysis

The probability of surviving an item at reach on t time is given by reliability function. The reliability function of transmuted 2 parameters RD is

$$R_{TR}(t) = P(T > t) = \int_t^{\infty} f(t)dt = 1 - F_{TR}(t)$$

$$R_{TR}(t) = e^{-\beta(x-\alpha)^2}(1 - \lambda(1 - e^{-\beta(x-\alpha)^2}))$$

RESULTS

Application

Participants and Experimental Design

12 elite male water polo players and 11 nonathletic males participated in Djelic *et al.,* 2014 [7] study. Students of University of Belgrade were recruited as non-athletes who exercised < 3 h/week. The participants were healthy, had normal blood pressure, non-smokers, were not under drugs or medication and did not show record of any endocrine disorders neither before nor during the period of study. They showed no genealogy of diabetes mellitus or adiposity. Participants who were studied knew the procedures before they provided written consent to take part in the activities which was accepted by Committee of Ethics, School of Medicine at University of Belgrade. In Djelic *et al.,* 2014 [7], participants completed a questionnaire on training history and the body composition test before the exercise. Blood samples before the exercise for athletes and non athletes were extracted at 9 AM. Each and every participant were fed rich in carbohydrates 2 hours before exercise testing. Treadmill was used to perform exercise tests. It was followed by gradual arrangement intending time span between 8 and 12 min. The treadmill arrangement for VO_2 max test started at a rate of 4 km/h at 0.0% incline. Treadmill momentum was raised by 1 km/h per minute. Level of effort attained was maximal if a plateau in VO_2 was found in spite of increase in the intensity of exercise and RER value > 1.10. VO_2 was observed constantly, and average value of 3 greatest consecutive 10-s values was defined as VO_2 max. Samples of blood were extracted instantly after exercise and 30 minute during recovery after treadmill running test. Ages of water polo players and controls were almost the same. Furthermore, lean body mass of water polo players appreciably elevated. The GH response of the maximum exercise test is shown in fig 1. GH concentrations before and after exercise were in population reference range. In water polo players, the aggregation of GH was appreciably elevated immediately after 30-min of recovery in comparison with baseline levels (+84.2 %; +107.0 %, respectively; $p<0.05$; Fig. 1). In, the GH concentration was appreciably elevated (+22.9%; $p<0.05$; Fig. 1) soon after exercise and was higher 30 min after exercising.

MATHEMATICAL RESULTS

The pdf of transmuted 2 parameters RD on GH levels of water polo players and sedentary controls

Fig.1 depicts the comparative analysis of GH levels in water polo players and sedentary controls using transmuted 2 parameters RD. In contrast to Djelic *et al.,* [7] the transmuted 2 parameters RD $f(x)$ plot shows elevated measure of GH in water polo players in comparison with non-athletic subjects in Pre-Ex condition. It is clear that, Post-Ex state of both GH levels were decreased from Pre-Ex level and gradually increased in after 30min recovery state from Post-Ex level in water polo payer and non-athletic subjects. In similar with Djelic *et al.,* the level of GH was appreciably increased in water polo players in comparison with non-athletic subjects after 30min recovery state. Effect of transmuted 2 parameters RD on SGH concentrations before and after exercise, after 30 min of recovery in water polo players and controls.

The Reliability function of the transmutation of 2 parameters RD.

Mathematical figure 2 shown, The Reliability functions of transmuted two parameters RD analysis on GH levels in water polo players and non-athletic controls. Transmuted two parameters RD probability function $f(x)$ and $R(x)$ plot reveals that the elevated Reliability rate in water polo players as compared to non-athletic subjects in Pre-Ex state, Post-Ex state, the $f(x)$ and $R(x)$ was significantly increased from Pre-Ex level and gradually increased in after 30min recovery state from Post-Ex level in water polo payers. The Reliability function notably increased in water polo players as compared to non-athletic subjects in after 30min recovery state. Subsequently, the Reliability functions in non-athletic control subjects were increased from Pre-Ex state. Transmutation of the 2 parameters RD of its Reliability function's graphical levels for the effect of on SGH concentrations before and after exercise, after 30 min of recovery in water polo players and controls.

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DISCUSSION

The results of the present study clearly demonstrated that the water polo players showed the increased levels of probability functions and significantly increased levels of Reliability function's as compared with sedentary subjects, suggesting that the regular exercise welfares the life span. One among the statistical distributions is distribution of extreme value which is inevitably used in modelling lifetime data. Here, two parameters RD is applied to analyse the life time data (GH levels) of Djelic *et al.,* 2014. The results reveals that, the GH levels in Post-Ex state were decreased from Pre-Ex level and gradually increased in after 30min recovery state from Post-Ex level in water polo payers. In similar with Djelic *et al.,* the level of GH was appreciably increased in water polo players in comparison with non-athletic subjects in after 30min recovery state. In addition, the GH levels in non-athletic control subjects were increased from Pre-Ex state and decreased after Post-Ex state. In contrast to Djelic *et al.,* the 2 parameter Weibull distributions $f(x)$ plot reveals the elevated measure of GH in water polo players in comparison with non-athletic subjects in Pre-Ex condition, signifying that acute exercise showed impact on GH levels. Mejri et al. 2005 [13] established that GH levels were greatest in the beginning and reduced gradually. Transmuted two parameters Rayleigh distribution reliability function $R(x)$ plot reveals that the elevated probability density functions in water polo players as compared to non-athletic subjects in Pre-Ex state and the reliability function was markedly increased in water polo players as compared to non-athletic subjects and the reliability function in non-athletic control subjects were markedly increased from Pre-Ex state to after 30min recovery state, suggesting that acute exercises increases reliability rate present study gives an adequate interpretation in the results of Deljic *et al.,* with appropriate understandings.

CONCLUSION

In the present study, we have explored a two parameter Rayleigh distribution for alternative approaches to analyse a life time data. The results confirm the outcomes of Djelic *et al.,* 2014 that acute exercise have impact on the GH levels. In addition, the two parameter RD predicts the probability $f(x)$ and reliability function $R(x)$ of the serum growth

hormone levels which reveals the elevated reliability rate in water polo players as compared to non-athletic subjects in Pre-Ex state. The reliability function was markedly increased in water polo players as compared to non-athletic subjects. These findings could be an additional contribution in understanding human endocrine physiology better in severe exercise conditions.

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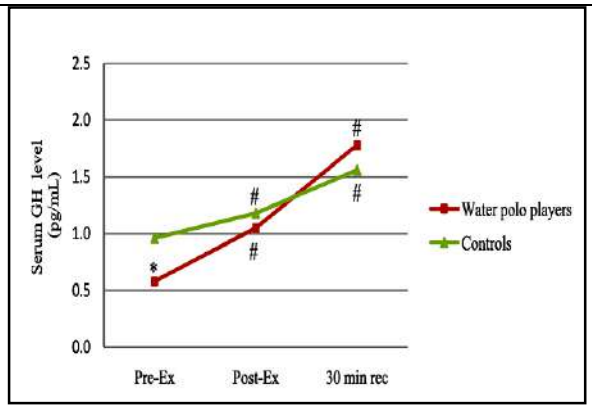

Fig. 1. Medical figure

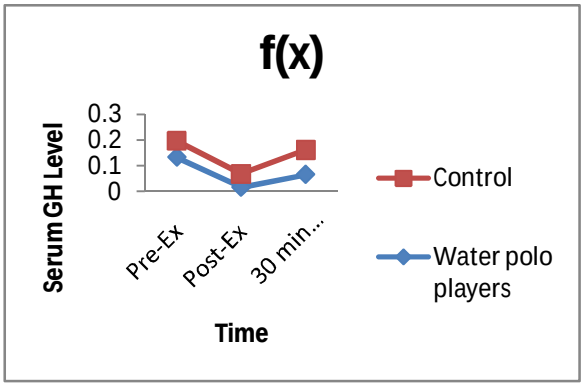

Figure: 2. Mathematical

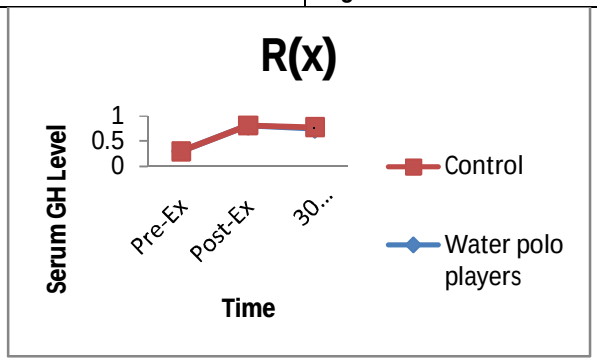

Figure: 3 Mathematical

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RESEARCH ARTICLE

Investigation of Drotaverine Hydrochloride by a Validated Molecular Absorption Spectral Technique using UV Spectral Technique

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ABSTRACT

The present method includes a simple, sensitive and specific UV method development and validation for the Drotaverine HCl by a validated molecular absorption spectral technique using UV and ATR-FTIR spectral techniques. The developed Differential UV-Spectrometric method utilizes 0.1N HCl as solvent which is cheaper as compared to other solvents and the quality of the developed method is accurate, precise, and easy as evident from the analytical and statistical parameters calculated. The UV - spectrum of Drotaverine HCl Pure and Formulation were recorded and the absorption maxima (λmax) were observed at 241nm. All the Aliquotes were scanned in 241 nm to compare the accuracy of the proposed method. Tablets showed linearity in the concentration range of 01-16 μg/ml. The validation of the proposed method was further confirmed by recovery studies at 50%, 100%, and 150%. The percentage recovery values were 98.89 w/w, 99.62% w/w, and 99.58% w/w and the average value is 99.36% w/w. These serve as a good index for the accuracy and reproducibility of the study.

Keywords: Drotaverine Hydrochloride, Uv-Spectrophotometer.

INTRODUCTION

Drotaverine is an antispasmodic drug that works by inhibiting phosphodiesterase-4 (PDE4). It is a benzylisoquinoline derivative that is structurally related to papaverine, although it displays more potent antispasmodic activities than papaverine. Drotaverine has been used in the symptomatic treatment of various spastic conditions, such as gastrointestinal diseases, biliary dyskinesia, and vasomotor diseases associated with smooth muscle spasms. It also has been investigated in dysmenorrhea, abortion, and augmentation of

labour. More recently, drotaverine gained attention in the treatment of benign prostatic hyperplasia, parainfluenza, and avian influenza viruses.Drotaverine is not approved by the FDA, European Medicines Agency, or Health Canada. It is approved for use in Thailand as oral tablets or intramuscular injections. Drotaverine is a selective inhibitor of phosphodiesterase 4 (PDE4), which is an enzyme responsible for the degradation of cyclic adenosine monophosphate (camp). Inhibition of PDE4 leads to elevated levels of camp, leading to smooth muscle relaxation. Drotaverine is reported to undergo extensive hepatic metabolism, which is its main route of elimination. It may also undergo biliary excretion to form conjugated metabolites. Proposed metabolic pathways and metabolites are based on limited animal studies: in rats, the major identified metabolites of Drotaverine are 4'-desethyl-drotaverine, 6-desethyl- Drotaverine, drotaveraldine, and 4'-desethyl-drotaveraldine, all of which are glucuronidated in the bile. Drotaverine is mainly eliminated via hepatic metabolism. About 67% of the drug is found in feces and 20% of the drug was eliminated with urine.

CHEMICALS AND REAGENTS

Drotaverine was soluble in 0.1N HCl. Sparingly soluble in water, soluble in ethanol(96%), freely soluble in chloroform, slightly soluble in acetone, practically insoluble in petroleum ether. 0.1N HCl have been found to be suitable solvents in the differential UV spectrophotometric method; its absorbance was 241nm, giving a single peak with maximum absorbance. Therefore, 0.1N HCl were chosen as the ideal solvent and were used for all experimental work.

INSTRUMENTATION

Shimadzu UV / Visible Spectrophotometer - UV - 1601.

DETERMINATION OF λmax

λmax is the wavelength of an absorption maximum. The standard drug was dissolved in 0.1N HCl to obtain a 10 µg / ml solution. The solution was scanned between 200-400 nm and the peak was found to show the maximum absorbance at 241 nm.

DETERMINATION OF MOLAR ABSORPTION CAPACITY:

The absorption constant 'a' is the ratio between the absorption of the sample and the product of the thickness of the medium and the concentration of the sample. Since the thickness of the medium is the same for different determinations, the absorption capacity depends on the absorbance and concentration of the sample. Due to an increase in the sample concentration, the absorbance, which is always constant, also increases or decreases.

Drotaverine standard solutions from 1 µg / ml to 16 µg / ml were prepared from the stock solution. The absorbances of various concentrations were measured at 241 nm. Using the formula, the absorbance of Drotaverine HCl was calculated.

INFLUENCE OF TIME ON THE STABILITY OF THE EXTINCTION:

The stability of the solution was checked by measuring the extinction at regular time intervals. It was observed that the absorbance remained stable for a period of 1 hour and then the absorbance decreased with increasing time.

PREPARATION OF STANDARD SOLUTION

Accurately weighed about 100 mg of Drotaverine hydrochloride was transferred to a clean dry 100 ml calibrated volumetric flask and dissolved in 0.1N HCl. It was shaken for few minutes and the solution was diluted to 100 ml with the same. From this various dilution were prepared to get final concentration of 16µg/ml solution. The absorbance was taken at taken at 241 nm.

PREPARATION OF SAMPLE SOLUTION

Accurately 10 tablets were weighed and average weight was taken, weight of mashed tablets 560mg was equivalent to 100 mg of Drotaverine hydrochloride tablets. It was taken in 100 ml standard flask and the sample was dissolved

in 0.1N HCl and was made upto 100 ml with the same and sonicated for about 10 mins. The solution was filtered through Whatmann filter paper No-1. From this further more dilution were prepared to get the concentration of 16 μg/ml.

VALIDATION OF THE DIFFERENTIAL UV SPECTROPHOTOMETRIC METHOD

ACCURACY

The precision of the proposed method was justified by performing recovery studies on known quantities of pure Drotaverine. A certain amount of a previously analyzed formulation sample was mixed thoroughly and the active ingredient content of the mixture was determined according to the suggested method.

Procedure

A formulation sample weighed accurately corresponding to 100 mg of Drotaverine was mixed with 50%, 100% and 150% of the pure Drotaverine drug. The amount of the mixture corresponding to 100 mg of Drotaverine was weighed out and a sufficient amount of 0.1N HCl was added to dissolve and the volume was made up with them. The solution was filtered using the Whatman filter and further analyzes were performed according to the procedure described in Establishing the standard absorbance curve. The absorbance was measured under different conditions and the standard deviation (S.D.) and the relative standard deviation (R.S.D.)

LINEARITY

Method

Exactly weighed 100 mg of Drotaverine were dissolved in the solvent 0.1N HCl in a given method. Then the absorbance from 1 μg / ml to 16 μg / ml was recorded. It obeys the area of the beer's law range.

RESULTS AND DISCUSSION

DETERMINATION OF λ_{max}

The λ_{max} of Drotaverine standard drug was determined using 10 μg/ml solution. The solution was scanned between 200 – 400 nm and found that the peak at 241 nm showed maximum absorbance. Further 1 μg/ml to 16 μg/ml concentration was also scanned between 200 – 400 nm. The λ_{max} of the Drotaverine was found to be 241 nm. The UV-Spectra of Drotaverine in 0.1N HCl, in various concentrations for standard and sample drug were recorded and shown in Figure No.2, and Figure No. 3. UV – Spectrum of Drotaverine hydrochloride in 0.1NHCL

Observation

The λ_{max} of Drotaverine hydrochloride was found to be 241 nm.

OVERLAY SPECTRUM OF DROTAVERINE IN DIFFERENT CONCENTRATIONS

The Drotaverine standard drug was dissolved in 0.1 to obtain 10 μg/ml solution. The solution was scanned between 200 – 400 nm and found that the peak at 241 nm showed maximum absorbance between 200 – 400 nm. Further 1 μg/ml to 16 μg/ml concentration was also scanned between 200-400 nm in the overlay mode. The overlay of the Drotaverine is shown in the Figure No.4 and Figure No.5. Overlay Spectrum of Drotaverine hydrochloride in 0.1N HCL

Observation

Figure showing the overlay spectrum of Drotaverine hydrochloride at 1 μg/ml to 16 μg/ml.

STANDARD CURVE DATA

Standard curve was plotted using the absorbance at different concentration at drotaverine from 1 µg/ml at 241 nm. Each dilution was prepared in triplicate and the average value was found out. The absorbance of different concentration was recorded at 241 nm and the results are shown in Table No.1.

DETERMINATION OF ABSORPTIVITY $A_{1\,cm}^{1\%}$

From 100 µg/ml Drotaverine, 1 µg/ml to 16 µg/ml of standard solutions were prepared. The absorbance of different concentration was recorded at 241 nm. The absorptivity $A_{1\,cm}^{1\%}$ of Drotaverine at 241 nm was calculated using equation. The absorptivity $A_{1\,cm}^{1\%}$ was found to 375.9. The absorbance of different concentration was recorded at 241 nm and the results are shown in Table No.2. The curve was plotted as absorbance vs. concentration and shown in Figure No.6.

Observation

The average absorptivity of Drotaverine was found to be 660.23

MOLAR ABSORPTIVITY OF DROTAVERINE HYDROCHLORIDE

The molar absorptivity ε_{max} was calculated from the absorbance of different concentration of Drotaverine solution. The molar absorptivity ε_{max} was found to be 29167.25. The results were shown in Table No.4

Observation

The average molar absorptivity of Drotaverine was found to be 29167.25.

ANALYSIS OF FORMULATION I (DROTIN)

Brand Name	= DROTIN
Label Claim	= 40 mg
Weight of 10 Tablets	= 2.25 gm
Average Weight	= 0.225 gm
Equivalent Weight of 10 Tablets	= 0.5625gm

Observation

The assay value was found to be 99.76%

VALIDATION OF PROPOSED

Accuracy of Drotaverine at 50%, 100% & 150%:

Label Claim	= DROTIN
Weight of 10 Tablets	= 2.25 g
Average Weight	= 0.225g
Equivalent Weight of 10 Tablets	= 0.5625g

Observation

The S.D. and R.S.D value was found to be within the prescribed limits.

Report

Spectrum shows overlay of accuracy of 50%, 100% and 150%.

STABILITY OF DROTAVERINE HYDROCHLORIDE IN 0.1N HCl

Stability of Drotaverine Standard

The stability of 100µg/ml Drotaverine of standard was prepared and checked by measuring the absorbance at 241 nm at regular intervals of time at room temperature. It was observed that absorbance remain stable for a period of about

3 hours then gradually decreased. The results were shown in Table No. 6. At 2 hours the absorbance decreased to about 99.89% and by 3 hours the absorbance decreased to about 99.82%**.**

Stability of Drotaverine sample

A stability curve was prepared for sample by carrying out stability study for about 3 hours taking 10µg/ml sample solution and checking its absorbance at certain intervals by leaving it on bench top. Results were shown in Table No.7.

INTERMEDIATE PRECISION

Assay of drotaverine hydrochloride Tablets in 0.1 N HCl

Three solutions were prepared and three replicates each of the same concentration of Drotaverine hydrochloride about 10µg/ml. the absorbance of all the 9 solutions were recorded at 241 nm. From the absorbance value, the amount of Drotaverine hydrochloride present in each Tablets was calculated**.**

CONCLUSION

The developed UV differential spectrometric method uses 0.1N Hydrochloride as solvent, which is more economical compared to other organic solvents, and the quality of the developed method is accurate, precise and simple, based on the analytical parameters and calculated statistics. The spectrum of Drotaverine hydrochloride Pure can be viewed and the formulation was recorded. Absorption maxima (λmax) were observed at 241 nm. All aliquots were scanned at 241 nm to compare the precision of the proposed method. The Tablet showed a coating in the concentration range of 0112 µg / ml. The proposed method was validated by recovery studies at 50%, 100% and 150%.Plus confirmed%. The recovery percentages were 98.89 w / w, 99.62% w / w. and 99.58% w / w and the mean value is 99.36% w / w. This serves as a good index of the precision and reproducibility of the study. The concentration of 112 µg/ ml was established and the calibration curve of concentration versus absorbance was plotted. It obeys Beer's law and the value of R^2 is 0.99. The developed method shows the SD, RSD at the prescribed (limit value minus 2%) Therefore, the developed method is exact and exact. The results obtained were considered satisfactory in the closed declaration. From this it was concluded that the method is suitable for the confirmation of Drotaverine hydrochloride in its pure form, as well as for its formulation due to the simple, accurate and inexpensive method of routine analysis

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Table No.1: Calibration Data of Drotaverine HCl

S.No.	Concentration (µg/ml)	Absorbance at 241 nm			Mean absorbance
		Trial – I	Trail – II	Trial – III	
1	1	0.0991	0.0986	0.0990	0.0989
2	2	0.1493	0.1491	0.1489	0.1491
3	3	0.1898	0.1891	0.1893	0.1894
4	4	0.2556	0.2551	0.2555	0.2554
5	5	0.3502	0.3500	0.3498	0.3500
6	6	0.3956	0.3953	0.3955	0.3954
7	7	0.4556	0.4552	0.4552	0.4553
8	8	0.5219	0.5216	0.5211	0.5215
9	9	0.5801	0.5800	0.5795	0.5798
10	10	0.6383	0.6381	0.6380	0.6381
11	11	0.6523	0.6522	0.6521	0.6522
12	12	0.7394	0.7394	0.7390	0.7392
13	13	0.782	0.7814	0.7818	0.7817
14	14	0.8358	0.8356	0.8352	0.8355
15	15	0.8964	0.8962	0.8961	0.8962
16	16	0.9790	0.9788	0.9785	0.9787

Table No.2: Absorptivity $A_{1\,cm}^{1\%}$ of Drotaverine in 0.1N HCl

S.No	Concentration (µg/ml)	Concentration in %	Absorbance at 241 nm				Absorptivity $A_{1\,cm}^{1\%}$
			Trial – I	Trail – II	Trial - III	Average	
1	1	0.0001	0.0991	0.0986	0.0990	0.0989	989
2	2	0.0002	0.1493	0.1491	0.1489	0.1491	745.5
3	3	0.0003	0.1898	0.1891	0.1893	0.1894	631.3
4	4	0.0004	0.2556	0.2551	0.2555	0.2554	638.5
5	5	0.0005	0.3502	0.3500	0.3498	0.3500	700
6	6	0.0006	0.3956	0.3953	0.3955	0.3954	659
7	7	0.0007	0.4556	0.4552	0.4552	0.4553	650.5
8	8	0.0008	0.5219	0.5216	0.5211	0.5215	651.8
9	9	0.0009	0.5801	0.5800	0.5795	0.5798	644.2
10	10	0.0010	0.6383	0.6381	0.6380	0.6381	638.1
11	11	0.0011	0.6523	0.6522	0.6521	0.6522	592.9
12	12	0.0012	0.7394	0.7394	0.7390	0.7392	616
13	13	0.0013	0.782	0.7814	0.7818	0.7817	601.3
14	14	0.0014	0.8358	0.8356	0.8352	0.8355	596.7
15	15	0.0015	0.8964	0.8962	0.8961	0.8962	597.4
16	16	0.0016	0.9790	0.9788	0.9785	0.9787	611.6
AVERAGE							660.23

Table No.3: Molar Absorptivity of Drotaverine in 0.1NHCl

S.No	Concentration (µg/ml)	Concentration in M C in M = $\frac{\text{C in µg/ml}}{\text{x } 10^{-3} \text{ Mol. Wt}}$	Absorbance at 241 nm				$\varepsilon_{max} = \frac{\text{Absorbance}}{\text{C (in M) x b (in cm)}}$
			Trial – I	Trail – II	Trial - III	Average	
1	1	2.3×10^{-6}	0.0991	0.0986	0.0990	0.0989	43000
2	2	4.6×10^{-6}	0.1493	0.1491	0.1489	0.1491	32000
3	3	6.9×10^{-6}	0.1898	0.1891	0.1893	0.1894	27449
4	4	9.2×10^{-6}	0.2556	0.2551	0.2555	0.2554	27760
5	5	1.1×10^{-5}	0.3502	0.3500	0.3498	0.3500	31818
6	6	1.3×10^{-5}	0.3956	0.3953	0.3955	0.3954	30415
7	7	1.6×10^{-5}	0.4556	0.4552	0.4552	0.4553	28456
8	8	1.8×10^{-5}	0.5219	0.5216	0.5211	0.5215	28972
9	9	2×10^{-5}	0.5801	0.5800	0.5795	0.5798	28990
10	10	2.3×10^{-5}	0.6383	0.6381	0.6380	0.6381	27743
11	11	2.5×10^{-5}	0.6523	0.6522	0.6521	0.6522	26088

12	12	2.7×10^{-5}	0.7394	0.7394	0.7390	0.7392	27377
13	13	2.9×10^{-5}	0.782	0.7814	0.7818	0.7817	26955
14	14	3.2×10^{-5}	0.8358	0.8356	0.8352	0.8355	26109
15	15	3.4×10	0.8964	0.8962	0.8961	0.8962	26358
16	16.	3.6×10	0.9790	0.9788	0.9785	0.9787	27186
AVERAGE							29167.25

Table No.4: Percentage of Drotaverine Present in 40 mg of each Tablets

S.No	Concentration (µg/ml)	Absorbance at 241 nm				Amount present in each Tablets	Percentage (%)
		Trial – I	Trail – II	Trial - III	Average		
1	2	0.1405	0.1496	0.1479	0.1460	39.84	99.6
2	4	0.2806	0.2806	0.2765	0.2792	39.92	99.8
3	6	0.4179	0.4386	0.4329	0.4314	39.96	99.9

Table No 5: Accuracy of Drotaverine at 50%, 100 % & 150 %

S.NO	Initial amount (µg/ml)	Amount added (µg/ml)	ABSORBANCE	% Recovered
1	2	1	0.1891	98.89
2	4	2	0.3951	99.62
3	6	3	0.5793	99.58
Average				99.36
S.D				1.75
R.S.D				1.3

Table No. 6: Stability of Drotaverine (Standard) in 0.1N HCl

S.No.	Time (In Minutes)	Absorbance of Drotaverine hydrochloride (standard) at 241 nm	
		Absorbance	% Decrease
1	0	0.6400	100
2	15	0.6396	99.93
3	30	0.6396	99.93
4	45	0.6395	99.92
5	60	0.6394	99.90
6	75	0.6393	99.89
7	90	0.6393	99.89
8	105	0.6393	99.89
9	120	0.6396	99.93
10	135	0.6394	99.90
11	150	0.6392	99.87
12	165	0.6390	99.84
13	180	0.6389	99.82

Table No.7: Stability of Drotaverine hydrochloride Tablets (Sample) in 0.1N HCL

S.No.	Time (In Minutes)	Absorbance of Drotaverine hydrochloride (sample) at 241 nm	
		Absorbance	% Decrease
1	0	0.6285	100
2	15	0.6285	100
3	30	0.6282	99.95
4	45	0.6283	99.96
5	60	0.6283	99.96
6	75	0.6281	99.93
7	90	0.6280	99.92
8	105	0.6278	99.88
9	120	0.6275	99.84
10	135	0.6274	99.82
11	150	0.6274	99.82
12	165	0.6273	99.80
13	180	0.6274	99.82

Table No.8: Precision – Intermediate Precision

S.No.	Conc. µg/ml	Analyst	Absorbance 271nm				Amount determined (mg)	Percentage Assay
			Trial 1	Trial 2	Trial 3	Mean		
1	10	JANARTHANAN	0.6379	0.6378	0.6375	0.6377	39.97	**99.93**
2	10	SUNDARARAJAN	0.6377	0.6375	0.6378	0.6376	39.92	**99.92**
3	10	MOOSA ABDUL RAHIM	0.6375	0.6375	0.6577	0.6376	39.92	**99.92**
LABEL CLAIM = 40mg						**AVERAGE =**		**99.92**
SD =								**0.557**
%RSD =								**0.009**

Table No.9: Precision – Interday Precision

S.No.	Conc. µg/ml	Analyst	Absorbance 271nm				Amount determined (mg)	Percentage Assay
			Trial 1	Trial 2	Trial 3	Mean		
1	10	JANARTHANAN	0.6371	0.6372	0.6369	0.6370	39.92	**99.82**
2	10	SUNDARARAJAN	0.6370	0.6369	0.6372	0.6370	39.92	**99.82**
3	10	MOOSA ABDUL RAHIM	0.6369	0.6370	0.6574	0.6371	39.93	**99.84**
LABEL CLAIM = 40mg						**AVERAGE =**		**99.92**
SD =								**0.557**
%RSD =								**0.009**

Table No.10: Precision – Intraday Precision

S.No.	Conc. µg/ml	Analyst	Absorbance 241nm				Amount determined (mg)	Percentage Assay
			Trial 1	Trial 2	Trial 3	Mean		
1	10	JANARTHANAN	0.6369	0.6374	0.6369	0.6371	39.93	**99.84**
2	10	SUNDARARAJAN	0.6371	0.6368	0.6372	0.6370	39.92	**99.82**
3	10	MOOSA ABDUL RAHIM	0.6368	0.6369	0.6571	0.6369	39.92	**99.91**
LABEL CLAIM = 40mg						**AVERAGE =**		**99.92**
SD =								**0.001**
%RSD =								**0.010**

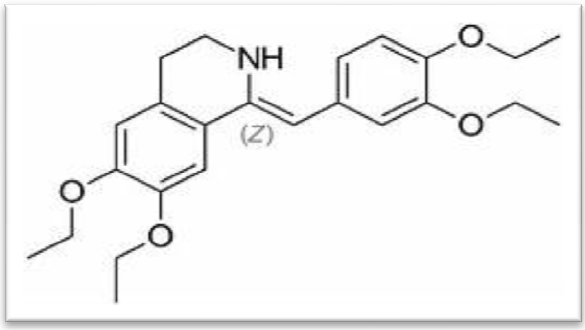

Figure 1: Drotaverine structure

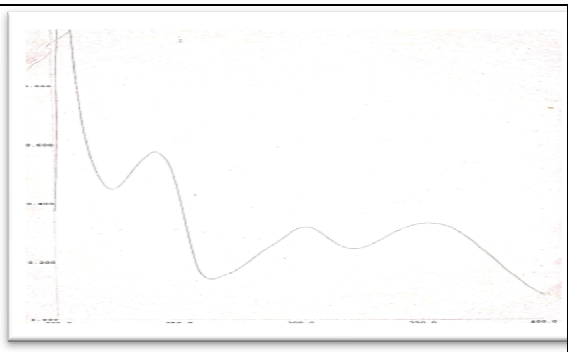

Figure 2: UV – Spectrum of Standard Drotaverine HCl

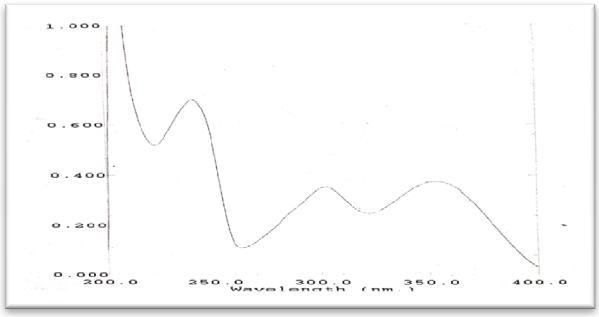

Figure 3 UV – Spectrum of Sample Drotaverine hydrochloride Formulation.

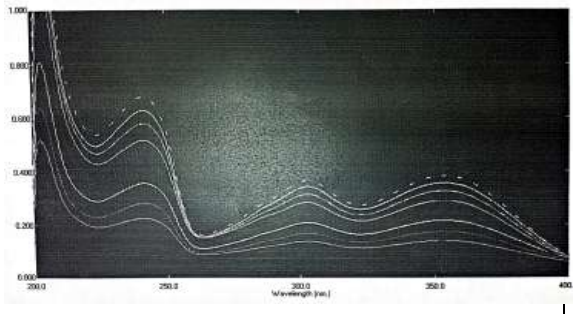

Figure 4. Overlay Spectrum of Standard Drotaverine HCl.

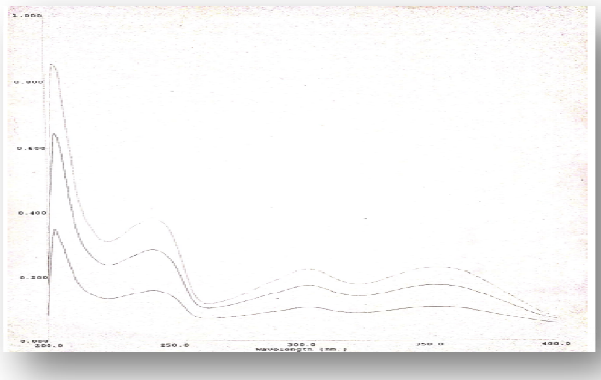

Figure 5. Overlay Spectrum of Formulation Drotaverine HCl Tablet

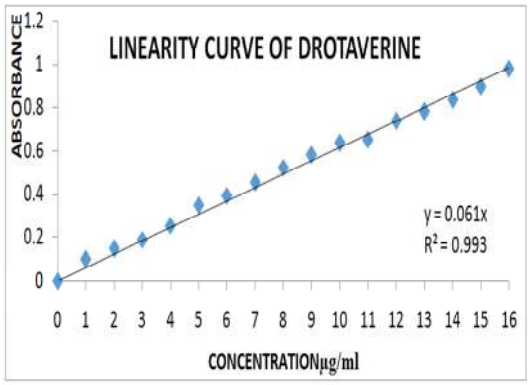

Figure 6. Standard Absorbance Curve of Drotaverine hydrochloride by UV – Method

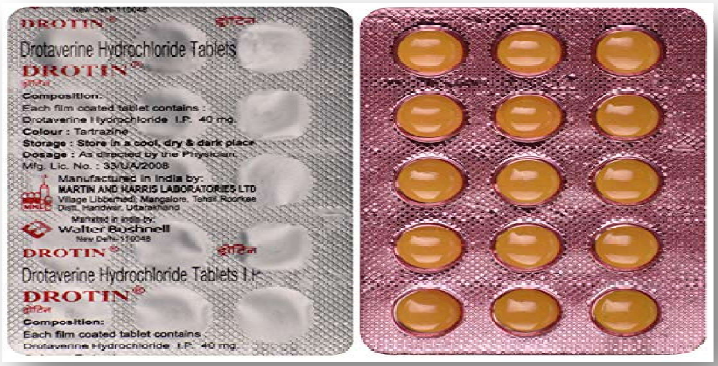

Figure 7. Analysis of Formulation I (Drotin)

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RESEARCH ARTICLE

Prediction of Indian GDP using Grey Forecast Model

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ABSTRACT

The Gross Domestic Product (GDP) of a country is an accurate indicator of the size of its economy. Judgments regarding whether an economy requires strengthening or moderation, under threats such as a recession or rampant inflation can be made based on the GDP. In this paper an implementation of the GM(1,1) model is proposed for the prediction of the Indian GDP. GM (1, 1) model (Grey forecast model) provides an efficient method to predict the future using known information. The statistical data of the Indian GDP between the years 2005 and 2017 is collected and provided as input to the GM(1,1) model to predict the future GDP. The statistical parameters such as small error probability, variance, correlation and regression measures with respect to the predicted data are analysed. It was found that the variance ratio and small error probability lie on Level 1 confirming that the analysis has good forecasting precisions. Further, the significant factors influencing the Indian GDP are analysed and the correlation degree of each factor are calculated and the corresponding regression lines are plotted. A comparative analysis is also made between the predictive and original GDP of India. The impact of COVID-19 pandemic in Indian GDP is also discussed.

Keywords: Grey forecast model; GDP; Variance; Correlation; Regression

INTRODUCTION

The Gross Domestic Product (GDP) of a country is an accurate indicator of the size of its economy. GDP is one of the most widely used single standard indicators to indicate the economic performance of a country. Calculating GDP is a complex process and countries across the universe adopt different methods to obtain their GDP. The social welfare of a country is determined by its GDP, if the GDP of the country is high, then the standard of living in that country will also be high. Many factors determine the economic growth of a country. Natural resources and technology may

boost the economic growth as this might increase the production activity curve of a country. Judgments regarding whether an economy requires strengthening or moderation, under threats such as a recession or rampant inflation can be made based on the GDP. A growing population can lead to higher workforces, boosting the economy of the country. On the downside having a large population leads to unemployment, stunting the economic growth. This effect of population on GDP of India has been analysed in detail by Palvi et al. [1] where the authors considered the change in GDP as dependent variable and population and inflation as independent variables. Poor health and low levels of education can lead to less productivity in various fields leading to a shift in the GDP. Many authors, studied the various factors that affect the GDP of a country and used different mathematical methods to find the impact of those factors on GDP. For example, Dhiraj et al. [2] studied the impact of various macroeconomic factors on GDP components using multiple regression analysis. Rudrani et al. [3] used principal component augmented time varying parameter regression approach to forecast growth rates for India with respect to trade and production as side-specific variables. Lall [4] studied the role of multinational enterprises in industrial development whereas Alfaro [5] analysed the impact of foreign direct investment in financial markets and economic growth. For further study on GDP of various countries readers may refer to Hansen [6], Srinivasan [7] and Wan [8].

In this paper, GM (1,1), a one variable, first-order grey model is used to predict Indian GDP. The statistical procedures followed in this model provides an effective method to predict uncertain systems with small samples and poor information. GM (1,1) model is widely used in many fields of science and engineering. For instance, Hui et al. [9] used GM (1,1) to study the growth of Japanese Larch and obtained the mean tree height. Yang et al. [10] predicted the incidence trend of typhoid and paratyphoid fevers in Wuhan City, China by employing GM (1,1) method. Li and Zhang [11] proposed an improved GM (1,1) model to predict the total energy consumption of Shanghai City in China and concluded that Shanghai's total energy consumption will increase in the forthcoming years. Chiu et al. [12] developed a multivariate grey prediction model (MGPM) for CO_2 emissions and employed neural-networks to adjust the predicted values obtained from the proposed MGPM. To analyse the trend of Taiwan's e- paper industry Huang et al.[13] adopted the three traditional grey models, GM (1,1), DGM (2,1) and Verhulst model. They compared the residual errors of the predicted series of all these models and determined the best forecasting method. Recently Liu et al. [14] proposed a new grey model based on fractional-order grey model and Verhulst model and obtained the final predictive result by using fractional-order GM(1,1) model and Verhulst model by weighting coefficients.A multivariate grey model optimized by a genetic algorithm was employed by Ye et al. [15] to analyse the carbon intensity forecasting in the Pearl river delta region of China. Urrutia et al. [16] compared the predictions obtained by Markov-Chain Grey Model with GM (1,1) and found that the Markov-Chain Grey Model is more precise than GM (1,1) forecasting model for analysing the electricity consumption in the Philippines.

Even though GM(1,1) model is widely used in many fields, in the literature it has been shown that GM(1,1) model is more accurate for the exponential data series than data series of saturation. Since the GDP of most of the countries is an increasing sequence, we adopt GM (1,1) model to predict the GDP of India by considering the following factors. Private Final Consumption Expenditure(PFCE) which includes, expenditure on durable goods, expenditure on non-durable goods and expenditure on services. Expenditure on various administrative schemes, welfare schemes, education etc. are considered under Government Final Consumption Expenditure (GFCE). In Gross Domestic Capital Formation (GDCF), investment on government projects and infrastructure are taken into account. Finally, net imports and net exports are also considered for the calculation of Indian GDP using GM (1,1) model. In this paper, the GDP of India for future years is predicted by employing Grey forecast methodology. The data for Indian GDP for the years 2005 to 2020 are collected from online sources and using the GDP values of the years 2005 to 2017, the GDP of India till 2022 has been predicted. It is of great interest for the economists over the world to analyze the GDP of various countries during the Covid-19 Pandemic. Monirul Islam et al. [17] analyzed the GDP of South Asian countries and concluded that India will be affected seriously followed by Bangladesh and Pakistan in terms of the GDP value. The authors also specify that the level of manufacturing of light/heavy products in India is large and hence the impact of COVID-19 outbreak in India will affect the economy five to eight times higher than the normal

situation.This is clearly reflected in the outcome of this paper since the actual Indian GDP of 2020 is 25,92,58,000 million dollars while the predicted GDP using GM (1,1) model is 32,24,40,000.

Basic Principles of GM (1,1) model

GM (1,1) model is a dynamic model that has been widely used for prediction in many research fields including finance, agriculture, transportation, economy and so on. This model provides a very effective method to determine the unknown information by using known information. The grey system prediction only uses little data to make quantitative forecast of the future. Here we first introduce the basic concept of Grey system and the algorithm of Grey Model-GM (1,1)(see Yanjun Li [18]).

The GM (1,1) model is illustrated below:

Let us consider the original data sequence composed by n elements,

$$x^{(0)} = \left[x^{(0)}(1), x^{(0)}(2), \dots, x^{(0)}(n)\right]$$

Generate the new data sequence

$$x^{(1)} = \left[x^{(1)}(1), x^{(1)}(2), \dots, x^{(1)}(n)\right]$$

so that$x^{(1)}(k) = \sum_{i=1}^{k} x^{(0)}(i)$, $k = 1,2,\dots,n.$

The derivative of the data sequence $x^{(1)}$ is defined as follows:

$$dx^{(1)}(k) = x^{(0)}(k) \ = x^{(1)}(k) - x^{(1)}(k-1),$$

and the mean value data sequence of the data $x^{(1)}$ is

$$z^{(1)} = \left[z^{(1)}(2), z^{(1)}(3), \dots, z^{(1)}(n)\right]$$

such that$z^{(1)}(k) = \frac{1}{2}x^{(1)}(k) + \frac{1}{2}x^{(1)}(k-1), k = 2, \dots, n.$

Hence the grey differential equation of GM (1,1) can be written as:

$dx^{(1)}(k) + az^{(1)}(k) = b$ or $x^{(0)}(k) + (az)^{(1)}(k) = b,$

where $x^{(0)}(k)$ is called the grey derivative, a is the evolution parameter, $z^{(1)}(k)$ is the white background valueand b is the grey effect amount.The above system of equations canbe represented as

$$\begin{bmatrix} -z^{(1)}(2) & 1 \\ \dots & \dots \\ -z^{(1)}(n) & 1 \end{bmatrix} \begin{bmatrix} a \\ b \end{bmatrix} = \begin{bmatrix} x^{(0)}(2) \\ \dots \\ x^{(0)}(n) \end{bmatrix}$$

and hence the GM(1,1) model can be written as : $Au = B,$

where $A = \begin{bmatrix} -z^{(1)}(2) & 1 \\ \dots & \dots \\ -z^{(1)}(n) & 1 \end{bmatrix}, u = \begin{bmatrix} a \\ b \end{bmatrix}$ and $B = \begin{bmatrix} x^{(0)}(2) \\ \dots \\ x^{(0)}(n) \end{bmatrix}.$

Now the least square method is used to calculate parameter vector u as

$u = [ab]=(A^T A)^{-1}A^T B,$

then substituting the calculated values, the time response equation is obtained as

$x^{\wedge(1)}(k+1) = \left[x^{(1)}(1) - \frac{\hat{b}}{\hat{a}}\right]e^{-\hat{a}k} + \frac{\hat{b}}{\hat{a}},$

Restore to the original data sequence we get,

$x^{\wedge(0)}(k+1) = x^{\wedge(1)}(k+1) - x^{\wedge(1)}(k) = (1-e^{\hat{a}})[x^{(1)}(1) - \frac{\hat{b}}{\hat{a}}]e^{-\hat{a}k}$------(1)

which is used to predict the future data.

Testing of GM(1,1) Model

The original data sequence is $x^{(0)} = \{x^{(0)}(1), x^{(0)}(2), \dots . x^{(0)}(n)\}$.

The forecast data sequence of GM (1, 1) model is

$x^{\wedge(0)} = \{x^{\wedge(0)}(1), x^{\wedge(0)}(2), \dots . x^{\wedge(0)}(n)\}$.

The following are the error data sequences

1. Absolute error data sequence is:

$\{x^{\wedge(0)}(1) - x^{(0)}(1), x^{\wedge(0)}(2) - x^{(0)}(2), \dots , x^{\wedge(0)}(n) - x^{(0)}(n)\}$;

2. Residual data sequence is:

$\{\varepsilon\varepsilon(1), \varepsilon\varepsilon(2), \dots \varepsilon\varepsilon(n)\} = \{x^{(0)}(1) - x^{\wedge(0)}(1), x^{(0)}(2) - x^{\wedge(0)}(2), \dots , x^{(0)}(n) - x^{\wedge(0)}(n)\}$;

3. Relative error data sequence is:

$\{\frac{x^{\wedge(0)}(1) - x^{(0)}(1)}{x^{(0)}(1)}, \frac{x^{\wedge(0)}(2) - x^{(0)}(2)}{x^{(0)}(2)}, \dots . \frac{x^{\wedge(0)}(n) - x^{(0)}(n)}{x^{(0)}(n)}\}$

4. The standard deviation of original data sequence is:

$S_1 = \sqrt{\frac{1}{n}\sum_{k=1}^{n}(x^{(0)}(k) - \bar{x})^2}$, where $\bar{x} = \frac{1}{n}\sum_{k=1}^{n} x^{(0)}(k)$

5. The standard deviation of absolute error data sequence is:

$S_1 = \sqrt{\frac{1}{n}\sum_{k=1}^{n}(\varepsilon(k) - \bar{\varepsilon})^2}$, where $\bar{\varepsilon} = \frac{1}{n}\sum_{k=1}^{n} \varepsilon(k)$

6. The standard deviation ratio of original data and absolute error data sequence is

$\frac{S_1}{S_2} = c$.

The inspection level standards of GM (1, 1) model precision are shown in the following table

For a given number c_0, when $c < c_0$ this model is called mean square deviation qualified model. Similarly, for a given number P_0, when $P > P_0$ this model is called small error qualified model where $P = P\left[\left|\xi(k) - \bar{\xi}\right| < 0.6745 \times S_1\right]$.

Prediction of Indian GDP Using GM (1, 1) Model

In this section, the GDP of India till the year 2022 is predicted using the current data using GM (1, 1) method. The statistical data of GDP of India for the years 2005 to 2020 are taken from the website [19,20] and is displayed in table 2. The Central Statistics Office (CSO), under the Ministry of Statistics and Program Implementation, is responsible for macroeconomic data gathering and statistical record keeping. The CSO coordinates with various federal and state

government agencies and departments to collect and compile the data required to calculate the GDP and other statistics. To predict the unknown GDP we use the GDP of the first 13 years from the following table 2.

Through the total data of Indian GDP, the original data sequence is represented as follows:

$x^{(0)}$ = {834218,949118,1238700,1224100, 1365370, 1708460, 1823050, 1827640, 1856720, 2039130, 2103590, 2289750, 2652250}

The mean value data sequence $z^{(1)} = [z^{(1)}(2), z^{(1)}(3), \dots, z^{(1)}(n)]$ is calculated as

$$z^{(1)} = \{1308777, 2402686, 3634086, 4928821, 6465736, 8231491,$$

$$10056836, 11899016, 13846941, 15918301, 18114971, 20585971\}$$

Now, the newly formed sequences can be arranged in the matrix form as $Au = B$.

Using the method of least squares in MATLAB we get u as

$$u = \begin{bmatrix} -0.121 \\ 22715.943 \end{bmatrix}$$

The time response equation is:

$\hat{x}^{(1)}(k+1) = \left[x^{(1)}(1) - \frac{\hat{b}}{\hat{a}}\right] e^{-\hat{a}k} + \frac{\hat{b}}{\hat{a}} = 1308777\, e^{1.0211k} - 13582000;$

Now, by using the time response equation the predictive GDP is obtained by simulation in MATLAB R2016b. The simulated value $\hat{x}^{(1)}(k+1)$ and the total predictive value of Indian GDP are shown in the table 3.

The absolute error sequence, the relative and the residual error sequences are given in Table 4.

The standard deviation of the original data sequence, S_1 = 515190.

The standard deviation of the absolute error data sequence, S_2 =105260.

The variance ratio $c = \frac{S_1}{S_2}$ = 0.2043.

Comparing the above values with actual data, we can find that the error is small enough that Grey Model-GM(1,1) can be actually used.

Now the small error probability ($P = P[|\varepsilon(k) - \bar{\varepsilon}| < 0.6745 \times S_1]$) can be calculated as follows:

For the data under consideration $\bar{\varepsilon}$ = 3029.23 and $(\varepsilon(k) - \bar{\varepsilon})$ = 185030.77 which is less than 0.6745 × S_1. Hence the small error probability is 1 > 0.95 and it falls under the category Level-1(refer Table-1).

The above results confirm that the model has good forecasting precisions and is useful to forecast the GDP of India. Indian GDP for the forth coming years is predicted using GM(1,1) model and the results are presented in the following table 5.

The scatter diagram of predictive GDP

In the graph (Fig.1) the $x - axis$ denotes the years and the y-axis denotes the GDP values (unit: one hundred million dollars). The red curve represents the original GDP of India and the blue curve represents the GDP predicted by the GM (1,1) model. It is observed that GM (1,1) model predicts the GDP with high accuracy.

Analysis with Different Factors

The following section analyses the five significant factors that influence the Indian GDP. The data is taken for the same period as in the previous section. The important factors that we consider are x_0(Export), x_1(Import), x_2(gross

domestic capital formation), x_3(government final consumption expenditure) and x_4(private final consumption expenditure). The Grey correlation coefficient between x_0 and x_1, x_0 and x_2, x_0 and $x_3$and x_0 and $x_4$are calculated. This leads us to analyse the significance of x_0 with respect to the rest of the factors which play a major role in the calculation of Indian GDP. We use the non-dimensional scaling to make the calculation simpler.The results after dividing each data sequence are given in the following table 7.

The Correlation Test

The correlation degree of any two sequence of the GM (1,1) model is R_i and the formula used to calculate the correlation coefficient is

$R_i = \frac{1}{n}\sum_{i=1}^{n} \xi_n(i)$, where $\xi_n(i) = \frac{min_{1\le i\le n}|x_0(i)-x_n(i)|+\rho\, max_{1\le i\le n}|x_0(i)-x_n(i)|}{|x_0(i)-x_n(i)|+\rho\, max_{1\le i\le n}|x_0(i)-x_n(i)|}$

here ρ ($0 < \rho < 1$) is called the resolution factor and we choose $\rho = 0.5$.

Using the table 8, the correlation degree of each factor is calculated and their values are $R_1 = 0.59$, $R_2 = 0.59$, $R_3 = 0.72$ and $R_4 = 0.6$. The maximum difference in the correlation degree of the components that are taken for study is less than or equal to 0.13. Hence, we have $0 < |R_i - R_j| \le .13$. Also the regression lines showing the dependency of x_0 with respect to the rest of the factors are given in Fig.2.

The figure 2 shows that there is a gradual growth between the export x_0 and the import x_1 and also, we can note that the same trend is followed between the export $x_0$and the gross domestic capital formation x_2. Further the graph shows that there is a good balance between the export x_0 and the government final consumption expenditure x_3. Also, there is a greater increase in the private final consumption expenditure x_4 compared to the other factors.

Impact of Covid-19 on Indian GDP

The economic impact of the coronavirus pandemic in India has been largely disruptive. In this paper we have considered various factors that affect the GDP of a country like export, import, Gross Domestic Capital Formation, Government Final Consumption Expenditure and Private Final Consumption Expenditure. We used the data of Indian GDP of the year 2005 to 2017 and predicted the GDP of India for the year 2005 to 2022. Due to the fall in the international markets because of the Covid-19 pandemic there is a decline in the exports of goods like chemicals, textiles, engineering goods, etc. whose demand has come down during this pandemic situation. As unemployment in the country has increased due to the Covid-19 pandemic the public couldn't invest money in assets which leads to a fall in the Gross Domestic Capital Formation (GDCF).Government Final Consumption Expenditure (GFCE) amount is dependent on investments sectors like defense, education and housing which are highly impossible to afford during this pandemic time. The expenditures of resident households and non-profit institutions serving households on goods and services are considered as Private Final Consumption Expenditure (PFCE). The production of products was halted due to lack of labors and raw materials which in turn lead to the high demand of daily essential. Due to the high demand and low supply, the wholesalers and distributors were unable to meet the requirements. All the above factors have affected the country's GDP and a huge drop in GDP has been observed. In our work the predicted GDP is 34,76, 10,000 million dollars for the financial year 2021 however as per the results by National statistical office under ministry of statistics the GDP of Q1 2021 is 26,89,55,600million dollars. The GDP of India has fallen down by 23.9%because of covid-19 pandemic. To recover this situation a strong leadership effort will be required from the government.

CONCLUSION

In this paper, the GDP from the year 2005 to 2017 is provided as input to the GM (1,1) model and the GDP from 2005 to 2022 is calculated. A detailed discussion in predicting and analysing the Indian GDP and the factors that influence the Indian GDP are also analysed. It has been observed that there is no significant difference between the original

GDP and the calculated GDP for the years 2005 to 2019 whereas the original GDP declined significantly in the year 2020 because of Covid-19 pandemic. As export is one of the important factor that influences the GDP of India, the correlation between export and other factors like Import, gross domestic capital formation, government final consumption expenditure and private final consumption expenditure are determined. It was found that the maximum difference between the correlation coefficients of these factors is positive (0.13). This confirms that export has direct impact on all other factors. Hence this study shows that GM(1,1) model is effective in the calculation of Indian GDP. The limitations of this study are, the GM (1,1) model is more efficient for an increasing or a decreasing data sequence than a non-monotonic sequence like GDP of a country. In future, similar prediction analysis can be carried out using Markov-chain Grey model or a second order Grey forecast model.

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Table 1 Inspection Level Standards of GM (1, 1) Model

Precision grade	relative error	The standard deviation ratio c_0	small error probability P_0	Corresponding correlation degree ρ
Level 1- very good	0.01	<0.35	>0.95	0.90
Level 2-qualified	0.05	<0.50	>0.80	0.80
Level 3- reluctant	0.10	<0.65	>0.70	0.70
Level 4- unqualified	0.20	≥0.65	≤0.70	0.60

Table 2Indian GDP from 2005 to 2020 (unit: one hundred million dollars)

Year	**2005**	**2006**	**2007**	**2008**
GDP	834218	949118	1238700	1224100
Year	**2009**	**2010**	**2011**	**2012**
GDP	1365370	1708460	1823050	1827640
Year	**2013**	**2014**	**2015**	**2016**
GDP	1856720	2039130	2103590	2289750
Year	**2017**	**2018**	**2019**	**2020**
GDP	2652250	2713170	2868930	2592580

Table 3 Predictive GDP

Year	Original GDP	$\hat{x}^{(1)}(k+1)$	Predictive GDP
2005	834218	834218	834200
2006	949118	1960000	1125500
2007	1238700	3173000	1213400
2008	1224100	4481000	1308200
2009	1365370	5892000	1410300
2010	1708460	7412000	1520400
2011	1823050	9051000	1639100
2012	1827640	10818000	1767100
2013	1856720	12723000	1905000
2014	2039130	14777000	2053800
2015	2103590	16991000	2214100
2016	2289750	19378000	2387000
2017	2652250	21951000	2573300

Table 4 Error Sequences

Year	Original GDP	Predictive GDP	Absolute Error	Residual Error	Relative Error
2005	834218	834200	0	0	0
2006	949118	1125500	176430	-176430	0.1859
2007	1238700	1213400	-25280	25280	-0.0204
2008	1224100	1308200	84060	-84060	0.0687
2009	1365370	1410300	44920	-44920	0.0329
2010	1708460	1520400	-188060	188060	-0.1101
2011	1823050	1639100	-183950	183950	-0.1009
2012	1827640	1767100	-60570	60570	-0.0331
2013	1856720	1905000	48320	-48320	0.0260
2014	2039130	2053800	14640	-14640	0.0072
2015	2103590	2214100	110530	-110530	0.0525
2016	2289750	2387000	97240	-97240	0.0425
2017	2652250	2573300	-78900	78900	-0.0297

Table 5 Predictive GDP of India

Year	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Original GDP	834218	949118	1238700	1224100	1365370	1708460
Predictive GDP	834200	1125500	1213400	1308200	1410300	1520400
Year	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Original GDP	1823050	1827640	1856720	2039130	2103590	2289750
Predictive GDP	1639100	1767100	1905000	2053800	2214100	2387000
Year	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Original GDP	2652250	2713170	2868930	2592580		
Predictive GDP	2573300	2774300	2990900	3224400	3476100	3747500

Table 6 Data of Different Factors (all figures are given in crore)

Year	Export x_0	Import x_1	Gross domestic capital formation x_2	Government final consumption expenditure x_3	private final consumption expenditurex_4
2005	5724	7415	240580	1,85,266	1925592
2006	6918	8933	281995	1,95,677	2089852
2007	7623	11310	324020	2,00,941	2270688
2008	11200	18790	382431	2,15,116	2479686
2009	17640	30550	429285	2,52,927	2656483
2010	16820	27430	449686	3,04,671	2855920
2011	20110	32700	467071	3,18,059	3105677
2012	29940	46140	471431	3,35,659	3351265
2013	29840	50040	674878	31,79,479	5179091
2014	31320	46750	716140	35,23,380	5557329
2015	31820	46290	774388	39,26,363	5912657
2016	31030	44790	906101	44,56,911	6351137
2017	26230	38100	980209	51,84,837	6812334

Table 7 Non-Dimensional Scaling

	2005	2006	2007	2008	2009	2010	2011
x_0	1.00	1.21	1.33	1.96	3.08	2.94	3.51
x_1	1.00	1.20	1.53	2.53	4.12	3.70	4.41
x_2	1.00	1.17	1.35	1.59	1.78	1.87	1.94
x_3	1.00	1.06	1.08	1.16	1.37	1.64	1.72
x_4	1.00	1.09	1.18	1.29	1.38	1.48	1.61
	2012	2013	2014	2015	2016	2017	
x_0	5.23	5.21	5.47	5.56	5.42	4.58	
x_1	6.22	6.75	6.30	6.24	6.04	5.14	
x_2	1.96	2.80	2.98	3.23	3.77	4.07	
x_3	1.81	17.16	19.02	21.19	24.06	27.99	
x_4	1.74	2.69	2.89	3.07	3.30	3.54	

Table 8. The Correlation Test

$\xi_1(i)$	1.00	0.99	0.79	0.57	0.43	0.50	0.46
$\xi_2(i)$	1.00	0.98	0.99	0.82	0.56	0.61	0.51
$\xi_3(i)$	1.00	0.99	0.98	0.94	0.87	0.90	0.87
$\xi_4(i)$	1.00	0.94	0.92	0.72	0.51	0.55	0.48
$\xi_1(i)$	0.44	0.33	0.48	0.53	0.55	0.58	
$\xi_2(i)$	0.33	0.40	0.40	0.41	0.50	0.76	
$\xi_3(i)$	0.77	0.49	0.46	0.43	0.39	0.33	
$\xi_4(i)$	0.34	0.41	0.41	0.41	0.45	0.63	

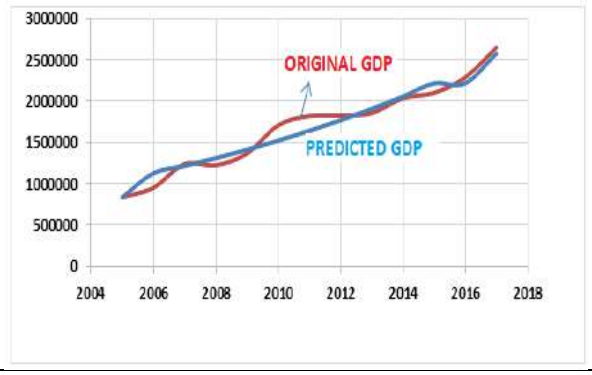

Fig.1.Predictive GDP of India

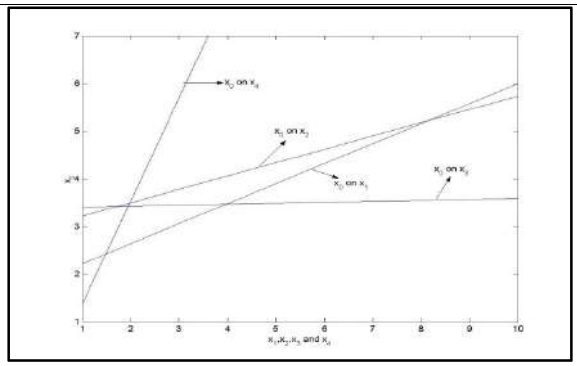

Fig.2.Regression lines of $x_0$on x_1, x_0 on x_2, x_0 on x_3 and x_0 on x_4

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RESEARCH ARTICLE

Effect of Matrix Rhythm Therapy and Myofascial Release on Pain and Disability Patients with Periarthritis Shoulder

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ABSTRACT

Periarthritis encounters many adults and older adults. This is one of the common shoulder conditions which affects shoulder movement and causes Disability. Although there are many managements for the periarthritis shoulder, none of them are found effective. Matrix rhythm therapy is a recent technique that gives vibrations that enhance functions and reduce pain. Studies are much limited with the use of matrix rhythm therapy. So, this study is conducted to identify the effect of matrix rhythm therapy versus myofascial release therapy on pain and Disability in periarthritis of the shoulder. This study is an experimental study with fifty participants referred to the physiotherapy department with complaints of shoulder pain and diagnosed as periarthritis shoulder. A clear explanation about the study was given to all the subjects, and all were divided into two equal groups by a computer-assisted method. In Group A, subjects underwent Matrix rhythm therapy for 15 minutes, followed by a range of motion exercises for 10 minutes. Group B subjects underwent Myofascial release therapy for 15 minutes, followed by a range of motion exercises for 10 minutes. The study was conducted three days a week for six weeks. The subjects underwent a total of 18 sessions. The study uses outcomes as pain and Disability. The pain was assessed using 10- cms visual analog scale, and disability was assessed using the DASH questionnaire. This study result shows about 35% of pain improvement between the groups and 70% of improvement on the shoulder disability between the groups. The student 't' value of the pain scale shows 8.64 ± 1.06, and the DASH is 23.81 ± 14.11, which is more than the table value. This study concluded that the application of

the Matrix rhythm therapy significantly improves the pain and disability compared with myofascial release therapy.

Keywords: Periarthritis Shoulder, Matrix Rhythm Therapy, Myofascial release therapy, Pain, DASH, Functional disability.

INTRODUCTION

Shoulder disorders are the third most common musculoskeletal injuries seen in general practice [1]. Shoulder-related problems significantly affect mobility and produce disability in general populations [2]. Periarthritis shoulder affects around 3%—5% of the general population and 20% of diabetic patients [3]. Shoulder disorders usually present with the symptoms of pain, stiffness, and restriction of motion in all planes may be with or without trauma [4]. Periarthritis (PA) or Adhesive capsulitis or Frozen shoulder is characterized by the gradual development of restriction of the shoulder motion with non-specific radiographical findings [5]. The actual cause for the PA is unknown, usually seen following trauma, hyperthyroidism, CVA, diabetes, or overuse [6]. This condition usually affects unilaterally with the inability to move the shoulder. PA causes stiffness around the shoulder, which adversely affects daily living activities and consequently impairs the quality of life [7]. Few works of literature support that PA shoulder resolves in 3 years, but around 20—50% of patients have long-lasting symptoms [8,9].

PA shoulder involves the rise of pain and disability in the upper limb, which lowers the quality of life. There is fibrosis in the glenohumeral joint capsule, which is accompanied by progressive stiffness of the shoulder joint, which restricts the range of motion [10]. It has a significant impact on the quality of life in the patients, which causes functional limitations in every stage of diseases [11]. A typical symptom consists of the restriction of the passive and active range of motions, and the first movement affected is the external rotation and abduction of the shoulder [12]. In general, there are a stage and severity in self-limiting, interfering in daily living, work, and leisure activities [13]. Management of the PA shoulder depends on the patient-specific and condition-specific. There are no universal algorithms in management. The main goal is to restore the shoulder range of motion, reduce the pain and promote the functional independence of the joint [14]. Physical modalities play a significant role in reducing pain, but there is still a lack of evidence on which modalities are helpful. Early mobilization within the painful limits is also recommended; however, the frequency and duration are controversia [l6]. Since PA shoulder remains an unsolved clinical problem, and no present physiotherapy treatment is universally accepted and effective. There is a need for solid research and the development of new strategies in management.

Matrix rhythm therapy (MaRhyThe) is a new innovative technique described by Dr. UG Randoll. This is applied using a simple equipment, it produces a combination of both mechanical and magnetic pulse with an electrically powered oscillator which provides a dynamic frequency of 8—12 Hz [15].This technique ensures an adequate supply of oxygen and nutrients to the cells and removes the metabolites and end products. It reaches the deeper tissue layers in the mild, specific, and guided pain freeway. It helps in restoring excellent tissue resonances. The oscillator produces the lifting action as a horizontal micro extension movement transferred to the inner organs, tissues, and bones [16]. Myofascial release therapy (MFR) is one of the soft tissue techniques which aids in treating soft tissue dysfunctions and removing tightness and restrictions [17]. MFR acts on the superficial and deeper fascial structures and reduces the limitations in the fascia, which causes tightness around the joint. Studies have supported that MFR plays an essential role in shoulder dysfunctions [18,19]. Studies comparing Myofascial release therapy with Matrix rhythm therapy are not much available. MaRhyThe as a treatment for shoulder dysfunction is also very less as this is one of the recent advancements in physiotherapy many have not understood the effectiveness. The effectiveness of MaRhyThe needs to be proved and needs to be addressed. So, the study aims to compare the effect of the MaRhyThe with MFR on pain and disability in individuals with peri arthritis shoulder.

MATERIALS AND METHODS

The study is an experimental study that collects the samples who visit the physiotherapy department with complaints of shoulder pain were included based on the selection criteria. A clear explanation about the study was given to all the subjects. Those who were willing to accept for the study, were noted down and given an appointment schedule. All the accepted subjects were screened for age, history, pain scale, shoulder disability, and quality of life. A precise selection criterion is framed, and the subjects were selected based on that, 45—60 years of age, both gender, pain in the shoulder for more than three months, Unilateral PA shoulder, Range of motion is not affected more than 50 % when compared with the standard shoulder, Pain scale is less than 6 (moderate pain) in the visual analog scale. The subjects who were not selected were with Diabetes, hypertension, any recent injury to the shoulder, old fracture around the shoulder, secondary PA shoulder, subjects who underwent regular treatment. The institutional ethical committee approved this study, and written consent was obtained from every subject before the beginning of the study. Fifty-five subjects that blinded evaluator selects were included based on the selection criteria. Random allocation of the participants was done with the computer-assisted method. Twenty-seven subjects were divided into two groups equally. In Group A, subjects underwent Matrix rhythm therapy for 15 minutes, followed by a range of motion exercises for 10 minutes. In Group B, subjects underwent Myofascial release therapy for 15 minutes, followed by a range of motion exercises for 10 minutes. The study was conducted three days a week for six weeks. A total of 18 sessions was undergone by the subjects. The study uses outcomes as pain and disability. The pain was assessed using 10-cms visual analog scale, and disability was assessed using the DASH questionnaire.

Matrix rhythm therapy was applied as described by Bhartiya 2017, and this was applied by a trained physiotherapist with certification in Matrix rhythm therapy. Subjects lie down in supine position with the arm abducted to the maximal and the MaRhyThe applied to the anterior aspect of the shoulder region (pectoral muscles, anterior fiber of deltoid muscle & biceps muscle). The next is to position the patient in the side-lying with armrest over the body, MaRhyThe applied over the lateral aspect of the shoulder to stimulate the middle fibers of the deltoid, and arm abduction applied to the inferior aspect of the shoulder region, i.e., Armpit or axillary region (above & below Armpit). Subject in prone lying with the arm abducted to the available Range. MRT applied to the posterior aspect of the shoulder region, including neck and upper back region (posterior fiber of deltoid muscle, supraspinatus & infraspinatus muscle, tricep muscle, latissimus dorsi muscle, serratus anterior muscle, and trapezius muscle from occiput to spine of the scapula.) 15 minutes to each position [20]. Myofascial release therapy was applied by the senior therapist who practiced the MFR for ten years. Subject positioned in supine, start with arm pull in abduction to the end range pull by the therapist by grasping the subject's wrist and hand. The stretch has to be held for 90 seconds. Focused stretch was given along with arm pull where there are restrictions in the arm abduction; during the movement, the subtle facial restrictions were identified and was released. Muscles focused on the release are Pectoralis major and minor, deltoid, subscapularis, and trapezius. Five repetitions were given for each technique, and a stretch was held for 90 seconds [17]. This study technique was carried out by the two senior physiotherapists who obtained certification in each technique. A piece of strong home advice was given to all the subjects. Each group has two subjects who are not continuing the treatment, so this study was completed with 25 subjects in each group. The blinded assessor collects the outcome data on day one and at the end of the study (6th week). All the data were analyzed using SPSS 20.0. The level of significance for this study is fixed as $\alpha < 0.05$.

RESULTS

All the collected data were evaluated using SPSS 20.0 and with MS excel. The effect of the groups was calculated through statistical analysis, and the demographical characteristics are displayed in Table I. The difference between the interventions was examined using the Student t-test, as shown in Table II& Table III. It was shown that the critical value is $p<0.05$. When comparing the pain values using the VAS within the groups, there was a significant difference obtained. The mean of the post-test values of the groups is not equal to the μo, and there was a good effect

size noted on both the post-test values like 1.92 in group A and 3.63 in group B. This shows that there were significant differences noted between the average and μo. Between-group comparison on the pain values shows that there is a marked difference obtained in group A when compared with group B. The effect size on pain was noted with Cohen's is 3.356. Within group comparison of the disability using the DASH, the scale shows a marked difference between the pre-test and post-test values. The observed post-test mean values of groups are not equal to the μo, so it was identified as there is significance noted on the effect size; the post mean values are 83.4 in group A, and 55.45 in group B shows there is a difference. This shows a marked difference between the average and μo. Between-group comparison on the disability shows that there is a marked difference obtained in group A when compared with group B. The effect size on pain was noted with Cohen's is 5.34. The findings of this study show that the magnitude of the difference between the average and is μ0 large, which shows there is an obvious difference obtained between the interventions.

DISCUSSION

The purpose of the study was to compare the effect of the MaRhyThe with MFR on pain and disability in individuals with peri arthritis shoulder. Periarthritis shoulder is a condition that is characterized by pain and limitation of range of motion [21]. It is unclear why there is an inflammation around the capsule and causes the PA [22].When the capsule is stretched, the adherent capsule causes pain when stretched suddenly and produces the mechanical restrain to the motions [7]. MaRhyThe is a relatively less explored model in the physiotherapeutic modality. It aids in improving the active and passive movements in the shoulder and improving sensory function [23]. MaRhyThe activates and rebalances the specific vibrations in the skeletal muscles and the nervous system [20]. A similar study was conducted by Naik et al., which showed that MaRhyThe improves the range of motion and reduces pain. It also releases the blocked neuromuscular process and restores the sympathovagal balance. MaRhyThe cause vibratory effects, which help to increase the lymphatic venous perfusion of the extracellular space in which an anti edematous product originates [20]. Improved circulation in the treatment areas receives oxygenated blood and encourages ATP synthesis, inductively relaxing the contracted muscles [24].

MaRhyThe promotes physiological metabolisms in the intercellular and the extracellular levels by maintaining the normal pH of the tissues by the micro mobilization. There is an increase in microcirculation within the tissues, which enhances the removal of metabolic waste products, reduces the edema, and improves the extensibility in the soft tissues [25]. Myofascial release reduces the fascia restrictions in the tissues, and it eases pressure in the fibrous bands of the connective tissues or fascia. The myofascial release's gentle and sustained stretching is believed to free adhesions and softens and lengthen the fascia [26]. Reducing the fascial restrictions improves circulation and improves the nervous system transmission. MFR elongates fascia and relaxes the tissues, enhancing the range of motion, flexibility, and pain reduction [27]. This study compares the effect of MaRhyThe with the myofascial release, which identifies that the MaRhyThe is produce superior results when compared with the MFR. When analyzing within groups, it shows that both the group results significantly. There was a significance of p=0.05 with 95% CI between the groups identified on both outcome measures. So, this study showed that the MaRhyThe has more significance which rejects the null hypothesis. There are a few limitations noted where the age distribution and duration of the symptoms are heterogeneous. This study has got limited literature since the MaRhyThe is a new concept in India. A longer-term follow-up was not made. The exercises which are advised to the participants are not well monitored.

CONCLUSION

This study concluded that MaRhyThe has a significant effect on reducing pain and improving function compared with myofascial release therapy. There were substantial differences between the groups' pre-test and post-test values; upon reaching the post-test values, it was identified as MaRhyThe produces a significant effect.

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Conflict of Interest

NIL declared by the authors

Source of Funding

NIL declared by the authors.

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Table 1: Demographic Analysis

	Mean (S.D)	p value
Age	51.98 ± 4.69	0.0048
Gender		
Male	52.16 ± 4.85	0.0011
Female	51.68 ± 4.53	0.0010
Side		
Left	52.90 ± 4.66	0.005
Right	50.47 ± 4.45	0.005

Table 2: Analysis of the Treatments between the Groups

Procedures	Pre-treatment (Mean ± SD)	Post-treatment (Mean ± SD)	Percentage of change	Paired 't' test values	Effect size	P value
VAS Group A	5.71 ±0.51	1.92 ±0.80	66%	18.29 ± 1.95	130%	0.05
VAS Group B	5.92 ±0.48	3.63 ±0.51	39%	17.04 ± 1.21	110%	0.05
DASH Group A	49.51 ±2.56	83.4 ±2.96	70%	37.59 ± 16.6	60%	0.05
DASH Group B	49.31 ±2.62	55.45 ± 4.8	70%	6.26 ± 4.89	40%	0.05

Table 3: Interventional Analysis

Procedures	Group A (Mean ± SD)	Group B (Mean ± SD)	Percentage of change	Z value	Student t test values	Effect size	P value
VAS	1.92 ± 0.80	3.63 ± 0.51	65%	6.92	8.64 ± 1.06	35%	0.001
DASH	83.4± 2.96	55.45 ± 4.8	40%	4.93	23.81 ± 14.11	70%	0.001

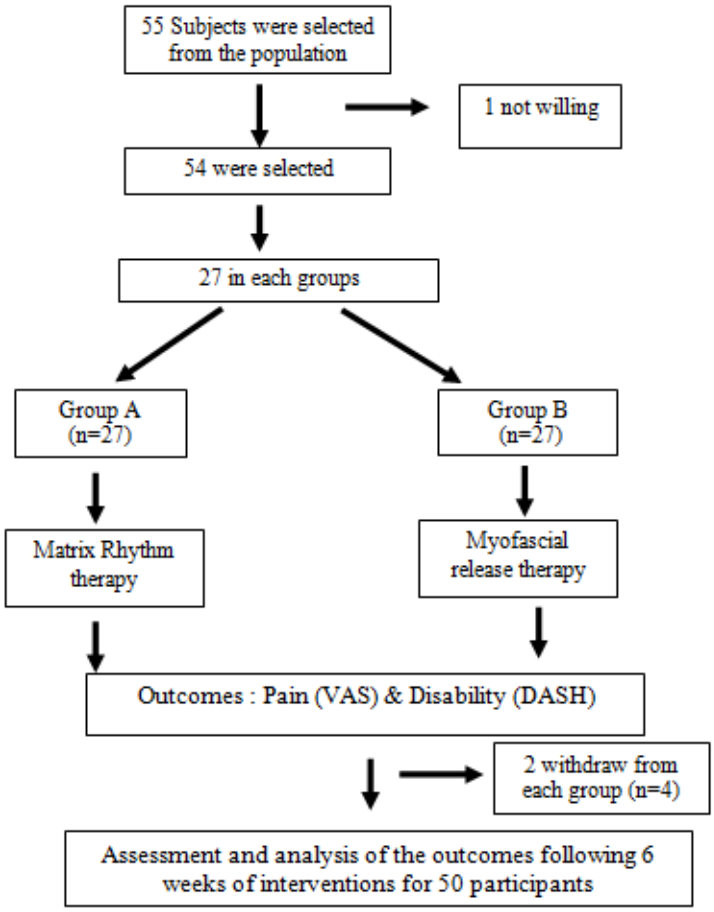

Figure 1: Flow Chart

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RESEARCH ARTICLE

Air Pollution in Opencast Coal Mine is Dangerous for Human Health: A Special Case study to Kalipahari Open Cast Project Patch-A, Kalipahari Colliery, Sripur Area, Raniganj Coalfield

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ABSTRACT

Coal is recognized to be the main source of energy for many decades. It contributes to about 30% of the world's commercial energy requirement. It is mainly excavated by two methods - opencast and underground mining. The geological structure and condition determines the method of excavating. Open cast coal mining is considered to be the common mining activity in the Raniganj coalfield. It causes significant effect on the air, water, land, human health as well as the entire environment. It massively alters the adjoining vegetation, soil and bedrock that ultimately leads to changes in groundwater levels, surface water and flow paths i.e. coal mining adversely affects the whole eco-system. Blasting, drilling, loading, and unloading transportation and burning of coal and wastes etc. are the reason for huge air pollution during open cast mining activity. Air pollution has significantly affected the community's health on the exacerbation of different diseases. The aim of the present study is to evaluate the air quality of Kalipahari open cast coal mine patch -A under Kalipahari colliery in the Sripur area, Eastern coalfield from October 2019 to September 2020 and how it affected the local inhabitants. The status of air pollution in the area was evaluated through Temtop M2000C Air Quality Monitor. It was observed during the said period: average PM2.5 44.46 ug/m3, PM10 63.89 ug/m^3, Particles 4741.2 per/L, atmospheric CO_2 567.58 ppm which were much greater than the World Health Organization guideline levels for the pollutant in atmospheric air. Simultaneously questionnaire survey and discussion were made among mineworkers, office staff, worker union leaders, inhabitants of surrounding areas, local physicians of Kalipahari Health Centre, E.C. Ltd. &Medical Officers of Paschim Bardhaman district. The survey and discussion data were analyzed to evaluate the critical situation arising out of the air

pollutants and its impact on human health i.e., the paper throws light on the burning issues of open cast coal mines and its impact on the environment and health of the local people.

Keywords: Air pollution; Eco-system; Human health; Open cast coal mining; Local inhabitants; World Health Organization guidelines.

INTRODUCTION

The globalization has conventionally been accompanied by rapid increases in energy demand (Kaygusuz, 2012). At the time of energy production from different sources, environmental pollution may occur in different ways at different degrees (Omer, 2008). Burning fossil fuels such as oil, natural gas and coal in energy production is still in use (Veziroglu and Sahin, 2008). Among all these, coal is the most cheap and vital resource still present under earth (Franco and Diaz, 2009). It generates about 42% of the world's electricity, provides near about 29.6% of global principal energy needs from 2001 to 2010 (World Coal Association, 2011). To fulfil the energy requirement, the overall coal production through coal mining activity has excessively increased in India. As per coal production, India ranks 3rd among the top ten coal-producing countries (World Coal Association, 2011). In India, coal mining was first initiated in the Raniganj Coalfield, Bengal province in the year 1774. Rich treasure of coal was found near Ethora (presently in Salanpur community development block) by John Sumner and Suetonius Grant Heatly of the British East India Company. The exploration and mining operations were haphazard in the early stage. Alexander & Co started regular mining in 1820. In 1835, after the collieries had been bought by Prince Dwarkanath Tagore the field was led by Carr Tagore and Company. From the entire 19th century to a major portion of the 20th century, Ranigunj coalfields contributed as the major producer of coal in our country (Chattopadhyay, 2001). But coal mining activity negatively affected the atmosphere, ambient air, land, soil, human health, ecology, and water system i.e. the surrounding entire environment of the mining area (Peplow and Edmonds, 2002; Younger& Wolkersdorfer, 2004). Surface mining and underground mining activity are the chief mining processes and surface mining activities are instrumental in causing the huge problem of air pollution actively or passively (Baldauf et al., 2001; Collins et al., 2001). Pollutants like particulate matter (PM), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon dioxide (CO_2) etc., are the most important emissions during coal mining and through active mine fires. Due to dry weather and lack of humidity, winter season is noticed to be the riskiest among all the seasons in the year in respect of respirable ambient air (Dash et al., 2020). The human health, flora and fauna are tremendously affected by coal mining areas by the air pollutants that deteriorate ambient air quality (Singh et al., 1991). It's also causing fatal injuries, associated in chronic health disorders, such as black lung disease, which causes permanent scarring of the lung (Schins and Borm, 1999). In mining activity drilling, blasting, loading-unloading of materials, overburden etc., are the principal factors that create massive air pollutants that cause respiratory diseases (Gautam et al., 2012).

The present study was conducted over a period of a year from October 2019 to September 2020 to quantify the air pollutant concentrations that deteriorated the ambient air quality in respect of PM2.5, PM10, Particles and atmospheric CO_2 in the vicinity of the Kalipahari open cast coal mining project area of Kalipahari colliery, situated in Raniganj coalfields of West Bengal. And simultaneously to sort out how the said deteriorated air quality affected on local inhabitants', mine workers' health through questionnaire survey was done. Discussion with mineworkers, colliery office staff, colliery worker union leaders, local people, local physicians and Medical Officers of district administrative level was made in this regard.

The present study site Kalipahari OCP patch-A, Kalipahari colliery is situated in Sripur area, Eastern coalfields ltd. (23.667389°N Lat and 87.012046°E Long) in Asansol sub division, Paschim Bardhaman district, West Bengal, India.

6 spots were considered for measuring the air quality which are located in the surroundings and inside the Kalipahari OCP patch-A, Kalipahari colliery.

MATERIALS AND METHODS

The air quality of Kalipahari OCP patch-A, Kalipahari colliery was measured in 6 different spots through Temtop M2000C Air Quality Monitor. Data on Temperature, Humidity, PM2.5, PM10, Particles and Atmospheric CO_2 of these spots were taken into consideration for the analysis from October 2019 to September 2020 during the afternoon hours. 10 readings were taken from each spot at a distance of 10 meters apart and the mean values were considered for statistical analysis

RESULTS AND ANALYSES

In the present study from October 2019 to September 2020 the ambient air pollution in respect of PM2.5, PM10, Particles, atmospheric CO_2 were measured and atmospheric temperature & humidity were also recorded at 6 different points, 3 spots were outside the OCP i.e. adjoining mining site, and 3 spots inside the mining area.

DISCUSSION

In opencast coal mining activity, a huge burden has to be removed to reach the coal deposits. This may require transporters, loaders, conveying belts, excavators etc., resulted in the massive discharge of fine particulates from the exhausting materials. Transportation, excavation, loading-unloading size reduction, stockpiling etc. are also required (Ghose, 1989). All of these generate there particulate matter including all kinds of air pollutants. The variety and volume of air-borne dust particles and pollutants in the ambient air are responsible for the deterioration of air quality in the surrounding mining areas. Cowherd (1979), has reported that in OCP vehicular traffic on haul road can contribute to 8O% of the dust emitted. Chadwick (1987), have estimated it as 25% for both during loading and unloading of a dumper while about 30% of total coal dust i.e., particles released during journey time on a bitumen haul road in the mining site. Drilling is perhaps an important source of fugitive dust particles. It is the major source of fugitive dust particles in wind erosion from coal stockpiles.

The present study is based on air quality deterioration and its impact on human health in Kalipahari open cast coal mine project, patch-A, Kalipahari colliery and its surrounding area. The source of air pollutants was recognized responsible for deteriorating the ambient air quality in the said mining site (Figure 6). Among these dust particles i.e., PM 10, PM 2.5 (suspended particulate matters) & CO_2 were considered for analysis. During the study period i.e. from October 2019 to September 2020 atmospheric temperature and humidity were also recorded. In the present study, the air pollution was measured through Temtop M2000C Monitor at 6 different points, 3spots were outside the OCP i.e. adjoining mining site, and 3 spots were inside the mining area (Figure 3). The study period was divided into three months interval and 10 readings were taken from each spot at a distance of 10 meters apart and the mean values were considered for statistical analyses. The data of pollutants revealed that SPM concentration and CO_2 level for almost all measuring spots were the most from October 2019 to December 2019 and from January 2020 to March 2020 because of the winter season & low humidity. Whereas during the pre-monsoon and monsoon period from April 2020 to June 2020 and July 2020 to September 2020 SPM concentration and CO_2 level were comparatively low, due to the removal of dust particles by rainwater (Table 1). The concentrations of the pollutants were high in winter in comparison to the pre-monsoon or the monsoon seasons due to industrial activities, indiscriminate open-air burning of coal by the local people, for vehicular traffic & cooking purposes etc. in Raniganj coalfield (Reddy, 2003). The annual average (from October 2019 to September 2020) PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spots of Kalipahari OCP patch-A, Kalipahari colliery were 44.46 ug/m3, 63.89 ug/m3, 4741.2 per/L, 567.58 ppm (Table 1)

respectively which is much more than WHO Air quality guideline values (Table 4). After an investigation in the vicinity of opencast coal projects, Basundhara Garjanbahal area, India, Sahu et al., (2018) reported more or less the same result i.e., PM 2.5 and PM 10 varied in range 41-71 ug/m3, 31-62 ug/m3 respectively. They also mentioned from their study site the major sources of air pollutants were road dust caused by vehicular movement, spillage of coal during transportation, spontaneous combustion of coal, and biomass burning in the village area. In Raniganj coalfield the rate of emission of a greenhouse is increasing day by day to the threatening level and has become harmful for local inhabitants because of the huge producing of CO_2. The annual rate of CO_2 emission is increasing by 0.4% per year in this coalfield (Goswami, 2014). From the above observation it is very much clear that the air quality in the vicinity of the said study site as well as in the mine's ambience is very much negatively altered. It was judged by the yardstick of Air Quality Guideline Levels laid down by the WHO.

To verify how much polluted ambient air impacted local inhabitants and mine workers' health, a survey and discussion through an ideal questionnaire was done throughout the studied period among the said mineworkers, colliery office staff & worker union leaders, 200 local people, physician, E.C. Ltd. Health Centre, Kalipahari and Paschim Bardhaman district-level administrative medical officers

Questionnaire format 1. At the time of survey among the local people and mineworkers of Kalipahari colliery and its surrounding areas to achieve air pollution perception in the open cast coal mine.

1. How would you rate the overall air quality in your area now compared to last year?
o Much better
o A little better
o About the same
o A little worse
o Much worse

2. What do you think are the main causes of air pollution in your area? Please select all applicable.
o Blasting in the mines for breaking coal chunks
o Mining sources/manufacturing facilities
o Loading unloading of coal
o Digging of pit
o Motor vehicles
o Household cooking and heating
o Population growth
o Smoke of cigarettes
o Waste disposal
o Burning of coal
o Pollution from other source(s)

Other (please specify)

3. How muchis the air pollution affecting you?
o Very much affected
o Affected a little
o Not affected at all
4. In which of the following ways are you affected? Please select all applicable.
o Breathlessness / having more difficulty in breathing
o Doing less outdoor activity
o Doing more to look after my skin
o Doing more to keep health
o Feeling depressed

- o Irritation to eyes/nose/throat
- o Skin problems
- o Cardiac problem
- o Diabetic
- o Blood pressure
- o Kidney problem
- o Tuberculosis
- o Wanting to move to other less polluted place
- o Asthma incidences
- o Poor visibility
- o Worrying about the living environment for children

Other (please specify)

5. Polluting companies should be penalized even if it puts some jobs at risk.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

6. Pouring and spraying of water to stop flying of dusting particles.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

7. Government should do more to promote and encourage a better environment.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

8. Administration should stop and check emission more frequently.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

9. Improving the environment is the responsibility of every citizen.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

10. Recycling programs should be put in place and promoted across the whole area.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

11. I am actively involved in cleaning up the surrounding environment.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

12. The pollution is out of my control and I cannot do anything to change it.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

13. I do not see the pollution as a problem.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

14. If I know how to contribute better to a cleaner environment, I would take action.

Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

Questionnaire format 2. At the time of discussion with a local physician, Kalipahari Health center, E. C. Ltd. and Paschim Bardhaman district administrative level of Medical Officers to achieve air pollution perception in the open cast coal mine.

1. How would you rate the overall air quality in your area now compared to last year?
   - o Much better
   - o A little better
   - o About the same
   - o A little worse
   - o Much worse
2. What do you think are the main causes of air pollution in your area? Please select all applicable.
   - o Blasting in the mines for breaking coal chunks
   - o Mining sources/manufacturing facilities
   - o Loading unloading of coal
   - o Digging of pit

o Motor vehicles
o Household cooking and heating
o Population growth
o Smoke of cigarettes
o Waste disposal
o Burning of coal
o Pollution from other source (s)

Other (please specify)

3.To what extent is the air pollution affecting the local people?
o Very much affected
o Affected a little
o Not affected at all

4. In which of the following ways the people are affected in this locality? Please select all applicable.
o Breathlessness / having more difficulty in breathing
o Doing less outdoor activity
o Doing more to look after my skin
o Doing more to keep health
o Feeling depressed
o Irritation to eyes/nose/throat
o Skin problems
o Cardiac problem
o Diabetic
o Blood pressure
o Kidney problem
o Tuberculosis
o Wanting to move to other less polluted place
o Asthma incidences
o Poor visibility
o Worrying about the living environment for children

Other (please specify)

5. Polluting companies should be penalized even if it puts some jobs at risk.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
6. Pouring and spraying of water to stop flying of dusting particles.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
7. Government should do more to promote and encourage a better environment.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
8. Administration should stop and check emission more frequently.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
9. Improving the environment is the responsibility of every citizen.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
10. Recycling programs should be put in place and promoted across the whole area.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
11. As a Doctor you are actively involved in cleaning up the surrounding environment.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
12. The pollution is out of my control and I cannot do anything to change it.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯

13. I do not see the pollution as a problem.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
14. If I know how to contribute better to a cleaner environment, I would take action.
Strongly agree ◯ Agree ◯ Undecided ◯ Disagree Strongly ◯ Disagree ◯
15. Do you think that the diseases caused by air pollution successively increased in comparison to previous years?
Yes ◯
No ◯
16. Please furnish the types of diseases caused by air pollution dominantly found in the locality.
17. Mention the percentage of the diseases of the patients you treated last year.

Inhaling contaminated air with these pollutants can be highly dangerous for human health, lungs, immune system, heart, reproductive system, brain, to some extent genetic material is also affected (Gasparotto & Martinello 2020). On the basis of survey and discussion with respondents, the ambient air quality of the said OCP site and its vicinity was found a little worse compared to last year due to various increases in mining activities. As a result, the mining workers and local inhabitants were affected by breathlessness, asthma, bronchitis, chronic obstructive pulmonary disorders, cardiovascular disorders, irritation to eye, nose, throat, skin, diabetics, hypertension, kidney problems, liver problems, poor visibility. Having consolidated response from different categories of respondents on various kinds of health disorders, a response percentage (issue or problem-wise) was taken into consideration and plotted in the bar graph (Figure 9).

All respondents were of the same opinion that to keep an eye on industrialization and development, mining activities/operations cannot be stopped for the sake of saving the environment only. A balance between mining activities and environmental management is the need of the hour. A well though of plan must be adopted by E.C. Ltd.'s authority to improve the ambient air quality of the said site and its vicinity. Coal as a major energy resource must be utilized with appropriate measures of safeguarding human health as well as environmental protection (Gasparotto & Martinello 2020).

CONCLUSION

Open cast coal mining creates a serious air pollution problem in Kalipahari OCP patch-A, Kalapahari colliery, Raniganj coalfield. The rapid development of industries and development of open cast coal mining are growing at a phenomenal rate in this area. Work zone and ambient air quality data from October 2019 to September 2020 collected and analysed reveal that dust particles i.e., PM 10, PM 2.5 (suspended particulate matters), CO_2 are the high pollution potential in the said project area and its surrounding localities. By means of survey and discussion through ideal questionnaires with mineworkers, colliery office staff & worker union leaders, local people, physicians and district-level administrative medical officers, it is recognized that due to massive pollutants for mining operations, the ambient contaminated air is found to have a great impact on local inhabitants' and mine workers' health. At the same time, it is also realised that open cast mining activity cannot be stopped in the name of protecting the environment or local inhabitants health only. A suitable balance between open cast mining activities and environmental management is needed immediately. Sustainable air pollution control measures involve master planning and its proper implementation in the said open cast coal mine to improve the ambient air quality in the mine and its vicinity.

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Table 1. Status of air quality in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spotsof Kalipahari OCP patch-A, Kalipahari colliery during October 2019 to September 2020

Spots	Temperature (°C)						Humidity						PM2.5 ug/m³					
	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average
1	16	36	35	33	30	31.19	38	44	65	52	49.75	49.14	51.1	42.2	33.5	41	42	44.46
2	17	35.5	35	34	30.25		36.5	43.2	66.2	51.3	49.3		54	41.2	34.1	43	43.125	
3	17	37	36	34	31		36	41.9	65	52	48.725		55.5	42	34	41	43.175	
4	18	37	36	34	31.25		37	42	66	51	49		58.4	43	35	44	45.125	
5	18	39	37	35	32.25		37.5	42.1	65.4	52.6	49.4		61.5	45.5	35.4	46	47.075	
6	17	39.1	37.5	36	32.4		35	41.9	66	51.8	48.675		59.2	44.9	34.9	46	46.275	

PM10 ug/m³						PARTICLES per/L						Atmos. CO_2 ppm					
Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Average	Area average
70.1	61.2	52.9	59	60.8	63.89	5321	3915	3002	4002	4060	4741.2	504	501	504	502	502.75	567.58
72.3	62	54	61.4	62.425		5617	4001	3021	4304	4235.75		505	502	509	548	516	
74.3	63	52.1	60	62.35		5754	3906	2902	4001	4140.75		524	526	521	541	528	
77.7	64.2	54.5	62	64.6		7205	5601	3554	5305	5416.25		650	624	600	596	617.5	
79.1	66.2	56.5	64	66.45		7028	5725	3601	5054	5352		647	641	642	584	628.5	
80	66.5	54.2	66.2	66.725		6695	5825	3502	4948	5242.5		651	652	547	601	612.75	

Table shows the annual (from October 2019 to September 2020) air quality status in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spots.

Table 2. Annual average value of air quality in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO2 at 6 spots of Kalipahari OCP patch-A, Kalipahari colliery during October 2019 to September 2020

Parameters	Spot1	Spot2	Spot3	Spot4	Spot5	Spot6
Temperature(°C)	30	30.25	31	31.25	32.25	32.4
Humidity	49.75	49.3	48.725	49	49.4	48.675
PM2.5ug/m^3	42.00	43.13	43.18	45.13	47.08	46.28
PM10ug/m^3	60.8	62.425	62.35	64.6	66.45	66.725
PARTICLESper/L	4060	4235.75	4140.75	5416.25	5352	5242.5
Atmos.CO_2ppm	502.75	516	528	617.5	628.5	612.75

Table shows the annual average air quality status in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spots of Kalipahari OCP patch-A, Kalipahari colliery, Sripur area

Table 3. ANOVA: Single Factor shows the annual (from October 2019 to September 2020) air quality status in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spots of Kalipahari OCP patch-A, Kalipahari colliery

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	94098197.3	5	18819639	279.684	1.88568E-20	2.62065
Within Groups	1614934.1	24	67288.921			
Total	95713131.4	29				

Notes: SS = Sum of Squares, df = Degree of Freedom, MS = Mean Sum of Squares, Fobs = statistical F, P- value = Probability, Fcrit = Critical F.

Table shows the annual (from October 2019 to September 2020) air quality status in respect of Temperature, Humidity, PM 2.5, PM10, Particles and atmospheric CO_2 at 6 spots of Kalipahari OCP patch- A, Kalipahari colliery. Here F > F crit, rejected the null hypothesis. Thisis the case, 279.684>2.62065. The means of annual air quality status of the said open cast coal mine project at the selected 6 spot are not all equal. At least one of the means is different.

Table 4. Annual average (from October 2019 to September 2020) PM 2.5, PM 10 and atmospheric CO_2 of Kalipahari OCP patch-A, Kalipahari colliery is compared to WHO Air quality.

	PM2.5 ug/m^3	PM10 ug/m^3	Atmos. CO_2 ppm
Kalipahari OCP patch-A area	44.46	63.89	567.58
WHO Air quality guideline values	10	20	400

Table shows the PM2.5, PM10 atmospheric CO_2 in the said mine's ambient air were much more than WHO Air quality guideline values

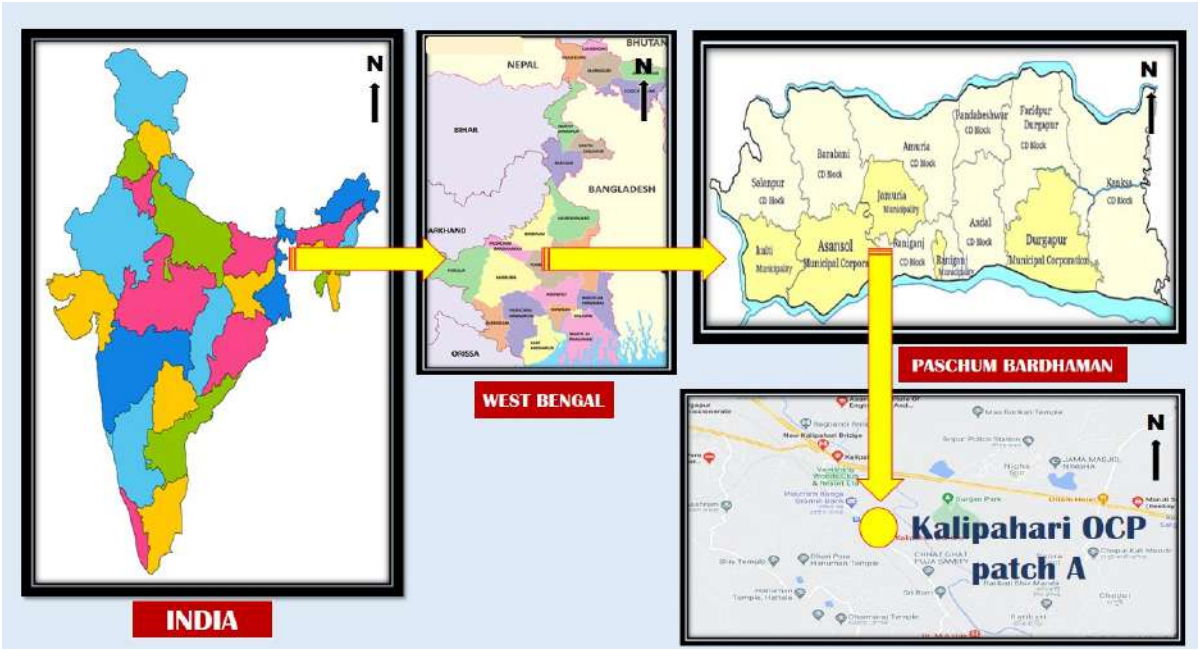

Figure 1. Geographical location of Kalipahari OCP patch-A, Kalipahari colliery
(source https://www.google.com/maps/search/Kalipahari+map/@23.6662728,86.8622994,11.48z)

Figure 2. Satellite view of Kalipahari OCP patch-A, Kalipahari colliery
(source https://www.google.com/maps/search/Kalipahari+map/@23.6662728,86.8622994,51609m/data=!3m1!1e3)

Figure 3. Air quality studied spots in Kalipahari OCP patch-A, Kalipahari colliery SPOT 1: Kalipahari Rly. Station area, SPOT 2: Kalipahari colliery office area, SPOT 3: Outside of Kalipahari OCP patch-A, Kalipahari colliery, SPOT 4: Entry point of Entrypoint of Kalipahari OCP patch-A, Kalipahari colliery, SPOT 5: Mining site of Kalipahari OCP patch-A, Kalipahari colliery, SPOT 6: Dumping site of Kalipahari OCP patch-A, Kalipahari colliery.

Figure 4. Air quality of Kalipahari OCP patch-A, Kalipahari colliery was measured at different spots through Temtop M2000C Air Quality Monitor

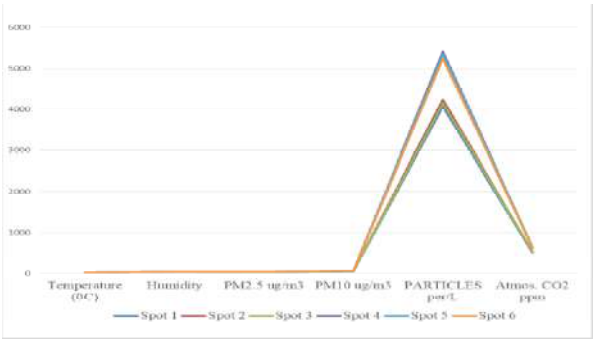

Figure 5. Annual (from October 2019 to September 2020) air quality status in respect of Temperature, Humidity, PM 2.5, PM 10, Particles and atmospheric CO_2 at 6 spots of Kalipahari OCP patch-A, Kalipahari colliery, Sripur area

Figure 6. The sources of air pollution were identified and different mining activities were also recorded in Kalipahari OCP patch-A, Kalipahari colliery, Sripur area. Various major processes during coal excavation, i.e. a: blasting, b: transportation of mining materials on a bitumen haul road in the mining site, c: Excavator loading dumper truck on mining site, d: Burning of coals in the mining site, e: At the time of transportation of coal or other mine materials, f: Burning of mining waste, g: the dusty, foggy atmosphere of the mining site, h: Dust particles on leaves at the adjoining mining site.

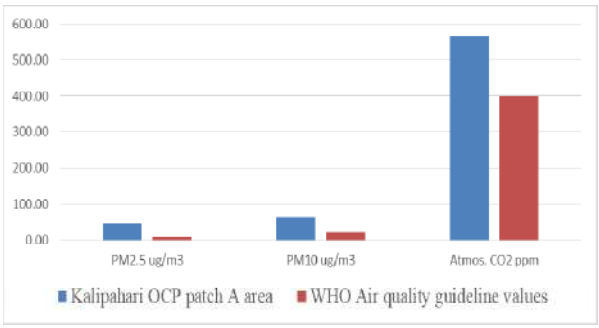

Figure 7. Bar graph of PM 2.5, PM 10 and atmospheric CO_2 of Kalipahari OCP patch-A, Kalipahari colliery in comparison to WHO Air quality guideline values.

Figure 8. At the time of questionnaire survey and discussion with a: Mineworkers, b: colliery office staff, c: colliery worker union leader, d: local peoples, e: Physician, E.C. Ltd. Health Centre, Kalipahari.

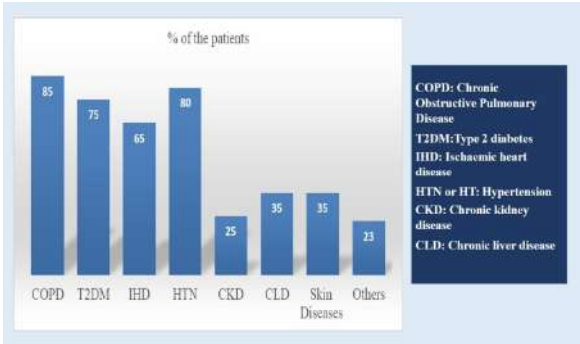

Figure 9. Percentage of the diseases of the patients caused by air pollution dominantly found in the Kalipahari colliery surrounding areas.

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RESEARCH ARTICLE

Assessing the Impact of Physico-Chemical Factors of Vaigai Reservoir Water in Theni District, Tamilnadu, India

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ABSTRACT

Water is the spirit of all existence. It is one of the most important major components of environmental resources. Water quality has become a major concern due to ever increasing human developmental activities that over exploit and pollute the water resources. The present study was focused to analyse the physico-chemical factors of vaigai reservoir water in Theni district. The vaigai reservoir water samples were collected from four different sites such as S 1 , S 2 , S 3 , S 4 during November-March, 2020. Evaluation of physico-chemical parameters was carried out to assess the quality of water. The physico-chemical factors such as, Temperature, pH, Electrical Conductivity (EC), Total Dissolved Solid (TDS), Total Hardness (TH), Magnesium Hardness (MH), Biochemical Oxygen Demand (BOS), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), Nirate, Nirite, Chloride and Iron were analysed. Each physico chemical factor was compared with the standard desirable limit prescribed by BSI. In the present investigation, the results revealed that, the physico chemical factors varied with different sites, such as S 1 and S 2 were recorded in the permissible limit, whereas the highest values of pollutants were recorded in S 3 and S 4 , by various anthropogenic activities such as discharges of domestic waste material, industrial and agriculture wastes.

Keywords: Water analysis, physical and chemical properties.

INTRODUCTION

Water is one of the majority vibrant wealth of the planet, and only 2.5 percentage of the total global water resource is fresh (Shiklomanov, 1998). Water is one of the abundantly accessible substances (Ravi et al., 2012). The healthy ecosystem depends on the physico chemical and biological characteristics of water (Venkatesharaju K., et. al., 2010).

Climate change, fast urbanization is affecting water quality and threatening the availability of freshwater sources causing water scarcity in many regions of the world (Eslamian, 2016; Frederick and Major, 1997; Sophocleous, 2004).Water quality is an essential indicator to determine the reuse potential. Water quality varies with geographic location, season, weather, human activities, site-specific conditions and the presence of pollution sources. Point source pollution like domestic or industrial wastewater loads can easily be identified, and therefore managed, whereas non-point source pollution like urban or agricultural runoff increases the complexity in finding and implementing quality improvement measures (Ongley et al., 2010; Shi et al., 2017; Tran et al., 2019, 2015; Wang et al., 2016). Land use and environmental land use conflicts represented by the use of land disrespecting soil capability is a source of water pollution that plays a pivotal role in determining water quality (Giri and Qiu, 2016; Junior et al., 2014; Pacheco and Fernandes, 2016). The Vaigai reservoir built across the majestic River Vaigai near Andipatti, the dam with a height of 111 feet can store 71 feet of water. It is 7 kms from Andipatti, 14 kms from Theni and 70 kms from Madurai, the Souther state of Tamil Nadu, India.

Good quality of water resources depends on a large number of physico chemical and biological characteristics. These parameters are essential to identify magnitude and source of any pollution load (Amadi, 2005). To assess these parameters is essential to identify the magnitude and source pollution load in the aquatic system. Many research are being carried out to suggest appropriate conservation and management strategies based on the physico and chemical characteristics of water (Rajesh K.K., et al., 2002, Jayaraman P.R., et al., 2003, Sharma M.R. and Gupta A.B., 2004, Rajasekar K. T., et al., 2005, Sridhar R., et al., 2006, Srivastav N., et al., 2009, Damodharan P., et al., 2010, Prasana M. and Ranjan P.C., 2010, Medona Mary R., et al., 2014). World Health Organization (WHO) is most important of parameters of drinking water quality like pH, temperature, Dissolved Oxygen, BOD, COD, Total Hardness, Total alkalinity. The aim of the present study was investigate the physico chemical parameters of Vagai Reservoir water samples were collected from October 2018 to March 2019.

MATERIALS AND METHODS

Location

Vaigaidam reservoir is located to the West of Periyakulam town at the Latitude, 77- 28'4''and Longitude of 10 o 7'45''at 400 MSL on the foot of Western Ghats of Palani hill range. It is 9 k.m. away from Periyakulam and 10 km away from Jayaraj Annapackiam College for women, Periyakulam. It supplies water to Periyakulam throughout the year. Irrigation under Vaigaidam system is about 2,865 acres. The capacity of maximum water level is 100 meter square feet. Area of water spread on maximum water level is 48.64 meter square feet.

Sample Collection

The water samples were collected from four different site of Vaigai Dam Reservoir during the month of April 2019 to September 2019. The samples were collected in precleaned polyethylene bottle as prescribed by standard methods (APHA, 2005). The parameters such as colour, odour, and water temperature were determined on the spot. After that the samples were kept in ice cold box before transporting to the laboratory. The sample was collected from different origin like S 1- upper stream dam water, S 2- lower stream dam water, S 3- nearest in bridge and S 4- nearest in entry point, and the samples were analyzed.

Water Analysis

Water analysis was carried out in Vaigai Dam, Theni District. The water quality parameters such as temperature, pH, acidity, alkalinity, hardness, macro and micro elements were analyzed.

Labelling the samples bottle for identification

The sample details such as collection site, date and time were marked by permanent glass marker containing indelible ink directly on the sample collection bottle.

Preparation of samples bottle for water collection

Before filling the river water sample, the bottle was brushed with phosphate free detergent and then rinsed three times with cold tap water, 10% percent hydrochloric acid and demonized water.

Determination of the Physico chemical parameters of the water samples

A Jenway model 4020 conductivity meter was used to determine the conductivity and TDS of the samples. A pH meter (Jenway model) and combination electrode was used to determine the pH level of the water samples. A turbidimeter (hatch model) was used to determine turbidity levels of water samples. Sample was vigorously shake and poured into turbidimeter sample cell to at least 2/3 full. An appropriate range was selected using the range knob. The turbidity value was then read. Nitrate, phosphate, fluoride and sulphate in dam water were determined by hydrazine reduction method, reaction with ammonium molybdate and ascorbic acid, spadns method, turbidimetric method respectively using UV/ Visible spectrophotometer in accordance with APHA 4500. Potassium and sodium were analysed with flame atomic absorption spectrophotometer (FAAS) in accordance with APHA (1998). The SPSS version 10.0 software was used to determine the correlation coefficient of the parameters using Pearson's correlation at two tailed.

RESULT AND DISCUSSION

Table 1 presented the physico-chemical characteristics of water samples from the vaigai dams reservoir, whilst Table 2 shows correlation between the parameters. The physico-chemical parameters obtained from the water samples fell within the World Health Organisation (WHO) (2008). Generally, pH values recorded from the samples ranged from 6.0 to 7.8 with a general mean value of 6.8±0.6 pH-unit. The pH is an important variable in water quality assessment as it influences many biological and chemical processes within a water body and all processes associated with water supply and treatment (APHA 1995). Electrical conductivity (EC) values measured from the vaigai dam reservoir water ranged from 215 to 240 μS/cm with a general mean of 230±0.81 μS/cm. EC values recorded in this study are similar to that of Kpieta and Laari (2014) who studied small-scale dams water quality and the possible health risk to users of the water in the upper west region of Ghana. The Nitrates values recorded from the samples ranged from 0.25 to 32.5 μg/l with a general mean value of 0.42±0.2 μg/l. However, the low nitrate levels can cause long-term exposure as low levels between 2,000 to 4,000 μg/l in community water supplies has been linked to bladder and ovarian cancer (Weyer et al., 2001). Hence, indigenes continue consumption of the dam water might not be free from disease burden. Chloride value recorded from the samples ranged from 0.39 to 41.3 μg/l with a general mean value of 0.39±0.01 μg/l.

Calcium values recorded from the samples ranged from 1.2 to 1.9 μg/l with a general mean value of 1.2±0.2 μg/l. Magnesium value recorded from the samples ranged from 0.4 to 1.4 μg/l with a general mean value of 0.48±0.048 μg/l. Fluoride value recorded from the samples ranged from 0 to 6 μg/l with a general mean value of 0±0.4 μg/l. Sulphate value recorded from the samples ranged from 0.4 to 2.35 μg/l with a general mean value of 0.4±0.15 μg/l. Turbidity value recorded from the samples ranged from 1.1 to 7.5 with a general mean value of 1.1±0.1 μg/l. BOD value recorded from the samples ranged from 1.13 to 5.7 with a general mean value of 1.13±0.08 μg/l. DO value recorded from the samples ranged from 4.5 to 7.5 with a general mean value of 4.5 ±2.0 μg/l. BOD is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic materials (e.g., iron, sulfites). Typically the test for BOD is conducted over a five-day period (Milacron Marketing Co.). DO is one of the most important parameter. Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients, stratification etc. (Premlata Vikal, 2009).

CONCLUSION

In conclusion, they attain by collection, testing and analyzing of river water samples and do water quality consideration and to review the water quality and monitor the water excellence periodically and analysis all the macro and micro nuritrians in waterwater quality plays a vital role in the distribution, abundance and diversity of insect. Physicochemical water quality parameters in receiving water bodies in taken "VAGAI DAM WATER" were assessed during last year. Insect were collected during one year three ways near the dam and middle of the dam deep water in dam these are analyzed were taken and the physical, chemical, water, soil parameter were analyzed. Signs of increasing water quality deterioration were evident in the result of the physicochemical analyses. The abundance of aquatic insect was higher during the period of rains and reduced during the dry period.

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Table. 1 Physical- chemical parameters of water sample from Vaigai dam during April, 2019 to September, 2019

Parameters	BSI Standard	Unit	Mean				STD			
			Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Electrical Conductivity	300	µS/cm	215.167	215.8333	230.3333	240.3333	2.316607	3.060501	0.816497	2.160247
pH	8.5	pH-unit	7.20167	7.206667	6.8	7.126667	0.108336	0.048442	0.609918	0.840159
Total Alkalinity CaCo3	200	µg/l	5.17833	5.288333	5.266667	4.85	0.23769	0.15536	0.206559	2.836723
Total Hardness CaCo3	300	µg/l	53.25	54	59.98333	75	1.837117	2.966479	1.040032	1.264911
Calcium	75	µg/l	1.30833	1.201667	1.86	1.9	0.149722	0.207886	0.187617	0.126491
Megnecium	30	µg/l	1.05333	0.481667	0.808333	0.836667	0.05164	0.047504	0.10852	0.129718
Iron	0.3	µg/l	0.58333	1.916667	0.523333	0	0.179741	0.147196	0.05164	0
Nitrate	45	µg/l	2.33667	32.5	1	0.4	0.156801	1.870829	0.509902	0.219089
Chloride	250	µg/l	41.3333	0.391667	29.5	27.58333	1.21106	0.016021	14.01071	13.34323
Fluride	1	µg/l	0.35167	0	6.031667	6.033333	0.132728	0	14.19154	14.19108
Sulphate	200	µg/l	2.35	2.083333	0.475	0.411667	0.731437	0.147196	0.140535	0.154326
DO	5	µg/l	7.50667	7.15	5.733333	4.55	0.090921	0.151658	2.569565	2.053047
BOD	5	µg/l	1.13333	1.25	2.333333	5.633333	0.08165	0.187083	1.21271	2.871701
Turbidity NT Units	5	µg/l	1.1	2.121667	4.233333	7.55	0.126491	0.103618	1.206096	0.773951

Fig.1. Vagai Dam River study Area

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RESEARCH ARTICLE

Nutrition-Parasite Interaction in Ruminants

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ABSTRACT

There has been increased interest in the interactions between nutritional status of the host and parasite establishment/rejection. However, insufficient research work on this area of clinical nutrition stimulated in part by the need to develop sustainable approach to control of parasitic infection in ruminants, which are less reliant of frequent chemotherapy intervention. Improving host resilience or resistance to infection through management practices which involve manipulation of nutrition can be one component in an integrated approach. Our current understanding on the pathophysiology of parasitic infection has increased awareness of the mechanisms responsible for the impairment and productive losses.

Keywords: establishment, chemotherapy, infection, awareness.

INTRODUCTION

Feed intake and nutrient supply have been recognized for long time as an important criteria for achieving the efficiency of animal production. There are a number of internal and external factors that affect the production performance of animals. Parasites and parasitism have been one of the major constraints to animal productivity throughout the world. Disease-nutrition interaction is an omnipresent part of the ecosystem and involves relation between and among organisms and their environment. The outcome of the interaction depends on a myriad of factors governing the host and invader pathogen interaction, the number of the invader organisms, genetic makeup of the animal and finally the nutritional status of the host. There has been increased interest in the interactions between nutritional status of the host and parasite establishment/rejection. However, insufficient research work on

this area of clinical nutrition stimulated in part by the need to develop sustainable approach to control of parasitic infection in ruminants, which are less reliant of frequent chemotherapy intervention, (Coop and Holmes, 1996). Improving host resilience or resistance to infection through management practices which involve manipulation of nutrition can be one component in an integrated approach. Our current understanding on the pathophysiology of parasitic infection has increased awareness of the mechanisms responsible for the impairment and productive losses.

Effect of nutrition on host resistance

The interaction between parasitism and nutrition is generally described to be mutually reinforcing *i.e.* impaired nutrition tends to decrease resistance to infection or modify the outcome to the benefit of the parasite and in turn, the parasite tends to impair the nutritional status of the host. The major complex interactive relationship of the nutritional status of an animal or bird to the immune system on one hand and the invader on the other hand. The immune system has a high priority for nutrients. Stimulation of the immune system through natural infection can result in impaired growth. Responding to an infection places added demands on the nutritional state that cannot always be met, especially in parasitic infection that may affect nutrient utilization.

Metabolic consequences induced by parasitism and effects on feed utilization by the host

The level of larval challenge influences the extent of metabolic impairment induced by a parasite predominantly and the numbers and species of worms, which establish (Van Hourt and Sykes, 1996).This, will be modified by host factors such as age, breed, nutritional and immune status. Most of our understanding of the impact of gastrointestinal nematodes on ruminant metabolism and specific mechanisms of impaired productivity has been derived from experimental infection in sheep.

In general GI nematodes reduce nutrient availability to the host through both reductions in voluntary feed intake/ or reductions in the efficiency of absorbed nutrients although the underlying mechanism of the depression in appetite have not been fully elucidated (Dynes *et al.,* 1998).The relative contribution of these two mechanisms to impaired production is to some extent dependent on the species of parasite and its location in the GI tract. For example, Experiments using pair-fed and adlibitum fed controls have shown that the major consequence of a continuous infection with *Ostertagia circumcincta* (abomasal) in growing lambs is a reduction in voluntary feed intake (Sykes and Coop,1977), the extent of which is related to the level of Ostertagia larval intake (Coop *et al.,*1982).The depression of appetite in chronic sub clinical infections can range from about 15-20% (Coop *et al*.,1982).When feed intake is expressed per unit of body weight the differences in relative intakes of infected and control animals acquire resistance to infection and there is evidence that the presence of worms is required to maintain anorexia as feed intake of *T.colubriformis* infected sheep returned to normal within a few days following anthelminthic treatment (Kyriazakis *et al.,*1996). The depression of appetite in chronic sub clinical infections can range from about 15-20% (Coop *et al.,* 1982). When feed intake is expressed per unit of body weight the differences in relative intakes of infected and control animals is less apparent (Sykes *et al.*, 1988). Food intake usually returns toward normality as animals acquire resistance to infection and there is evidence that the presence of worms is required to maintain anorexia as feed intake in *T.colubriformis* infected sheep returned to normal within a few days following anthelminthic treatment.

A parasitized host can be compared to similar non-parasitized control given access the same amount of food, so that the consequences of parasitism on food utilization can be directly quantified. This is achieved by pair feeding of parasitized and control animals, so that the direct consequences on food utilization of the voluntary reduction in food intake, which accompanies the majority of parasitic infections, are overcome (Kyriazakis *et al*., 1996). Parasite, which invades body tissue, will contribute to inefficient food utilization and to additional nutrient requirement. Most of the evidence comes from blood-borne parasites such as *Trypanosomes* and *Babesia* spp. These would not be expected to have any effect on digestion and absorption in the GI tract. Similarly, no marked effect would be expected from such parasitism on nitrogen metabolism, as confirmed by the fact that pair-fed parasitized and control animals have an identical N balance during the course of infection with *Trypanosoma vivax* (Akinbamijo *et al.,* 1992).

Effect of nutrition on acquisition of immunity

The duration of the phase of acquisition of immunity during which the immune system recognizes the parasite and the immunological changes that precedes an effective immunological response are highly varying, could range from a few days in protozoan infections to several weeks in helminth infections. This may largely depend on the degree of contact of the parasite with the host system and the rate of infection (Dobson, *et al*., 1990).Nutrition of the host could have the potential to effect how rapidly immunity is acquired and the effect would be expected to be seen by best in helminth infections, in which the rate of acquisition of immunity is relatively lengthy. Under normal nutritional circumstances, changes in nutrient intake would not be expected to affect the early rate of acquisition of immunity to parasite in young animals. However, there is experimental evidence to suggest initial establishment of nematodes in young non – immune hosts is not affected appreciably by dietary supplementation (Coop *et al*., 1995). At the early rate of acquisition of immunity to severely undernourished animals that loose protein mass is severely impaired.

Effect of nutrition on expression of immunity in growing animals.

Acquisition of immunity is a crucial mechanism in resistance and if developed adequately, controls the impact of gastro intestinal nematode parasitism on life time productivity of the grazing animals. However, sheep may not develop full resistance for gastro intestinal parasites until 8-24 months of age. There is a large inter animal variation in the rate of development of resistance both within the same breed and between breeds which appear to have a strong genetic basis. There is considerable activity to respond to parasite challenge and within any group of outbreed animals maintained on a similar plane of nutrition. The role of host genes in susceptibility or resistance to many an infectious disease including parasitic infection is not very well understood.

Expression of acquired immunity in the host can affect parasites in various ways. In nematode species, acquired immunity has effects on parasite establishment, development, survival and fecundity. Recent experimental evidence reaffirms a supposition that host nutrition has the potential to affect the degree of expression of acquired immunity. Much of the experimental evidence comes from the studies on Haemonchus, Trichostrongylus, Ostertagia and Nematodirus spp. groups of gastrointestinal nematodes and there is little or no information on protozoan infections due to difficulty in inducing chronic or sub-clinical infection.

Host protein and energy nutrition can affect greatly the expression of immunity in sheep (Coop *et al.,* 1995), cattle and goats (Singh *et al.,* 1995) infected with trickle dose of gastro-intestinal nematodes. For example, supplementation of hay diet with 50 or 1000 g of fishmeal per day affects the role of worm expulsion after 10 weeks of trickle infection with *T. colubriformis* and the rate of expulsion was related to the level of supplementation (Van Houtert *et al.,* 1995). Wallace *et al.* (1995) found no difference with number of worms carried by sheep infected with *H. contortus,* but supplementation of a basal diet with soyabean meal had significant effect on worm fecundity. This finding is consistent with the view that function of growth is prioritized over that of expression of immunity and hence the latter will greatly be affected by host nutrition. This may imply that animals with higher requirement of growth (breeds of higher potential for growth) will express acquired immunity to a lesser extent than animals of lower requirements given access to the same level of nutrition.

Whether nutrition has any specific role in influencing the reflector immune functions and the expression of immunity is yet another question that cannot be assertively answered. However, the limited information on this subject may only suggest that different effector arms of the immune system will have different requirements for specific nutrients. Although, there is some evidence that nutrition can affect the levels of circulating antibodies in bacterial and viral challenge (Sinclair and Reed, 1998), only a minor role can be envisaged in the immune response towards these gastro-intestinal nematodes. The Trichostrongylus experimental model (Van Houtert and Sykes, 1996) shows that fish meal supplementation resulted in an increase in the concentration of mast cell proteinases and elevation of levels of circulating eosinophils with no changes in the levels of specific antibodies or non-specific antibodies.

Information on effect of micronutrients such as minerals and vitamins on parasitism is limited, although it is well known that the immune system has specific requirements of micronutrients such as zinc (Chandra, 1993). Studies on the relationship of phosphorous (P) with *T. vitrinus* worm burden in sheep suggest that increased 'P' supply from 1.88 to 2.75 g kg-1 dose decreases mean worm burden (from 11000 to 3,000) over 11 weeks (Coop and Field, 1983). Trace elements like copper, molybdenum and selenium are known to influence the host resistance to nematode infection.

Nutritional impairment due to blood parasite infection

Infection of *T.vivax* in African dwarf goats (Verstegan *et al*., 1991) resulted in fever, reduced appetite and increased metabolic rate. Dry matter intake was reduced after two weeks post infection. On an average feed intake per unit metabolic body size reduced by 13 to 24% in goat two weeks after infection with *T vivax* (Verstegan *et al*., 1991).In other studies too, the reduction of feed intake during febrile trypanosomiasis is of the same order (about 20% in goats (Akinbamijo *et al.,* 1992) and sheep (Akinbamijo *et al.,* 1994).

Significant factor contributing to increased energy expenditure would include high basal metabolic rate and a high maintenance requirement due to fever. A 15% rise in metabolic rate (Blaxter, 1989) and 25% rise in maintenance requirement (Verstegen *et al*., 1991) for every degree rise in temperature have been reported. The maintenance requirements in infected and control goats were calculated as 464 and 375 kJ ME/W kg $^{0.75}$, respectively and overall heat production was higher by 15% in infected goats (Verstegen *et al*, 1991). Similarly, Dam *et al* (1998) estimated higher maintenance requirement (406 kJ) in infected goats (*T.vivax*) than that in control (335 kJ/W kg $^{0.75}$). In another experiment, (Zwart *et al*., 1991) heat production of dwarf goats was increased by 15.6 kcal/d/kg or about 16%. The increase in heat production was greater during night (22 kcal/d/W kg $^{0.75}$) than during the day (14 kcal/d/W kg $^{0.75}$). Energy and N retention reduced during 2-4 weeks post infection. Serum thyroxin and T3 were also reduced by infection. The serum metabolites and insulin levels also reflected negative energy balance in infected groups. However, the relations between energy and nitrogen retention was not affected due to infection.

CONCLUSION

The interaction between the host and nutrition can be broadly discussed from two interrelated perspectives. Firstly, the effects of nutrition on the metabolic disturbances and pathophysiology induced by parasitism; and secondly the influence of nutrient availability on the ability of the host to mount on effective response against parasite establishment and/or development and to induce parasite rejection. The level of nutrition can thus influence the resilience and resistance of host to parasite infection. If resilience can be considered as the host ability to maintain a reasonable level of productivity upon parasite challenge and resistance is measure of the host's ability to limit the establishment, growth rate, fecundity and the persistence of parasite population. The growing interest in host nutrition-parasite interaction per se implies the improving host resilience and/or resistance to infection through management practices.

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RESEARCH ARTICLE

Treatment of Domestic Sewage Waste Water by *Phormidium sp*

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ABSTRACT

Phormidium sp of microalgae was identified and isolated from the ditch contains the Domestic Sewage Waste Water (DSWW) and it was allowed to treat the DSWW in a controlled lab environment. The treated DSWW and the untreated DSWW samples were subjected for various characterization process to evaluate its quality. The important water quality parameters such as Total Dissolved Solids (TDS), Nitrate, Phosphate, Total Hardness, Calcium, Magnesium, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were performed in both microalgae meditated treated DSWW and untreated DSWW sample. From this analysis, the results confirmed that the collected microalgae *Phormidium sp* has very good efficiency in treating the DSWW which was strongly evidenced by the water quality parameters.

Keywords: *Phormidium sp.,* Microalgae, Domestic Sewage Waste Water, coliform, Biochemical Oxygen Demand

INTRODUCTION

Exposure to contaminated water in the day to day life, possess serious issues to public health, agriculture and environment (Shakoor et al., 2017). Discharge and treatment of domestic sewage waste is still being a challenging issue mainly in the urban area (Topare et al., 2011).The improper waste disposal can cause various illness to public health. Stagnant or slowly moving sewage waste water in open system may enhance the growth of mosquitos, coliforms and other pathogenic microorganisms such as *Salmonella*, *shigella, E.coli, Pseudomonas, mycobacterium, Giardia lamblia* (Dumontet et al., 2001; Straub et al., 1993). In most of the places the outlet of sewage water is connected with river flow which causes serious issue to the aquatic organisms ((Marathe et al., 2017; Xu et al., 2019). Initially the accumulation of certain bacterial species in the sewage water increases the degradation process But, later

that accumulated bacterial species will causes infection to the aquatic organisms(Shannon et al., 2007). At some places the discharge of medical wastes along with the sewage creates drug resistant coliforms which are another serious issue to the environment(Straub et al., 1993).Throughout the world more than 80% of the waste water was directly mixing with the water bodies without adequate treatment (UN WWDR, 2017). The foremost approach of sewage waste water disposal in huge cities, towns, sub urban and rural areas are depends extra on sub surface disposal. At some places the waste water was collected from a particular cite and taken into the treatment site. In the treatment site, the collected waste was treated by conventional methods which includes, primary, secondary and tertiary methods. Though it is effective, different kind of strategies are required that are eco-friendly and cost effective. (Lloyd Jones et al., 2000). Untreated or Improperly treated waste water contains more nutrients and minerals which invites coliforms and other pathogenic microorganisms(Aditi et al., 2017; Latrach et al., 2018).The accumulated microorganisms were competitively growing in the sewage waste water with one another by utilizing the biodegradable matters. Microalgae may be the best competitor to the pathogens which can degrade the sewage waste water effectively(Mahapatra et al., 2013). There are numerous microalgal species such as *Chlorella sp*., *Arthrospira sp*., *Scenedesmus sp*. and *Nannochloropsis sp*. are naturally involves in the degradation of waste similar to bacteria but, few of them studied regarding the efficacy of waste water degradation(Al-Jabri et al., 2021).In this study it was planned to isolate the microalgal species from the sewage stream and will be evaluated for its waste water degrading efficacy.

METHODOLOGY

Collection of Microalgae

Generally algae is growing by two different forms in the rivers (Floating algae and Suspension algae). The Microalgae was collected in the ditch located at Nelson Road, Thiruvanaikovil, Tiruchirappalli (near to Srimad Andavan Arts and Science College) and the location was geographically identified at latitude 10°57'0" and longitude 10°57'0''(Figure 1).

Isolation and Identification of Microalgae

The collected algae samples were washed well in order to remove the impurities associated with it. Cleaned microalgae was inoculated in the fresh water containing adequate nutrients and marked as stock culture. The collected algae samples were further inoculated in differential media such as Blue green-11 medium, Tris-acetate-phosphate medium, Chu's medium No.10, Euglena medium, were used to isolate fresh water algae such as *Chlorella, Scenedesmus, Nostoc, Anabeana, Phormidium, Oscillatoria, Euglena, chlorogonium.* The isolated pure cultures were enumerated under light microscope(Lee et al., 2010)

Collection of Sewage Water Sample

Sewage water sample were collected from fifteen metres away from urbanized area. The domestic sewage water collected as a sample for treatment. A sterile bottle made up of plastic used to collect sewage water sample. In depth of fifteen centimetres the sample should be collected because of to avoid the surface accumulation of larvae of *Tubifex* (commonly called as sludge worms or sewage worms) and aerobic contaminants (Olds et al., 2018)

Pre Treatment of Sewage Water Sample

Sewage was contaminated with many living and non-living things. These things were macromolecules which were definitely eliminate before going to treatment. Generally elimination process was carried out by two basic steps:

Preliminary treatment: Raw untreated sewage water sample contaminate with suspended solids which were large on size. These suspended solids such as woods, rags, faecal objects were removed on this treatment under mechanical separation.

Primary treatment: Settled things present on sewage water removed by this method. After preliminary treatment the sample water (sewage) was placed with undisturbed for 24 four hours. After twenty four hours the solids which were settled and separated by filtration process using muslin cloth (Samer, 2015).

Treatment of domestic sewage water using microalgae

The isolated microalgae was introduced at 10% concentration in the pre-treated sewage sample (500 mL) for further degradation of wastes. For effective treatment process the setup was maintained in the pH of 7.1 at 20 to 31 °C. Constant agitation was provided (50 rpm) and the set up was exposed to 16:8 hours Light and dark conditions. The growth of microalgae was measured by UV-Vis spectroscopy at 680 nm (Seo et al., 2014).

Assessment of water quality

The quality of the domestic waste water was examined both before and after treatment of *Phormidium*. To examine the quality of water, important parameters like Total Dissolved Solids (TDS), Nitrate, Phosphate, Total Hardness, Calcium, Magnesium, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Faecal Coliforms level were quantified by Standard Methods for the Examination of Water and Wastewater of the American Public Health Association (APHA), 2017.

RESULTS AND DISCUSSION

In this conducted study, *Phormidium sp*. and *Chlorella sp.* of microalgal species were identified and *Phormidium sp*. was isolated as mentioned in the methodology section (Figure 2). During the treatment of raw sewage sample *Phormidium sp*. reaches its decline phase in 5 days. In specific, the growth of *Phormidium sp*. was rapid in first 48 hours.(Tricolici et al., 2014)documented that *Phormidium sp*. has very effective in the treatment of dairy industry waste water. From that report it was clearly stated that, *Phormidium sp*. takes 9 days to reach its decline phase. In this study the main reason for this rapid growth of *Phormidium sp*. is may be due to the richness of various nutrients available as biodegradable organic and inorganic waste material present in the domestic sewage waste water (DSWW).The quality of treated domestic sewage waste water was assessed by comparing it with before treatment. Inorganic substances such as (Sulphate, Nitrate and Phosphates, Calcium and Magnesium etc.,) are one of the vital components which causes drastic increase in the level of Total Dissolved Solids (TDS) and Total Hardness(Durairaj et al., 2012).In the current study the DSWW sample collected before treatment shows elevated level of TDS and Total Hardness after the treatment with *Phormidium sp* both TDS and Total Hardness were highly reduced up to 47 % and 43 % respectively (Figure 3). Further the level of Nitrate and Phosphate were also checked in the collected DSWW to support the obtained TDS and Total Hardness value. From the result (Figure 4), it was identified that the level of both nitrate and phosphate was drastically reduced 74.5 % and 67.6 % respectively(Ajala and Alexander, 2020).

In continuation, the important water quality parameters such as, Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) ware analysed (Figure 5). BOD is the best methodology which helps to quantify the amount of oxygen required for the breakdown of organic matters by the microorganisms whereas, the COD is helpful to quantify oxygen demand for the degradation of both biodegradable and non-biodegradable organic matters using chemical (Hami et al., 2007). In the conducted study, the percentage of BOD and COD reduction is 65 % and 74.4 % respectively which shows *Phormidium sp.* effectively reduced the level of BOD and COD after the treatment duration. This reduction ratio is far better than the previously published report (Wood et al., 1989). The growth of any living organism in the waste water is mainly depends upon the available of nutrients and oxygen. Apart from the utilization of nutrients and oxygen from domestic sewage, microalgae may degrade the biodegradable organic matters which may compete the growth of coliforms present in the domestic sewage(COLBOURNE and BROWN, 1979) In this study, initially in the DSWW, 100, 86 and 78 coliform colonies were recorded for 10^{-6}, 10^{-7} and 10^{-8} CFU respectively (Figure 6). After the treatment with *Phormidium sp.* the number coliform colonies were drastically reduced to 25, 17 and 10 for 10^{-6}, 10^{-7} and 10^{-8} CFU which clearly indicates that the growth of *Phormidium sp.* is actively controlling the growth of coliforms in DSWW(Abdel-Raouf et al., 2012).

CONCLUSION

From the ancient days to current situation, treatment of waste water is being a tough task to handle. Though various methods or suggestions given by the peoples, a novel and effective method or tool is required to treat the waste

water effectively at low cast and in eco-friendly manner. Microalgae mediated treatment of waste water is one of the novel, effective and non-problematic method which was clearly evidenced from this study. Yet, few more studies are in process to evaluate the toxicity of microalgae used in DSWW treatment.

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CONFLICT OF INTEREST

Author(s) of the manuscript declare(s) that there is no conflict of interest

Highlights

- Analysis of Domestic Sewage Waste Water (DSWW) quality
- Isolation and Identification of Microalgae from ditch
- Treatment of DSWW using microalgae *Phormidium* sp
- Qualitative Analysis of treated Domestic Sewage Waste Water (DSWW)

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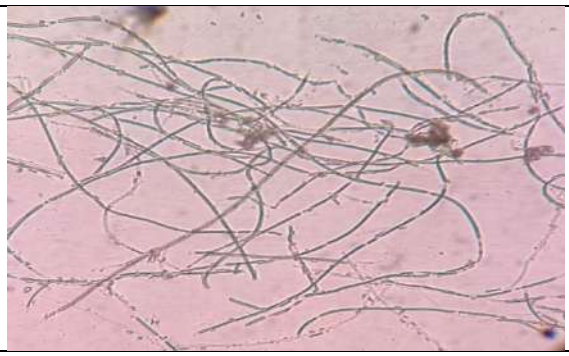
Figure 01



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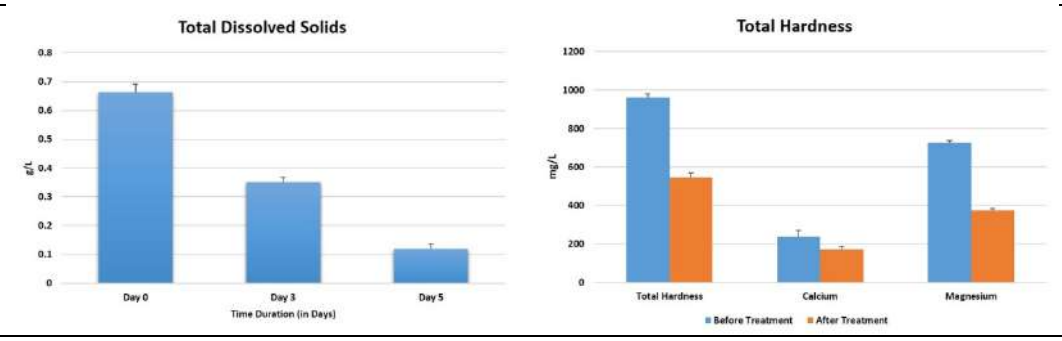

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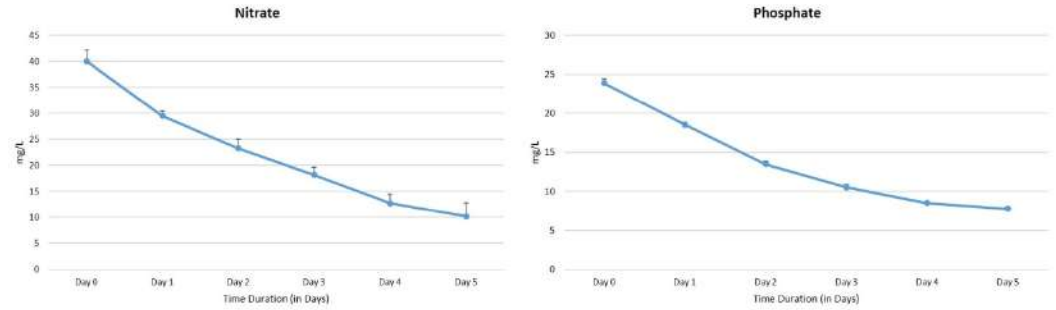

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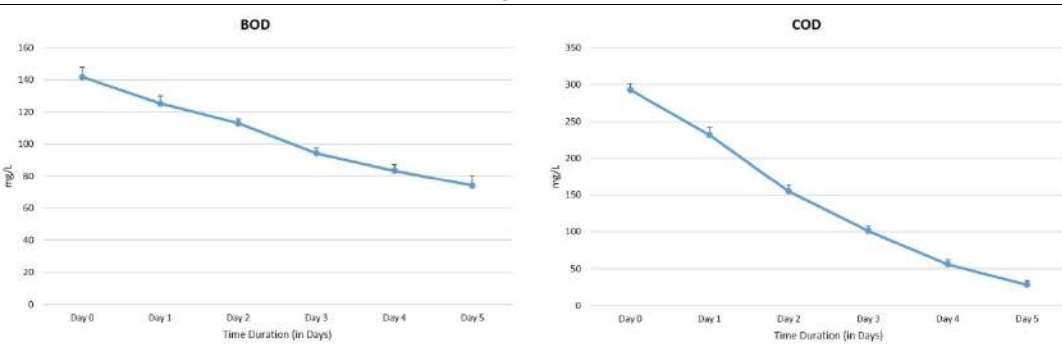

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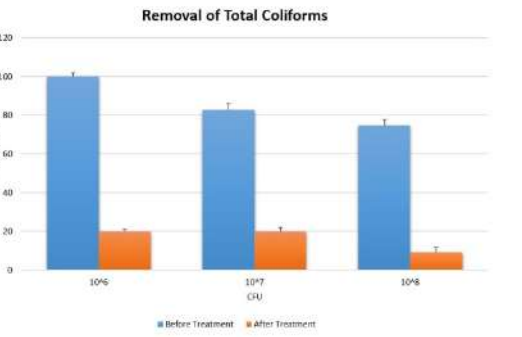
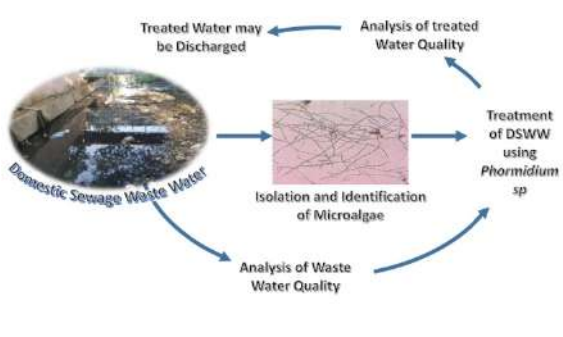


Figure 06



Graphical Abstract

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RESEARCH ARTICLE

Effect of Physostigma in Steroid Induced Glaucoma in Rabbits

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ABSTRACT

In Homoeopathy, Basic experimental studies in Glaucoma are not done with various potencies. Hence, this study is intended to verify the specific effect of Physostigma, 30CH, 200CH and 1M In Steroid Induced Glaucoma in Rabbits .Experimental model is proposed to achieve measurements to prove or disprove the hypothesis. To study the efficacy of physostigma30CH, 200CH and 1M in reducing glaucoma on steroid induced glaucoma models of male albino rabbits. To measure IOP after the induction of steroid which is treated on physostigma30CH, 200CH and 1M To compare and contrast the effect of different potency of physostigma.

INTRODUCTION

Glaucoma is a group of eye diseases which result in damage to the optic nerve and vision loss. Vision loss from glaucoma, once it has occurred, is permanent. About 6 to 67 million people have glaucoma globally. The World Health Organization has estimated that India has a 1% prevalence of blindness. [1] of the estimated 8.9 million blind in India, 12.8% are due to glaucoma. The problem is expected to reach alarming proportions by the turn of the century [2]. While there are excellent population-based data available from the West [3,4,5,6,7,8,9] such data from South Asia, especially India, are lacking. Risk factors for glaucoma include increased pressure in the eye, a family history of the condition, migraines, high blood pressure, and obesity. The homeopathic approach to treating eye disease is not new and there's a strong history of homeopathy in ophthalmology. The New York Ophthalmic Hospital was a homeopathic hospital in 1852 and it was under homeopathic management until 1867. In 1931 it treated over 31,000 patients. The American Homeopathic Ophthalmology and Otology Society existed from 1877, and was still in existence in 1941.Here are standard works on ophthalmology by homoeopaths. For example Homeopathic Therapeutics in Ophthalmology, published in 1916 by John L. Moffat, M.D., and Ophthalmic Diseases and Therapeutics, which was published in 1872 by A.B. Norton, M.D.

Homeopathic constitutional treatment will take good care of glaucoma cases. As glaucoma is progressive destructive disease, with homeopathic medication complaints will reduce and it will arrest the further progression of disease without any side effects. The most common type is open-angle glaucoma with less common types including closed-angle glaucoma and normal-tension glaucoma [9]. Open-angle glaucoma develops slowly over time and there is no pain.1 Side vision may begin to decrease followed by central vision resulting in blindness if not treated [9]. Closed-angle glaucoma can present gradually or suddenly.[10] the sudden presentation may involve severe eye pain, blurred vision, mid-dilated pupil, redness of the eye, and nausea. Regular eye examinations by your ophthalmologist are the best way to detect glaucoma. Your ophthalmologist will measure your eye pressure with Tonometry. Inspect the drainage angle of your eye with Gonioscopy. Evaluate your optic nerve with Ophthalmolscopy and test the visual field of each eye with Perimetry. Optic nerve evaluation and visual field testing are performed at regular intervals to monitor the effects of glaucoma. The information from these tests provides an indication of the effectiveness of the treatment being used and whether further treatments may be necessary. Eye pressure is measured in millimeters of mercury (mm Hg). Normal eye pressure ranges from 12-22 mm Hg, and eye pressure of greater than 22 mm Hg is considered higher than normal.

Suresh S, Ganesh Lakshmanan, Manonmani had done pilot study which attempts to study the direct action of homeopathic remedies on ciliary muscles of the eyes by testing the efficiency in myopic individuals. Random cases of 15 myopic individuals of different ages were selected from the primary clinic; the lowest age was 7 years, highest 35 years. An ophthalmologist tested the errors and noted them: a patient value ranging from -6 to -1 was treated with potentized physostigmavenenosum. Based on Stuart thorough protocols of potency selections were made. Repetitions of remedy based on potency were used. Parameters were verified after 24 weeks. Homeopathic physostigma showed positive changes in 11 out of 15 cases and no improvements in for 4 cases. Fair improvements were noted in 8 of 15, mild improvements were noted in 3 cases, which were free from symptoms. There was an overall improvement in 73.34% and no improvements in 26.66% of the cases. Moderate to good improvements were noted in 53.33%, mild improvement in 20% of the cases. This study clearly exhibits that potentized physostigma is most effective in treating short-sight, acting over ciliary muscles, evidence based in myopia [11].

Abed H. Pathan, Syed Ayaz Ali reported that the anti-glaucoma activity of aqueous methanolic ginger extract (*Zingiber officinale*) against carbomer induced experimental glaucoma in rabbits. Aqueous methanolic extract of *Zingiber officinale* was orally administered to carbomer induced glaucomatous rabbits. Pilocarpine 2% eye drop was used as a standard drug. Intraocular Pressure (IOP) levels were determined after oral administration of a dose of *Zingiber officinale* (200 mg/kg, p.o.) in glaucomatous rabbits. IOP were determined for four weeks after oral administration of aqueous methanolic extract of *Zingiber officinale* (200 mg/kg, p.o). An aqueous methanolic extract of *Zingiber officinale* was found to reduce intra ocular pressure in carbomer induced experimental glaucoma in rabbits. Sufficient reduction in IOP was observed from second week of administration of ginger extract. A significant decrease in IOP ($p<0.01$) was observed in animals treated with standard pilocarpine and aqueous methanolic ginger extract. The effect of extracts of *Zingiber officinale* on serum pseudocholineterase was also measured. A significant decrease in the level of pseudocholinestrase ($p<0.01$) was observed in the rabbit serum treated with aqueous methanolic extract of ginger [12].

Prabhakar Adake, H. S. Somashekar, C. G. Gokul, Abhishek Acharya, M. Naveen Kumar and R. Santosh reported that Glaucoma was induced in rabbits (N=18) by bilateral topical instillation of 1% prednisolone eye drop (10 µl) twice a day for a period of 40 days. Before the induction of glaucoma, baseline intraocular pressure (IOP) in both the eyes of all rabbits was measured under sedation (i.v midazolam) by Schiotz tonometer. At the end of 40 days induced IOP was measured for all rabbits and rabbits were divided into three groups of six rabbits in each. Right eyes of group A, B and C rabbits received 0.5% diltiazem, 0.1% verapamil, and 0.5% timolol eye drops twice daily for 12 days respectively. Whereas, left eyes of all rabbits received distilled water hence represented as control. IOP was measured in all rabbits on every 4th day till 12 days of treatment period. Intra-group comparisons of IOP changes were made by paired't' test. And unpaired 't' test for inter group comparisons. One way ANOVA was used for

multiple group comparisons followed by post-hoc Tukey's test for group wise comparisons. In 0.5% diltiazem treated eyes, the mean IOP significantly reduced from 22.9±1.9 mmHg (10%) on 4th day to 16.9±1.1 mmHg(S, *P*<.001) on 12th day (34%). Similarly, mean IOP in 0.1% verapamil treated eyes significantly reduced from 22.7±1.3 mmHg (7%) on 4th day to 15.5±1.4 mmHg(S, *P*<.001) on 12th day (37%). Whereas, mean IOP significantly reduced from 22.4±1.9 mmHg (14%) on 4th day to 16.4±1.4 mmHg (S, *P*=.001) on 12th day (36%) in 0.5% timolol treated eyes [13].

METHODS

Animals

Rabbits, weight 1.5 to 2.5kgs are included in this present study. The Rabbits are procured from Sri Venkateswara Enterprises Bengaluru. The Rabbits are imbared in the central Animal house of the Department of pharmacology, Vinayaka Missions College of Pharmacy, Salem. Physostigma Homoeopathic medicines are purchased from Reputed Pharmaceutical Industry which is preparing under Indian Homoeopathic pharmacopeia, 25 Rabbits are divided into 5 groups ,Each group consist of 5 Rabbits. Four Rabbits in each cage, they are randomly housing at a controlled temperature 21±3c With a 12 hours light, 12 hours dark cycle. Base line Intra Ocular Pressure (IOP) for both eyes of all Rabbits are measured before induce glaucoma, To induce glaucoma in Rabbits steroid model instilled with 10µl of I.V prednisolone eye drops twice a day for a period of 40 days.

Drugs

1% prednisolone acetate (steroid) eye drops for induce glaucoma.
Physostigma 30C, 200C, 1M for test drug for glaucoma (Group wise).
0.5% Timolol eye drops is standard drug to reduce glaucoma.(Test group of Rabbits)
4% xylocaine eye drops for anaesthetic before measuring IOP by Schiotz tono meter.
Midozolam IV for sedation of Rabbits.

Study Procedure

Before starting the research work first get clearance from Institutional Animal Ethical Committee (IAEC). Totally 25 Albino Rabbits (n=25) is using for research study. By lateral topical instillation of 1% prednisolone for induce elevation of IOP in both eyes of all Rabbits above base line level (Once a day). Induce IOP measuring at the end of 40 days by Schiotz tonometer. The cortico steroid induce glaucoma is well known in human and closely resemble the human disease in clinical feature as well as in the underline mechanism [13]. After the induction of glaucoma Rabbits were divided into 5 groups of 5 Rabbits in each potency.

Group I : Normal Group Without any medication
Group II : 2 Drops of Timolol (Standard drug) into both eyes 2 times per day in left Eye.
Group III: 2 Drops of physotigma 30CH Eye drops gives 2 times a day in left Eye.
Group IV: 2 Drops of physotigma 200CH Eye drops gives 2 times a day in left Eye.
Group V : 2 Drops of physotigma 1M Eye drops gives 2 times a day in left Eye

IOP is measure in both eyes for all Rabbits on every fourth day till end of the twelfth day of treatment period. Before measuring IOP giving sedation of all the Rabbits with intravenous (marginal ear vine) Midozolam in a dose of 0.5 – 1.0 mg/kg and cornea is anaesthetized with topical 4% xylocaine drops. For this study conversion table used to derive the IOP in millimeter of mercury (mm Hg) from the scale reading and plunger weight. To avoid Diurnal variations of IOP all Tonometeries perform at the same time of the day preferably in the morning hour (Around 9 AM).

Statistical Analysis

Collect the IOP reading are express as mean ±SD. Intra group comparison of IOP changes measuring by paired 't test and unpaired 't test for intergroup comparison. One way ANOVA is using for multiple group of comparison followed by post-hoc Tukey's test.

RESULTS & DISCUSSION

In this study there are five groups are considered with the four types of days they are as follows Group 1: Normal no any medication, Group 2: Standard drug timolol maleate Group 3: Physostigma 30ch Group 4: Physostigma 200ch Group 5: Physostigma 1m, and the days are classified as day 0, day 4, day 8 and day 12. So based on the prospective studies the samples have been collected. The number of samples collected in this study are 25 sampled observations.

Null Hypothesis H_{01}: There is no significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m.

Null Hypothesis H_{02}: There is no significant differences among the days of day 0, day 4, day 8 and day 12.

Alternative Hypothesis H_{11}: There is significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m.

Alternative Hypothesis H_{12}: There is significant differences among the days wise of drugs.

The analysis and results are tabulated and given in Table 1. It gives information regarding the descriptive statistics which gives the measures like mean, standard deviation and the number of sample observations. The table 2 gives the result of the two-way ANOVA while the study having two independent variables like group of the drugs and the different types of days of study. The mean of Groups significant value that is p-value which is lesser than the 0.05. Hence the null hypothesis of this study is rejected and it's concluded that there are significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m. The mean of Days significant value that is p-value which is lesser than the 0.05. Hence the null hypothesis of this study is rejected and its concluded that there are significant differences among the days of drugs.

The mean of interaction effect of Groups and Days significant value that is p-value which is less than the 0.05. Hence the null hypothesis of this study is rejected and its concluded that there are significant differences among the groups and days of drugs. There for the null hypothesis is rejected by testing the significance difference through the two-way ANOVA. So the next proceeding is to check the interaction levels through the multiple comparisons tests (Tukey test) to identify the effects of iterations in the interaction levels. The analysis and results are tabulated in Table 3. It gives the mean value 34.061 for the study with the standard error of 0.180 and the confidence interval has been built with the 95% of lower bound is 33.703 and the upper bound is 34.420.

In table 4 while considering the interactions effects with in the group while considering the Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m having significant difference with the Normal no any medication. But there is no significant difference between Physostigma 30ch, Physostigma 200ch, Physostigma 1m having significant difference with the Normal no any medication. From the table 5 while considering the interaction effects with in the days while considering the day 0, day 4, day 8 and day 12 have the significant difference among them. Figure 1, shows the means of drugs of the Physostigma 30ch works in parallel with drugs Standard drug timolol maleate. When drugs of Physostigma 200ch and Physostigma 1m are compared with drug Standard drug timolol maleate is only a small difference.

CONCLUSION

Timolol maleate is the Standard Medicine for Glaucoma in Allopathy. In Homoeopathic System of Medicine Physostigma shows good improvement in Steroid Induced Glaucoma. In that, Physostigma 30 CH shows more improvement comparatively with other Potencies. Based on the statistical analysis Physostigma shows improvement in Glaucoma.

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Table 1. Descriptive statistics

Group	Days	Mean	Std. Deviation	N
Normal no any medication	0 Day	45.7600	1.42934	5
	4th DAY	45.7600	1.42934	5
	8th DAY	45.7600	1.42934	5
	12th DAY	45.7600	1.42934	5
	Total	45.7600	1.31165	20
Standard drug timolol maleate	0 Day	45.7600	1.42934	5
	4th DAY	34.7580	3.33284	5
	8th DAY	23.4400	1.39571	5
	12th DAY	17.9800	1.17771	5
	Total	30.4845	11.13056	20
Physostigma 30ch	0 Day	45.7600	1.42934	5
	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	18.1400	.96073	5
	Total	30.8590	11.22279	20
Physostigma 200ch	0 Day	45.7600	1.42934	5

	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	20.7000	.91924	5
	Total	31.4990	10.49242	20
Physostigma 1m	0 Day	45.7600	1.42934	5
	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	21.5200	.88431	5
	Total	31.7040	10.27374	20
Total	0 Day	45.7600	1.30480	25
	4th DAY	37.7972	4.74737	25
	8th DAY	27.8680	9.26147	25
	12th DAY	24.8200	10.82570	25
	Total	34.0613	11.15430	100

Table 2. Two-way Analysis of Variance from Groups and Days

Source	Sum of Squares	df	Mean Square	F	Sig.
Group	3440.601	4	860.150	264.793	.000
Days	6864.378	3	2288.126	704.389	.000
Group * Days	1752.583	12	146.049	44.960	.000
Error	259.871	80	3.248		
Total	128334.649	100			

Table 3. Estimated Marginal Means

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
34.061	.180	33.703	34.420

Table 4. Multiple Comparisons from the Groups

Group	Group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Normal no any medication	Standard drug timolol maleate	15.2755*	.56995	.000	13.6848	16.8662
	Physostigma 30ch	14.9010*	.56995	.000	13.3103	16.4917
	Physostigma 200ch	14.2610*	.56995	.000	12.6703	15.8517
	Physostigma 1m	14.0560*	.56995	.000	12.4653	15.6467
Standard drug timolol maleate	Normal no any medication	-15.2755*	.56995	.000	-16.8662	-13.6848
	Physostigma 30ch	-.3745	.56995	.965	-1.9652	1.2162
	Physostigma 200ch	-1.0145	.56995	.392	-2.6052	.5762
	Physostigma 1m	-1.2195	.56995	.214	-2.8102	.3712
Physostigma 30ch	Normal no any medication	-14.9010*	.56995	.000	-16.4917	-13.3103
	Standard drug timolol maleate	.3745	.56995	.965	-1.2162	1.9652
	Physostigma 200ch	-.6400	.56995	.794	-2.2307	.9507
	Physostigma 1m	-.8450	.56995	.577	-2.4357	.7457
Physostigma 200ch	Normal no any medication	-14.2610*	.56995	.000	-15.8517	-12.6703
	Standard drug timolol maleate	1.0145	.56995	.392	-.5762	2.6052
	Physostigma 30ch	.6400	.56995	.794	-.9507	2.2307

	Physostigma 1m	-.2050	.56995	.996	-1.7957	1.3857
Physostigma 1m	Normal no any medication	-14.0560*	.56995	.000	-15.6467	-12.4653
	Standard drug timolol maleate	1.2195	.56995	.214	-.3712	2.8102
	Physostigma 30ch	.8450	.56995	.577	-.7457	2.4357
	Physostigma 200ch	.2050	.56995	.996	-1.3857	1.7957
**. The mean difference is significant at the .05 level.*						

Table 5. Multiple Comparisons from the Days

Days	Days	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 DAY	4th DAY	7.9628*	.50978	.000	6.6252	9.3004
	8th DAY	17.8920*	.50978	.000	16.5544	19.2296
	12th DAY	20.9400*	.50978	.000	19.6024	22.2776
4th DAY	0 DAY	-7.9628*	.50978	.000	-9.3004	-6.6252
	8th DAY	9.9292*	.50978	.000	8.5916	11.2668
	12th DAY	12.9772*	.50978	.000	11.6396	14.3148
8th DAY	0 DAY	-17.8920*	.50978	.000	-19.2296	-16.5544
	4th DAY	-9.9292*	.50978	.000	-11.2668	-8.5916
	12th DAY	3.0480*	.50978	.000	1.7104	4.3856
12th DAY	0 DAY	-20.9400*	.50978	.000	-22.2776	-19.6024
	4th DAY	-12.9772*	.50978	.000	-14.3148	-11.6396
	8th DAY	-3.0480*	.50978	.000	-4.3856	-1.7104
**. The mean difference is significant at the .05 level.*						

Table 6. Mean Drugs of Groups and Days

Days	0 Day	4th Day	8th Day	12th Day
Normal no any medication	45.75	45.75	45.75	45.75
Standard drug timolol maleate	45.75	34.76	23.44	17.98
Physostigma 30ch	45.75	36.16	23.38	18.14
Physostigma 200ch	45.75	36.16	23.38	20.7
Physostigma 1m	45.75	36.16	23.38	21.52

Table 7. Comparison on 12th Day

Rabbit Sl. No	Group: A (Normal No Any Medication)	Group: B (Standard Drug Timolol Maleate)	Group: C (Physostigma 30 CH)	Group: D (Physostigma 200 CH)	Group: E (Physostigma 1M)
01	46.9	18.0	17.3	21.3	21.9
02	45.8	19.6	18.5	19.6	20.1
03	45.8	16.5	17.3	20.1	21.3
04	46.9	17.3	19.6	21.9	21.9
05	43.4	18.5	18.0	20.6	22.4

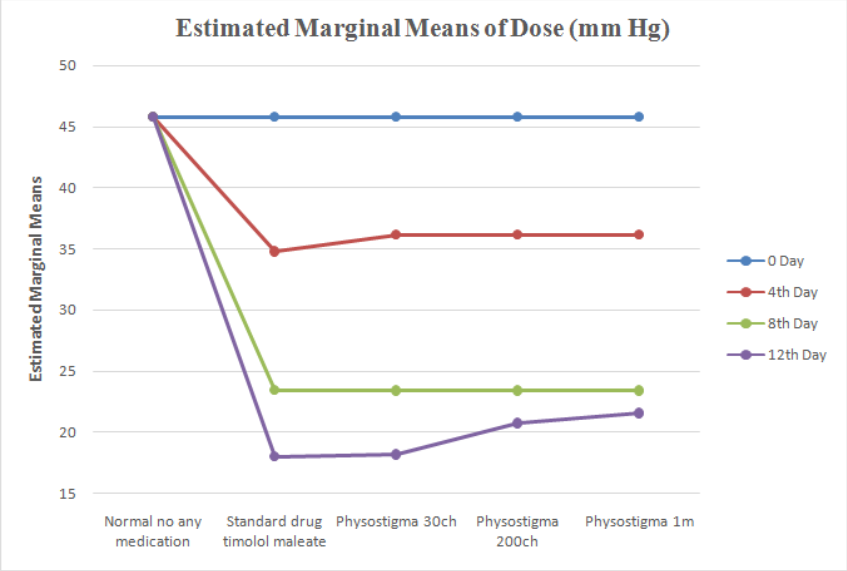

Figure 1. Estimated Marginal Means of Dose (mm Hg)

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RESEARCH ARTICLE

Production and Localization of Methionine -ɤ- Lyase from Estuarine Bacterial Communities and Evaluation of Its Methionine Utilization Potential

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ABSTRACT

Methionine -ɤ- lyase is widely distributed in the three domains of life and plays a central role as an anticancer drug. Seven different species of methionine ɤ lyase producing bacterial strains were isolated from the estuarine environment. Based on their Physiological and Phylogenetic characteristics, the organisms were identified as *Bacillus sp, Microbacterium sp, Pseudomonas sp, Stenotrophomonas sp, Acinetobacter sp, Paucisallibacilli sp, and Staphylococcus sp* and 16s r RNA sequences were submitted to the Genbank. Demethiolation activity was quantified for all the seven isolates. Further, the localization of Methionine-ɤ- lyase was carried out by cell fractionation technique. The activity of Methionine -ɤ-lyase was found to be abundant in the extracellular fluid followed by the cytoplasm, periplasmic space, and bacterial membrane. This of maximum enzyme productivity of 62U/ml was found in *Stenotrophomonas sp* and was further characterized by using Atomic force microscopy imaging.

Keywords: Methionine gamma lyase, Subcellular localization, Methionine Quantification, Estuary bacteria, *Stenotrophomonas*, Atomic force microscopy

INTRODUCTION

Methionine is the sulfur containing essential amino acid that acts as a precursor for cysteine, taurine, homocysteine, and cystothionine biosynthesis (Stipanuk and Martha H 2004).Biological processes such as methylation of DNA, polyamine synthesis and mammalian protein synthesis are mainly determined by the level of methionine. Excessive methionine dependence was observed in many types of cancer cells for their survival and proliferation. Hence, methionine restriction is a principle approach in metabolic control of cancer cells (Cavuoto *et al.,* 2012). Malignant and transformed cell lines of breast, bladder, colon, melanoma, prostate was observed as methionine dependent in nature. Methionine restriction leads to considerable extension of lifespan in fruit flies (Cellarier E*et al.,* 2003).Artificially this methionine restriction and depletion can be attained by certain lyases which can deform methionine. From archea to plants excluding humans have an idiosyncratic enzyme methionine gamma-lyase (MGL, EC 4.4.1.11) which depend on pyridoxal-L-phosphate (PLP) to transfigure sulfur containing amino acids such as methionine and cysteine to alpha-keto acids, ammonia, and volatile thiols by α,γ - elimination and γ replacement reactions. The enzyme was first discerned in rumen bacteria by Weisendanger and Nisman in 1953. Among five distinct fold of PLP-dependent enzymes, MGL belongs to fold type 1 Aspartate Aminotransferase (AAT) family. It was effectively indicated as a dynamic curative agent against numerous sorts of tumors (Sato, Dan and Tomoyoshi Nozaki). *In vitro* methionine limitation by Methionine -ɤ -lyase arrests the cancer cell growth in the late S/G2 phase (Lu *et al.,* 2000). Wide-ranging pre-clinical studies have been carried out with *Pseudomonas pudita* methioninase expressed in *E.coli* (Ohigashi K *et al.,* 1951) Bacteria such as *Pseudomonas ovalis , Aeromonas*, *Brevibacterium linens*, *Citrobacter freundii* and fungus *Aspergillus flavipes*, *Trichoderma koningii,* are the extensive methioninase producers which were reported earlier. However, Methionine -ɤ -lyase from microbial communities possesses high immunogenicity and shorter life span. Hence, there is a persistent demand to establish and promote neoteric microbial communities to procure high yield of pharmacologically potential Methionine -ɤ -lyase without any imperfection (El-Sayed and Ashraf S., 2010). It is noteworthy that bacterial population present in the estuarine environment holds potential enzymes and high specific affinity for amino acids. Therefore, estuary will be the striking source for the isolation of microbial communities with superior Methionine -ɤ -lyase production. In all the earlier studies, researchers concluded L-methioninase as an intracellular enzyme in bacteria and extracellular in fungi. Although methioninase is well documented, only very few reports are available on its existence inside other organelles such as periplasmic space and bacterial membrane. Hence, the subcellular localization plays a crucial role in proper bioprocess development for the acquisition of an enzyme. In this article, we report a wide range screening of 113 estuarine bacterial isolates to evaluate their methionine -ɤ -lyase production potential and localization of the neoteric enzyme.

MATERIALS AND METHODS

Site Description and Sample Collection

Fort Kochi is a small estuary located in Kerala, South India connected east side to the Arabian sea and the west side to the Vembanad Lake. (Fig-1) Five different sites such as Fort Kochi- Vipin Ferry terminal (9°58'08.95N and 76°14'38.87''E), Ernakulum (9°58'38.79''N and 76°15'54.97''E) Fort kochi beach 9°58'10.50''N and 76°14'21.62''E), Azhimugam (9°57'55.78''N and 76°13'20.67'' E), Vypin beach (9°58'24.81"N and 76°13'2.98"E) were chosen and sample collection was performed in the month of August 2014. Water samples were taken from approximately 15 cm below the surface of Estuary at five different sites. (Faust, Maria A., 2010 and Jackson, C. R., 2005) and the temperature of surface water and pH were measured. All water samples were routinely filtered through Whatman no. 1 filter paper to remove larger particles and further filtered by 0.22 μM pore size filter. Salinity, TSS, dissolved oxygen (DO) and BOD, was measured by standard methods (Dixit et a l., 2013).

Isolation and Screening of Microorganisms

For the enrichment of bacterial communities, 1 ml of estuary water sample was inoculated in estuarine saltwater agar medium composed of (gl^{-1}) yeast extract -1; Peptone -1; NaCl-10.0; KCl-0.25; $MgSO_4.6H_2O$ - 0.23; Final pH -7.2 (Goulder R., 1976) and the final concentration of added salts was 10.5%. The flasks were incubated on an orbital shaker at 30°C for 48 hrs. After incubation, the samples were serially diluted and inoculated on estuarine agar (EA) as described by ZoBell (MacLeod *et al.,* 1954), with modified salinity by adding estuary water instead of sea water. Inoculated plates were incubated at 15, 24, 30 and 36°C. Morphologically different colonies were sub cultured after 12, 18, 24 and 48 hours incubation and pure cultures were obtained. Isolated colonies were tested for the production of Methionine-ɣ-lyase in minimal methionine agar blogs(8mm diameter) containing (g/L) L-Methionine -10;KCl -2.5; K_2HPO_4 -5;$FeSO_4.7H_2O$-0.01;$ZnSO_4.7H_2O$ -0.01;NaCl-0.1 and Phenol red-0.12.

Physiological Characterization and Molecular Identification of Bacteria

The isolated MGL producers were characterized by their morphological and biochemical features according to Bergeys Manual of Determinative Bacteriology (Lechevalier, H. A., 1989). They were tested for catalase, hydrogen sulphate, oxidase, amylase production and nitrate reduction. Enzyme activities such as proteolysis, lipolysis, deamination, and decarboxylation were also tested. Utilization of various carbon sources were studied by using complex medium containing glucose, lactose, rhamnose, glycerol, sucrose, mannose and arabinose as a carbon source. Genomic DNA was isolated by CTAB-NaCl method (Wilson and Kate., 1987). The 16S rRNA genes from bacteria were amplified through 27F-1492R primer combinations (Weisburg., 1991). Amplification reactions were carried out with 10 pmol μl^{-1} of primers, 50 ng of template DNA, 5 µl of 10X reaction buffer, 1.5 mM $MgCl_2$, 200 µM dNTP mix, 5U Taq polymerase (Genet bio- HS Prime Taq) with the standard conditions . The amplicon was sequenced and the partial bacterial 16S rRNA sequences were compared with the existing sequence by using NCBI BLAST-N (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to classify the sequence with supreme similarity and also to determine their approximate phylogenetic affiliations. The Phylogenetic tree was constructed by MEGA 6.0 version using neighbor joining (NJ), of Tamura –Nei model. The obtained 16 S rRNA sequences were submitted to NCBI Genbank.

Assessment of Methionine Degradation Potential of the Isolates

Methionine utilization was quantified to ensure the methionine degradation potential of the isolates. Isolates were inoculated on modified M9 Medium containing the following components (gL^{-1}) L-methionine - 4, glucose -2, Na_2HPO_4 - 6, KH_2PO_4- 3,MgSO4.7H2O -0.24, NaCl- 0.5, $CaCl_2$- 0.011 and the final pH of the medium was adjusted to 7 and incubated from 12 h to 4 days to quantify day wise methionine degradation (Ruiz-Herrera et al.,1969 and El-Sayed *et al.,* 2012). After incubation, cell -free supernatant was obtained by centrifugation and remaining methionine in the media was quantified by modified method of Rose WC *et al.,* NaOH (0.5 ml of 5N) was added to the culture filtrate followed by the addition of 50 µl of 10% sodium nitroprusside solution with complete mixing. The tubes were incubated in a water bath at 37°C for 10 min and then chilled in an ice bath for 5 min. The reaction mixture was incubated for 10 min at room temperature at still condition. Glycine (1 ml of 3%) was added with vigorous shaking for 10 -15 min . To each tube, 1 ml of HCL and ortho phosphoric acid mixture (1:9) was added with proper stirring and incubated for 20 min. The reaction mixture was incubated for 10 min for color development and the OD was measured at 540 nm.

Quantification of End Products

The demethiolating activity was determined by quantifying the amount of free thiol liberated by using Ellman's reagent with the formation of yellow complex (Laakso, S., 1976). Quantification was carried out in 50mM potassium phosphate buffer holding 20mM methionine, 0.1mM pyridoxal phosphate, and 0.25 mM DTNB in a final volume of 1 ml. Reaction blanks were prepared separately without enzyme and substrate. Ethanethiol was used as an internal standard. The entire reaction mixture was incubated for 30min at 37°C in a closed vial. The optical absorbance was recorded at 412nm (Ferchichi, M *et al.,* 1976). Keto acids released during the enzymatic reaction were estimated

according to the method of Tomoaki Takakura *et al* 2003. α - keto acids released was quantified by preparing the reaction mixture consists of 100mM potassium Phosphate buffer containing 0.01% DTT, 10mM EDTA, 10µM PLP, 25 mM methionine. The vials were sealed tightly after the addition of 100µl of cell free supernatant enzyme. The overall enzymatic reaction was stopped by the addition of 100 µl 10% TCA. The reaction mixture was centrifuged at 10000 ×g for 15 min at 4°C and 100µl of supernatant was incubated with 140 µl of 0.1M sodium acetate buffer at pH 5.0 containing 1.33 mM 3-Methyl-2-benzothiazolinone hydrazine (MBTH) hydrochloride at 50°C for 30 min. The amount of α-Keto glutarate released was quantified spectrophotometrically at A_{335} and the standard curve was plotted with α-Keto glutarate (Sun *et al.,* 2013).

Subcellular Localization of Methionine and Methionine -ɤ-Lyase

Cultures were grown in methionine enriched semi synthetic medium till the attainment of stationary phase and centrifuged at 10000×g for 6 min at 4°C. The cell free supernatant was assessed for extracellular enzyme activity. The cells were suspended and washed with 50mM potassium phosphate buffer (pH 8.2). Half of the cell mass was suspended in 50mM potassium phosphate buffer containing 1mM PMSF, 1mM EDTA, and 0.02 mM PLP (Dias *et al.,* 1998). The content was ultra sonicated at 20 MHz, 40% amplitude for 5 cycles (2mins/cycle with 1.5s on and 0.5 sec off). The lysed cells were kept undisturbed for further 10 min at 4°C and centrifuged at 13000×g for 1 hour at 4°C. Intracellular cytoplasmic enzyme activity was assessed after collecting the supernatant (Amarita *et al.,* 2004). The cell debris was suspended in 3 ml of the above buffer and divided into three equal parts. To each tube 0.0001% (w/v) SDS, 1% Triton X 100 and 1mM EDTA were added respectively and incubated for 10 min. The mixtures were ultrasonicated and incubated with shaking at 180 rpm at 20°C for 30 min. The samples were ultra-centrifuged at 20,000 ×g for 1 hour at 4°C and the membrane bound enzymes were extracted and the activity was quantified from the supernatant. Periplasmic enzyme fluid extraction was carried out as described by Nossel and Heppel (Guyer *et al.,* 1985). Additional half of the cells were taken and re-suspended in 10 volumes of periplasmic fluid extraction medium consisting of 30% of sucrose dissolved in 33 mM potassium phosphate (pH 8) amended with 1mM EDTA (Kumar et al.,2010). The mixture was agitated with the stirrer for 20 min at 25°C and then centrifuged at 10000×g for 4°C for 15min.The supernatant was decanted and the pellet was well drained and dispersed in distilled water (40ml/gram). The suspension was incubated at -20°C for 15 min with vigorous agitation. The cells were centrifuged at 20000×g for 20 min at 4°C. The supernatant was analyzed for periplasmic Methionine -ɤ -lyase by estimating the end products namely, ethanethiol and keto glutarate. Unit enzyme activity (U) was designated as the amount of enzyme required to release 1µmole of methanethiol or Keto acids under optimal assay conditions. The total protein was quantified in each organelle according to Lowry et.al (1951) using BSA as an internal standard. Specific activity was expressed as units/mg of protein

RESULTS

Isolation and Identification of Methionine - ɤ-Lyase Producing Microorganism

Water samples were collected from five different places of Fort Kochi by random sampling physiochemical parameters (Table 1). The enrichment of the water sample promoted the revival capacity of the microbial communities present in the water. A total of 128 strains were isolated from estuarine water and analyzed for methionine -ɤ-lyase production. During screening, methionine was amended as the only nitrogen source to restrict the non-methioninase producers. As a result, 7 strains showed the production of ɤ-lace which was evident from the color change of the methionine agar blocks from yellow to pink. The efficient enzyme producing strains were further selected and identified by physiological (Table 2) and morphological characterization and by 16s rRNA sequencing (Figure 3). Based on the 16s rRNA sequencing, the isolates were identified and designated as, *Stenotrophomonas maltophilia* DMTMM1 (Accession No-KM588914.1), *Acinetobacter* sp., DMTMMS001 (Accession No-KJ850908), *Microbacterium* sp (Accession No –KJ850909) *Paucisalibacillus* sp DMTS003 (Accession No KJ850910)., *Staphylococcus* sp DMTMMS004 (Accession No KJ850910), *Pseudomonas* sp DMTMMS005 (Accession No KJ850912)., and *Bacillus* sp DMTMM006 (Accession No-KJ850913). The maximum amount of the enzyme was produced by *Stenotrophomonas*

maltophilia DMTMM1 which was less studied and morphological data was very less reported. So the atomic force microscopy image (Figure 2) revealed that the cells of the strain DMTMM1is rod shaped bearing lophotrichous flagella and forms Diplobacilli. The strain formed a flat patched layer on the agar surface with distinct cells being visualized as well. The bacterial dimensions characterized are as follows: (L) = 1.98µm, width (D) = 0.72 µm, roughness(R) = 2.11 µm.

Assessment of Methionine Degradation Potential of the Isolates

Depletion of methionine was quantified for every 6 hours up to 72 hours of incubation (Figure 1). None of the bacterial strains utilized methionine within 6 hours of incubation. However, gradual decrease in methionine level was observed after 12 h of incubation and complete degradation after 72 h methionine was observed with *Pseudomonas sp,* and *stenotrophomonas* sp. The rate of methionine utilization was very high between 24 to 48 h of incubation. Only a meager amount of methionine utilization (0.1mg) was observed with *Bacillus sp, Microbacterium sp, Pseudomonas sp,* and *Stenotrophomonas* sp even after 12 h of incubation.

Methanethiol and Keto-glutarate Quantification

Demethiolation lead to the production of methanethiol confirming the synthesis of methionine-ɤ-Lyase synthesis by the isolates. All the seven bacterial strains that utilized methionine were quantitatively assayed for methanethiol and keto-glutarate. Results obtained shows that *stenotrophomonas maltophilia* DMTMM1 possess the highest methanethiol and keto-glutarate producing capacity under optimum assay conditions. Unit enzyme activity and specific activity was quantified and tabulated based on the µmoles of methanethiol or α-keto acids released under the assay conditions.

Localization of Methionine -ɤ-Lyase

Subcellular localization was carried out with a view to establish an worthwhile bioprocess for the yielding of methionine - ɤ-Lyase. Whole cells of *Bacillus sp, Microbacterium sp, Pseudomonas sp, Stenotrophomonas sp, Acinitobacter sp, Paucisalli bacilli, and Staphylococcus sp* were subjected to organelle fractionation. Being Gram positive bacteria with a profuse cell wall, only a trace amount of extracellular enzyme activity was detected in the cell free extracts of *Bacillus sp, Pausicilibacillus, Staphylococcus* and *Microbacterium.* The quantity of extracellular enzyme high in *Stenotrophomonas*, *Pseudomonas* and *Acenitobacter sp.* The membrane bound enzyme was isolated using SDS, Triton X – 100 and EDTA. The maximum activity of membrane bound fractions was achieved with 0.001 % SDS when compared to Triton X 100 and EDTA. The extracellular enzyme activity was found to be high in gram negative organisms which possess the low periplasmic enzyme. No periplasmic and membrane bound enzyme activity was observed with *Staphylococcus sp*.

DISCUSSION

Estuary water accommodate well build biological and chemical grade with enormous nutrients from the surrounding watershed (USEPA 1993). Decomposition of organic matters releases inorganic compounds which can be used by autotrophs and heterotrophs for growth and metabolism .The estuarine environmental factors such as organic matter quality, climate, presence and absence of oxygen and wide range of salinity intensifies microbial growth and activity. Fortkochi estuarine eco system is unexplored and may supply a rich source of microbial populations yielding a novel and efficient enzymes and metabolites. Hence there is an extensive hope to pick out and characterize novel estuarine bacterial communities for L-Methioninase production. The bacterial strains described in this study were isolated as predominant utilizers of methionine from the subsurface estuarine water from Fort kochi. The wide range of bacteria able to utilize and degrade methionine includes genera such as *Achromobacter*, *Aeromonas, Brevibacterium, Citrobacter, Pseudomonas, Clostridium and Micrococcus*. Our present study broadens this range by including the genus *Stenotrophomonas, Acenetobacter* and *Pausacilibacillus*. The strains belonging to this genus have never been reported to have methioninase production .Of 128 isolates 7 exhibited methionine production

.Methionine utilization potential was observed from first hour of incubation to 72 hours and the activity of methioninase get increased with the depletion of methionine content in the medium.The maximum L-methioninase productivity (31.28 U/mg) with complete methionine degradation after 72 hours and the maximum biomass of 2.82 g/l was attained by *Stenotrophomonas* Sps., followed by *Pseudomonas* Sps., which is already well studied for methioninase with the activity of 26.65 U/mg with the biomass of 2.73 g/l. The least enzyme productivity and methionine utilization capacity were deducted in the cell free supernatants of *Staphylococcus* Sps and *Microbacterium* Sps. No Published reports have discussed about the subcellular Localization of methioninase in Periplasm and cell membrane. The extracellular release of methioninase without cell lysis probably involves a highly selective permeation process which depends on cell wall composition From this proceeding screening and Localization attempt it is clear that the *Stenotrophomonas maltophilia* DMTMM1was the most auspicious isolate for the production of L-Methioninase .Consequently, it was preferred for sub sequential experiments and the clear structure and size was measured by atomic Force imaging.

CONCLUSION

Methionine -ɤ-lyase is a potent therapeutic agent against various types of cancer, but leads to immunogenic downsides which induce the quest of new sources with high therapeutic efficacy with low immunogenic effects. The existing study focused on the search of novel methionine gamma lyase producers from estuarine environment which possess higher enzyme activity. A total of 128 bacterial strains were isolated and screened for enzyme production. Seven effective methionine decompose strains were identified and their sequence submitted in gene banks. *Stenotrophomonas maltophilia* was found to be the most potential isolate. Perhaps this is the first report of methioninase production and localization in *stenotrophomonas*.

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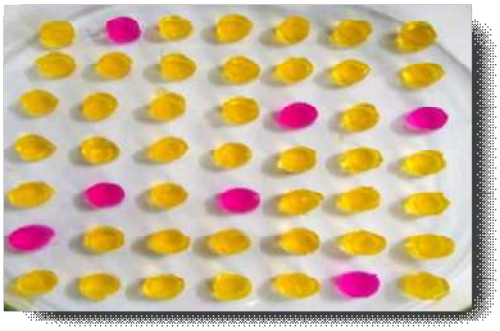

Figure 1- Screening of minimal methionine agar blogs for methionine -ɤ-lyase production. Blogs turned to pink because of ammonia release which raises the pH

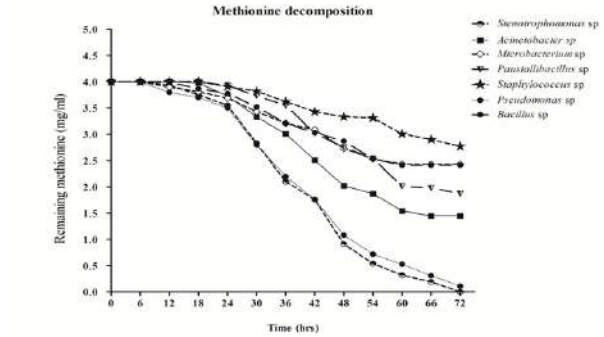

Figure 2- Decomposition of methionine from 12 to 72 hours of growth

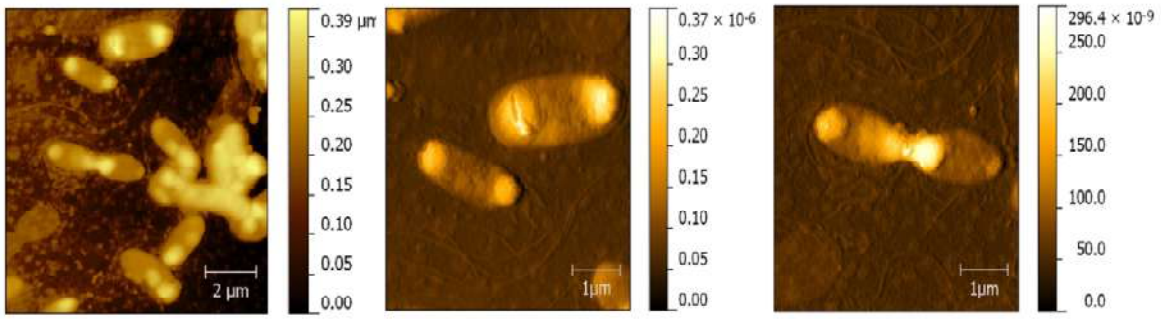

Figure 3- Atomic force imaging of DMTMMS001

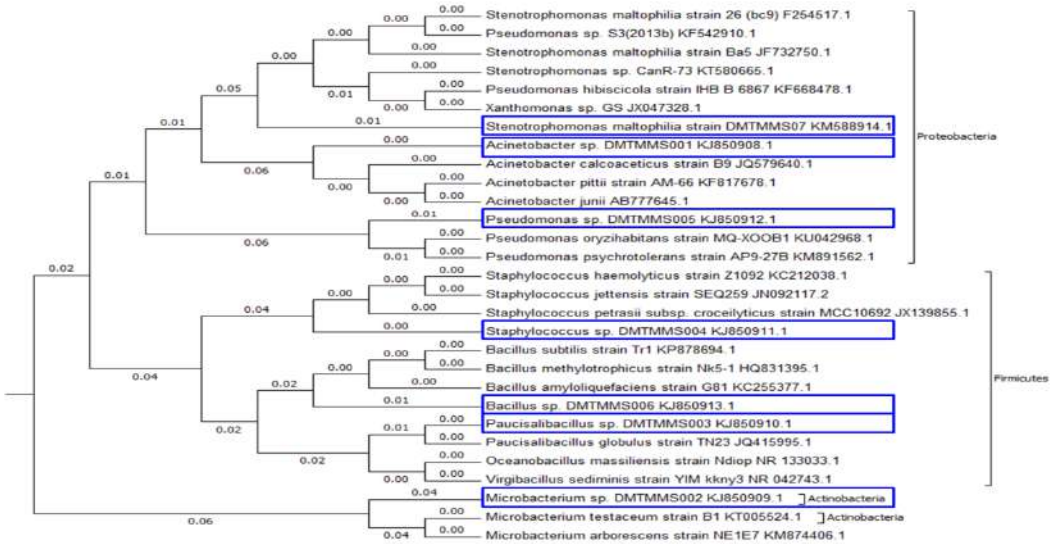

Figure -4 Phylogenetic tree constructed by MEGA-6.0

Site No	Sampling Site	pH	Temperature (°C)	Salinity (%)	Total Dissolved solids (mg/L)	Dissolved Oxygen (ml/L)
1	Fort kochi –Vipin ferry terminal	8.11±0.32	28.2±2	10.21±0.37	15.06±0.98	3.8±0.17
2	Ernakulam	7.81±0.19	29.7±1.7	9.38±0.89	27±0.219	4.65±0.98
3	Fort kochi	8.32±0.17	27.8±1.2	11.37±0.35	32±0.28	5.47±0.19
4	Azhimugam	8.77±0.71	29.9±2	18.62±0.76	19±0.913	4.34±0.76
5	Vypin beach	8.01±0.52	30.5±1	15.98±0.87	21±0.76	3.39±0.19

Table 1-Physico chemical parameters of Estuarine water samples from different spots

Strains	Cell biomass (g/l)	Extracellular		Cytoplasm		Periplasm		Membrane bound with SDS	
		Methioninase activity (U/ml)	Specific activity of methioninase (U/mg)	Methioninase activity (U/ml)	Specific activity of methioninase (U/mg)	Methioninase activity (U/ml)	Specific activity of methioninase (U/mg)	Methioninase activity (U/ml)	Specific activity of methioninase (U/mg)
Stenotrophomonas sp	2.73 ± 0.13	59.52±0.71	31.83±1.28	12.50±0.71	3.49±0.19	1.01±0.16	25.41±4.17	0.74±0.04	13.09 ±0.86
Acinetobacter sp	0.84 ± 0.06	12.84±2.40	13.10±0.81	1.02±0.23	0.47±0.107	0.35±0.03	7.13±0.61	-----	-----
Microbacterium sp	0.40 ± 0.03	1.52±0.23	1. 19±0.18	2.99±0.12	1.03±0.04	0.31±0.04	10.55±1.53	0.072±0.045	3.6±0.23
Paucisalibacillus sp	0.44± 0.06	5.60±0.81	9.66±1.31	9.96±0.18	7.84±0.14	1.12±0.17	11.23±1.76	0.87±0.10	2.56±0.30
Staphylococcus sp	0.32±0.056	0.48±0.08	0.561±0.09	1.69±0.16	1.08±0.10	-----	-----	-----	-----
Pseudomonas sp	2.82±0.13	52.77± 2.69	26.65±1.03	12.92±0.31	3.43±0.08	1.24±0.22	1.59±0.29	0.13±0.05	5.53±0.50
Bacillus sp	0.42±0.04	2.12± 0.213	1.36±0.13	8.50±1.14	4.27±0.577	0.36±0.046	5.68±1.79	-----	-----

Table 2-Sub cellular localization of methionine ɣ lyase from various strains (Each value =mean± value S.D)

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RESEARCH ARTICLE

Screening of Anticancer Activity in Methanol Extract of *Murdannia sahyadrica* against 1,2 Dimethyl Hydrazine Induced Colon Cancer

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ABSTRACT

Colon cancer is one of the deadly disease and second among the major disease worldwide. Although several synthetic drugs have been employed to cure the diseases, they exhibit toxicity/side effects. Recently, plant derived chemotherapeutic agents are used as an alternative source of drug. Anticancer activity of *M. sahyadrica* against 1,2-dimethylhydrazine (DMH) induced colon cancer was carried out in Sprague Dawly rats. *M. sahyadrica* methanol extract (250 and 500 mg/kg b.wt.) significantly ($P<0.01$) restored body weight, hematological parameters, protein and lipids profiles, serum enzyme activity (GOT, GPT, ALP) and serum antioxidant enzyme (SOD, CAT, GPX, GSH, LPO) levels compared with disease control (DMH) and normal control rats. Therefore, it could be concluded that, the methanol extract of *M. sahyadrica* is considered as one of the anticancer agents.

Keywords: *M. sahyadrica,* methanol extract, DMH, anticancer activity.

INTRODUCTION

Medicinal plants are used generally against a series of malignant disease and may affect various organs of the body. One among such diseases is cancer, characterized on the basis of rapid uncontrolled formation of abnormal growth of cells into the formation of tumor or proliferated cells. It is known that oxidants enhance the cell division to mutagenesis and one of the factors for carcinogenesis. Hence, antioxidants may exert their protective role by reducing oxidative DNA damage and abnormal growth of cell division. In the present scenario, the life style and food habit are one of the risk factors for the development of colon cancer. However, the other risk factors are genetic, chronic inflammation like ulcerative colitis, chronic disease, diabetes, etc. (Watson and Collins, 2011). The prevention of colon cancer could be possibly cured by the consumption of fruits and vegetables that are rich source of antioxidant properties and hence, protective role against colon cancer development (Arikawa and Gallaher, 2008).

Phytochemicals from plant sources are natural antioxidant such as polyphenols, flavonoids, alkaloid, *etc.* were found to inhibit tumors (Link *et al*., 2010). The species of the family Commelinaceae were reported to possess more content of anthocynanins, lutein and beta carotene with various biological activities including cancer. There has been great interest to develop the plant metabolites as novel chemotherapeutic agents that are more effective against colon cancer with minimum adverse side effects. No scientific records on anticancer potential were available in the genus of the study species. Therefore, the present study was aimed to evaluate the anticancer effect of *M. sahyadrica*, a new species from Commelinaceae.

MATERIALS AND METHODS

Plant collection and identification

Murdannia sahyadrica A. Ancy & Nampy of the family Commelinaceae was collected from Walayar, Palakkad, Southern Kerala, a small part of Southern Western Ghats, India and identified from the Department of Botany, Bharathiar University, Coimbatore, India.

Preparation of *M. sahyadrica* extract

Defatted plant samples were extracted with methanol using soxhlet apparatus and filtered with Whatman No.1 filter paper. The filtrate was dissolved with 1 per cent W/V of carboxymethyl cellulose (CMC). It was prepared freshly each time of administraion.

Experimental Design

Reagents and Chemicals were procured from Sigma-Aldrich chemical company, Bengaluru, Karnataka and were kept under refrigeration at 4°C until further use. The solvents were of analytical grade.

Animals

Male Sprague–Dawley rats weighing approximately 250-500 g were procured from KMCH College of Pharmacy, Coimbatore, Tamilnadu, India. All the rats were acclimatized to laboratory conditions at 25±1°C room temperature with relative humidity of 45-50% followed by 12 hrs of dark cycle. They were allowed to have free access to food and water. The present study was approved by Institutional Animal Ethic committee (IAEC No: KMCRET/Ph.D/11/2018-19), KMCH College of Pharmacy, Coimbatore. The current study was performed as per OECD-423 guidelines.

Acute toxicity study

Male Sprague Dawley rats were kept for overnight fasting prior to the administration of drugs. Animals were grouped to 4 (n=6) and received drug doses of 500, 1000, 1500 and 2000 mg/kg b.wt. of methanol extract of *M. sahyadrica.* Gross behavioral changes, hypersensitivity activities and mortality if any, were observed for one weeks (Ecobichon, 1997). Based on the LD_{50} values, 250 and 500mg/kg b.wt. were considered for the anticancer study.

Preparation of DMH

One gram of DMH was dissolved with 1Mm EDTA (pH-6.5). Once in a week for 4 weeks, DMH solution was administered/injected subcutaneously in the right thigh of all rats at a dose of 20 mg/kg (b.wt.) and then kept for 15 weeks (Nalini *et al*., 2004).

Preparation of Standard Drug, 5-Flurouracil solution

5-Flurouracil was dissolved in 10 ml of 1 per cent W/V of carboxy methyl cellulose (CMC) at the concentration of 20 mg/kg. b.wt.

Experimental Design

After an acclamitation period, a total of thirty male Sprague- Dawley rats were used in the present study. All the rats were classified into 5 groups with six animals each (n=6) as given below:

Group I: Control + 1 ml saline p.o every day for the entire period of study

Group II: DMH control, rats received (20mg/kg.b.wt) injections subcutaneously once a week for 4-consecutive weeks and the animals kept without any further treatment for 15 weeks.

Group III: DMH treated rats (20mg/kg.b.wt) once in a week subcutaneously for 15 weeks + treated further with *M. sahyadrica* methanol extract at the concentration of 250 mg/kg .b.wt by intraperitonial injection for 15 weeks.

Group IV: DMH treated rats (20mg/kg.b.wt) once in a week subcutaneously for 15 weeks + treated further with *M. sahyadrica* methanol extract at the concentration of 500mg/kg .b.wt by intraperitonial injection for 15 weeks.

Group V: DMH received rats (20mg/kg.b.wt) once in a week subcutaneously for 15 weeks + 5 flurouracil (100 mg/kg.b.wt intraperitionial injections (ip) for 15 weeks.

After 15 weeks, 5 fluorouracil at the concentration of 100 mg/kg.b.wt was injected intraperitonially to all the rats as it was considered as neoplastic stage. All the rats were anesthetized at the end of 15th week and the blood samples from respective groups were collected by Retro-orbital sinus bleeding. It was immediately centrifuged and the serum was separated from the plasma. The serum obtained was used to study the biochemical evaluation and the blood samples were used for haematological studies. Later, all the rats were sacrificed, and their colons were dissected out for histopathological observations. After compilation of the experiment, all the rats were sacrificed through cervical decapitation and entire liver and colon were perfused immediately. These organs were carefully excised trimmed free of extraneous tissue (Perse and Cerar, 2005).

Preparation of Tissue Homogenate

After the removal of colon tissues from rats, the tissues were weighed and 10% homogenates were prepared with 0.025 m Tris-HCl buffer at the pH of 7.5. At the end of 15th week, blood samples were collected from animals and serum was separated. In this study, body weight changes, hematological parameters (Nayak and Pattabiraman, 1981), enzymatic antioxidant assays such as SOD, CAT, GSH, GPx and LPO etc. (Melekh *et al*., 2017) were estimated from serum. Besides serum, protein (Lowry *et al*., 1951; Wolfson, 1948) and lipids profiles (Ihedioha *et al*., 2013), serum enzymes like GOT, GPT (Reitman and Frankel, 1957) and ALP (King and Amstrong, 1934) were investigated in all the experimental rats.

RESULTS

Table 1 depicted the effects of *M. sahyadrica* extract on normal group of control rats in comparison with DMH colon cancer induced control, colon cancer rats treated with plant extracts at the doses of 250and 500 mg/kg. b. wt and standard drug, 5 Flurouracil treated rats at the concentration 100 mg/kg.b.wt, respectively. The changes in the initial and final body weight changes for normal group of control rats were found to be 131.50 ± 1.96 gm and 154.55 ± 1.18gm respectively. Whereas in DMH induced colon cancer control rats and it was 133.50 ± 1.64 and 121.54 ± 1.96gm. Colon cancer induced rats treated with the plant extracts of *M. sahyadrica* extract at the doses of 250 and 500 mg/kg.b.wt were 144.56 ± 1.29 and 132.65 ± 1.55 during initial day and it was retrieved to near normally on the final day. These changes were similar to that of the standard drug, 5 Flurouracil treated rats (158.60 ± 2.96 gm).The administration of *M. sahyadrica*methanolic extract at the doses of 250 and 500 mg/kg b.wt significantly ($P<0.01$) changed the body weights. The final body weights in all the group of rats were found to be significantly increased and were comparable with the normal control group of rats.

The hematological parameters such as Hb, RBC and WBC in normal, DMH induced colon cancer, drug treated rats at the doses of 250 and 500 mg/ kg. b. wt and 5 Flurouracil treated rats were presented in Table 2. The haematological parameter, Hb was found to be significantly (P >0.01) lower in DMH induced rats compared with normal control rats. Whereas, WBC values were found to be increased significantly (P<0.05), DMH induced rats. However oral administration of plant extracts of *M. sahyadrica* at the dose range of 250 mg/kg b. wt and 500 mg/kg b. wt significantly attenuated these changes to normally which was comparable with that of the standard drug, 5 Fluorouracil treated rats.

The Table 2 represents the differential counts such as lymphocytes, neutrophils and eosinophils in all the experiment treated group of rats. The lymphocytes (%) count in DMH induced colon cancer rats was decreased significantly (P<0.01). Later, DMH group treated with two concentrations of *M. sahyadrica* extract at the dose of 250 and 500 mg/ kg. b. wt were found to be increased significantly (P< 0.05). The similar trend was found in standard drug, 5 Flurouracil treated rats and the values were brought towards normal control values. The neutrophils and eosinophil counts were consistently increased in DMH induced colon cancer cells compared to normal control rats. However administration of *M. sahyadrica* extracts at the dose of 250 mg/ kg. b.wt and 500 mg/ kg. b.wt reversed these changes towards normal control values (52.26 ± 1.56) & (6.16 ± 0.71) (49.16 ± 1.13 and 4.11 ± 0.27) . Similar results were found in rats treated with the standard drug, 5 Flurouacil. These results showed that the higher dose of *M. sahyadrica* extract was found to be effective in controlling the neutrophils and eosinophils count and their effects were comparable to that of the standard drug 5 Flurouracil.

The Table 3 depicted the serum protein profiles of all the experimental group of rats. The protein profiles viz., T-protein, albumin and globulin levels were found to be significantly decreased in DMH induced rats (P < 0.05) compared to normal control groups. However after administration of *M. sahyadrica* extract to DMH induced colon cancer rats. The protein profiles were found to be increased in both the plant drug and 5 Fluorouracil treated group of rats. The ratios of A/G were also found to exhibited similar trend at different group levels. The enzymes viz., SGOT, SGPT and ALP were found to be significantly (P< 0.01) increased in DMH induced rats and later administration of plant extract at 2dose levels viz., 150 and 300 mg/kg. b.wt. and standard groups decreased these values to near control group levels.

The Table 4 represents the lipid profile in all experimental groups. In DMH induced colon cancer rats, the lipid profiles were found to be increased compared to normal rats. In colon cancer induced rats, the triglyceride (TC), total glycerides, low density lipids, very low density lipids and PL increased significantly (P< 0.01 and P< 0.05) except high density cholesterol (HDL). Whereas, oral administration of *M. sahyadrica* extract to DMH treated group of rats, decreased the lipid profile and it enhanced HDL levels. In standard 5 Flurouracil, a standard drug treated group of rats the lipid profiles was found to be reduced to near normal group of rats.

The serum antioxidant enzymes such as superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) and lipid peroxidase (LPO) levels were investigated in all the group of rats (Table 5). In this experiment, the levels of antioxidant enzymes decreased significantly (P < 0.05) in DMH colon cancer induced rats as compared to normal control groups; whereas, LPO levels increased significantly (P<0.05). Administration of *M. sahyadrica* extract at the dose concentration of 250 and 500 mg/kg b. wt. significantly enhanced SOD, CAT and GPX levels (P < 0.01) and their values were comparable to that of normal group of rats as well as in standard drug, 5 Fluorouracil treated group V (P < 0.01) respectively.

DISCUSSION

The present results clearly indicated that the administration of procarcinogen DMH in the presence of *M. sahyadrica* extract brought profound alternations in changes of body weights, haematological parameters, protein and lipid

profiles, serum enzyme and enzymatic antioxidants in the colorectal cancer rats. The initial and final body weight of DMH treated rats showed significant low gain in the body weight in the experiment as compared to the control rats. The decreased body weight gain could be due to the tumor burden in the colon that leads to very low food intake whereas, the supplementation to DMH- treated rats significantly almost improved the weight gain as compared to DMH administered rats revealing the benefit of *M. sahyadrica* extract against DMH induced colon cargenogenesis. It either may be due to the inhibitory effect of the anti-proliferation potential of the extract. DMH is metabolized in liver to azoxymethnale to a methyl-free radical which generated OH radicals in the presence of metal ions and initiated the formation of LPO and MDA is a product of LPO function as mutagen.

DMH is one of the class of hydrazine and a strong alkylating agent. It is used as a carcinogen to induce colon cancer in many animal models (Karthickkumar *et al*., 2020). Here, DMH treated rats showed reduced weight gain when compared to other groups I, III and IV due to the decreased food intake. Besides, the glucose metabolism is altered and increased hepatic gluconeogensis depleted the energy sources for the cell metabolism that leads to the significant weight loss in the control group II rats. It is known that carcinogenic features of DMH is responsible for the methylation of the DNA base pair of all organ especially epithelial cells in the proliferative sites of the crypt and resulted in a great loss of colonic cells and increased proliferation (Ahmed *et al*., 2021). These alternation could produce inflammatory growth and oxidative stress. (Kamisah *et al.,* 2012). The anticolorectal cancer activity of *M. sahyadrica* against DMH induced colon cancer in rats resulted in the weight gain at the doses of 250 and 500 mg/kg body weights and standard drug 5 fluorouracil compared to control. The chemopreventive effect of *M. sahyadrica* could be flavonoids, phenolic compounds and Glycosids and phenols. Like this, several plant species were found to exhibit anti-cancer activities. Venkateswarlu *et al.,* (2014) and Oomoregie *et al* (2020) have investigated the anti-cancer activities of *Annona muricata* leaf and stem and *Phyllanthus amarus* respectively in DMH induced colon cancer and stated that it significantly decreased the aberrant crypt foci (ACF) weight of organs and increased weight gain and apoptosis index. Annonacin, an antitumourous compound found in the *A. muricarta* was effective against various in vitro cancer cell lines and these are selectively toxic to cancer cells but not to the healthy cells. In the cancer therapy weight loss is considered to be the weight loss when it is higher, that leads to the shorter the survival rate. Rojapathakota (2016) evaluated *Aegle marmelos* for anticolon cancer activity in DMH-induced rats and stated that this plant reduced the number of crypts and increased the weight gain and apoptotic index compared to weight gain and apoptotic index compared to DMH treated group.

When DMH induced rats were treated with *A. muricata* plant extract it was found to promote weight gain and reduced mortality rate in oral rats. In the present study also administration of methanolic extract of *M. sahyadrica* at the dose range of 250 and 500 mg/kg b wt. significantly inhibited the colorectal cancer in rats induced with DMH. The standard drug, 5 Flurouracil also inhibited the weight loss and decreased the number of cancer cells in the epithelium of the rat's colon. The obtained chemopreventive activity of the herbal plant extract could be due to the enhancement of xenobiotic metabolizing enzymes coincident with decreased ß-catein protein related with oxidative stress (Dadkhah *et al.,* 2014). In this study, DMH induced a few alterations in the hematological profiles of the rats and it showed a significant decrease in the counts of RBCs, WBCs, neutrophils, lymphocytes and monocytes. Here, the eosinophil's count was found to be significantly increased as compared with the control rats. It was suggested that the oxidative stress in the erythrocytes may played a vital role in hematological abnormalities that leads to carcinogenesis (Childress, 2012). It is known that DMH induced due to the inhibition in erythrocyte count due to the inhibition of RBC production and erythrocyte destruction in hemopoietic organs. Thus recruited to inflamed loci response to mediums and then released at the injury site.

Generally it was reported that toxin intoxicated rats showed elevated levels of serum enzymes, bilirubin and lipid peroxidation of liver tissues and reduction in serum protein profile (Nishanthini *et al.,* 2012). The liver injury caused by hepato toxicants were recognized as a major problem associated with the functions of gastrointestinal tract. In liver, all toxicants generate free radicals which bind with macromolecules and induce peroxidase degradation of membrane lipids. These series of events lead to form peroxidases protein synthesis and serum marker enzymes viz.,

SGOT, SGPT and ALP that become elevated. The SGPT activity is involved in the cell membrane damage and SGOT is considered as an indicator of mitochondrial damage (Daba and Rahman, 1998). It is due to the formation of highly reactive metabolite, methyldiazonium ion by DMH which lead to lipid peroxidation as oxidative stress and decreased the antioxidants in colonic cells. In the present study also, all the serum enzymes enhanced significantly ($P< 0.01$) in group II rats intoxicated with DMH. Oral administration of both 5-fluorouracil and methanolic whole plant extract of *M. sahyadrica* decreased SGOT, SGPT and ALP enzyme levels to normal control values. It was speculated that the extract exerted protective role on mitochondria and hepatocytes and could have normalized the enzyme levels by enabling hepatoprotective role. It is due to the formation of highly reactive metabolite, methyldiazonium ion by DMH which lead to lipid peroxidation as oxidative stress and decreased the antioxidants in colonic cells (Hamiza *et al.,* 2012).

The protein profile such as T. protein, albumin, globulin levels were decreased in rats intoxicated with DMH significantly ($p<0.05$) and restored to near normal levels in the rats administrated with both extracts of *M. sahyadrica* and standard drug. The same mechanism could have exerted in the protein profile also. In this study serum antioxidant enzymes in group I was compared with group II followed by group III and IV respectively. Wacy *et al.,* (2012) have already stated that antioxidants SOD, CAT, GSH and MDA were involved in the anticarcinogenesis mechanism against DMH-induced rats. The higher level of LPO in serum in DMH induced rats accounted for induction of oxidative stress and depletion of GSH. DMH also could have inhibited the activities of SOD and CAT. However, the treatment with extracts seems to be elevated these activities (Manal *et al.,* 2018). Whenever the antioxidant defense system decrease that leads to increase in the lipid peroxidation which caused a cell membrane distortion. 5 Flurourasil drug is generally applied drug for the management of colonic cancer. The antioxidant properties of 5 Flurourasil has proved that it increase GSH content, CAT activity and increased SOD activity. These enzymes could have protective role against several endogenous toxic substances like ROS and a few chemical carcinogens (Nimse and Pal, 2015). The decreased antioxidant enzyme activities were found to be enhanced after the artesunate treated in DMH induced rat colon cargenogenesis (Sazal *et al*., 2017). Our results also found to be similar compared to control group. These results suggest antioxidant activity of the extract (Arivalagan *et al*., 2016). It is evident that increase in levels of LPO and a decrease in antioxidant enzymes are characteristic features of tumor genesis (Sazal *et al*., 2017).

It confirmed the anticarcinogenesis activity against the action of DMH. Wacy *et al.,* (2012) have also stated that antioxidants SOD, CAT, GSH, etc. were involved in the anticarcinogenesis mechanism against DMH-induced rats. Besides GSH and LPO levels were statistically increased in DMH-induced group II rats compared to control rats treated with extracts and 5-fluorouracil standard drug. According to NurLinna MD Nasir *et al.,* (2017) Azoxymethane-induced colon cancer in rats involved in modulation of the colonic antioxidant system duce the occurrence of flavonoids in *Muntingia calabura* leaves. They have confined the action of AOM in rats and it triggered the oxidative system by causing DNA drug dose its reactive methyldiazonium ion and induction of microbiological changes and aberran crypts formation in rats induced with AOM/DMH followed by an irregular glandular architecture of epithelial cells developed into colorectal cancer (Caderni *et al*., 1995). The colorectal carcinogenicity was related to the formation of reactive metabolite, methyldiazonium ion AOM-induced rats and responsible for lipid peroxidation which led to antioxidant stress. The microbial β-glucuronidase enzyme activates the DMH metabolites to toxic carcinogenic transformation and promoted carcinogenesis in DMH treated rates of colon. These lead to transformation of normal epithelial cells of colon into cancerous ones. (Moore and Moore, 1995).

The depleted high density lipoprotein in DMH-induced group II of rats, increased significantly after the administration of extract and standard drug played a major role in glucose and lipid homeostasis. It has been involved in uptake, oxidation and consumption of free fatty acids, cholesterol and phospholipid synthesis and serrate plasma lipoproteins (Brown *et al.,* 1993). During the intoxication of rats lipolysis cold have stimulated in adipose tissue and liver become inactivated/damaged resulted in decreased activity of lipoproteins lead to hydrolysis of lipids and increased serum triglycerides (Brown *et al.,* 1979). The increase in triglycerides (TG) is due to

lack of insulin and when it is activated the lipoprotein lipase and TG come to normal level. The increase production of LDL and VLDL in DMH induced rats due to the over production of LDL and VLDL by liver. It may be due to the synthesis of hepatic triglycerides as a result of fatty acid influx (Coppack *et al.,* 1996). The HDL in DMH induced group II, group of rats indicated a rich for the development of atherosclerosis (Bopanna *et al.,* 1997).

CONCLUSION

Therefore the present study involves the methanolic extracts of *M. sahyadrica* could be considered as a natural potential source for anti-colon cancer activity and further studies are to be undertaken for further biologically active substances and their characterization.

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Table 1: Effect of *M. sahyadrica* extract on body weight changes in DMH induced colon cancer treated rats

Treatment	Dose	Initial Body weight (Gm)	Final Body weight (Gm)	Mean weight Gain (G↑) / loss(L↓) (Gm)	% Difference
Normal Control	0.9% Saline	131.50±1.96	154.55±1.18	23.05↑	17.52
CC induced Control	0.9% Saline	133.50±1.64	121.54±1.96**	11.96↓	8.95
CC induced Animal + MSM Extract	250(mg/Kg)	144.56±1.29	154.95±2.16* a	10.39↑	7.18
	500(mg/Kg)	132.65±1.55	169.54±3.65ns aa	36.89↑	27.81
Standard Drug (5 Flurourasil)	100(mg/Kg)	137.16±3.84	158.60±2.96ns aa	21.44↑	15.63

Values are mean ± SD of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to Colon Cancer induced control :a P<0.05 ;aa P<0.01 - as compared with Colon Cancer induced control to drug treated animal NS: not significant

Table 2. Effect of *M. sahyadrica* extract onhaematological parameters in DMH induced colon cancer treated rats.

Treatment	Hb (gm%)	RBC (million/mm^3)	WBC 0^3 cells/ mm^3)	Differential count		
				Lymphocytes	Neutrophils	Eusinophil
Normal Control	14.04±0.31	4.71±0.36	9.12±0.27	51.13±1.96	44.92±1.27	4.36±0.75
CC induced Control	8.36±0.27**	3.16±0.18.*	13.16±0.36*	32.16±0.93**	60.26±1.56**	7.13±0.18*
CC induced Animal Given MSM extract250 and 500(mg/Kg)	11.22±0.33a	3.46±0.19	12.76±0.13ns	42.16±1.23*	52.26±1.56*	6.16±0.71
	12.67±0.96a	3.93±0.26	10.14±0.16a	46.16±1.31a	49.16±1.13a	4.11±0.27
Standard Drug (5Fu) 100(mg/Kg)	13.16±0.16aa	4.09±0.11	9.21±0.27a	51.56±1.39a	40.27±1.23a	7.64±0.73

Values are mean ± SD of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to Colon Cancer induced control: a P<0.05; aa P<0.01 - as compared with Colon Cancer induced control to drug treated animal NS: not significant

Table 3: Effect of *M. sahyadrica* extract on serum biomarker levels DMH induced colon cancer treated rats.

Groups	Parameters							
	Dose	T.Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Normal Control	0.9% Saline	8.26±0.21	4.28±0.17	3.98±0.19	1.0:1	11.56±1.73	14.54±1.19	156.39±3.24
CC induced Control	0.9% Saline	6.71±0.31*	3.84±0.17ns	2.87±0.18*	1.3:1	49.56±2.96**	54.93±5.11**	229.56±5.67**
CC induced Control + MSM Extract	250(mg/Kg)	7.84±0.31	4.13±0.16	3.71±0.14	1.1:1	23.93±0.98*	26.39±1.06S*a	184.93±4.13
	500 (mg /kg)	8.16±0.15	4.43±0.14	3.73±0.15	1.1:1	17.31±0.91nsa	20.56±1.13aa	169.56±3.18aa
Std Drug (5 Fluorouracil)	100 (mg/Kg)	8.21±0.16a	4.56±0.13	3.65±0.16	1.2:1	12.16±0.81nsa	18.33±1.93aa	156.31±2.93aa

Values are mean ± SD of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to Colon Cancer induced control :a P<0.05 ;aa P<0.01 - as compared with Colon Cancer induced control to drug treated animal NS: not significant

Table 4: Effect of *M. sahyadrica* extract on serum lipid profile in DMH induced colon cancer induced rats.

Groups	Parameters						
	Dose	TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
Normal Control	0.9% Saline	124.36±1.84	91.36±1.34	46.31±1.13	59.99±1.15	18.27±1.26	178.68±0.14
CC induced Control	0.9% Saline	166.54±1.92**	126.56±1.29**	29.15±1.36**	112.08±1.30**	25.31±1.12ns	216.22±0.19*
CC induced Control + MSM Extract	250 (mg/Kg)	157.26±1.36*	113.64±1.17*	34.54±1.29nsa	100.02±1.20*a	22.72±1.26	207.96±0.15*
	500 (mg /kg)	132.93±1.54a	104.56±1.29a	38.23±1.64aa	73.79±1.60aa	20.91±1.29	186.30±0.21a
Std Drug (5 Fluorouracil)	100 (mg/Kg)	128.56±1.29a	94.96±1.54a	41.54±1.33aa	68.04±1.332aa	18.98±1.18	182.41±0.23a

Values are mean ± SD of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to Colon Cancer induced control :a P<0.05 ;aa P<0.01 - as compared with Colon Cancer induced control to drug treated animal NS: not significant

Table 5: Effect of *M. sahyadrica* extract on the serum antioxidant activity in the normal, Colon Cancer induced and drug treated rats.

Groups	Parameters					
	Dose	SOD (Unit/min/mg protien)	Catalase (Mmoleof H2O2 consumed/ min/mg protien)	GPX (u mole of glutathione oxidized/ min/mg protien)	GSH (Ug/ min/mg protien)	LPO (n mole of MDA/mg protein)
Normal Control	0.9% Saline	8.24±0.13	4.34±0.73	3.81±0.23	11.56±1.13	14.56±1.36
CC induced Control	0.9% Saline	6.43±0.31*	3.81±0.16*	2.66±0.17*	32.63±2.16**	41.36±1.16**
CC induced Control + MSM Extract	250 (mg/Kg)	7.84±0.56ns	4.06±0.84	3.33±0.23ns	23.16±1.36*	26.56±1.54*
	500 (mg /kg)	8.0±0.51a	4.19±0.16a	3.66±0.14a	15.16±1.17aa	20.31±1.39aa
Std Drug (5 Fluorouracil)	100 (mg/Kg)	8.13±0.34a	4.26±0.27a	3.74±0.73a	13.73±1.36aa	16.56±1.31a

Values are mean ± SD of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *P <0.05;**P <0.01 as compared with Normal Control to Colon Cancer induced control :a P<0.05 ;aa P<0.01 - as compared with Colon Cancer induced control to drug treated animal NS: not significant

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RESEARCH ARTICLE

Size Dependent Chromosome Positioning using Gene-Density-Based Model

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ABSTRACT

Chromosomes are not distributed randomly inside eukaryotic cell nuclei in interphase. There are mainly two chromosome positioning schemes – one is based on gene density and the other is based on chromosome size which is studied here. Computer simulation shows that the size dependent radial positioning of chromosomes appears because of the inhomogeneous distribution of activity across chromosomes which is originated because ATP consuming processes required in chromatin remodelling and transcription are distributed non-uniformly on each chromosome. Result obtained from computer simulation is in good agreement with the experimental data.

Keywords: chromosome positioning, out of equilibrium, active matter, computational biology, iopolymer

INTRODUCTION

Recent studies suggest that chromosomes are not organised randomly within the nucleus at interphase. Gene rich chromosome 19 in human cells is generally located near the centre of the nucleus whereas similarly sized but gene poor chromosome 18 takes the peripheral position [1,2]. In general, gene-rich chromosomes are found more centrally than gene-poor chromosomes which provides gene-density-based positioning scheme for all chromosomes [3].It has also been reported that in some human cell types, chromosomes are distributed within the nucleus based on their size with the centre of mass of smaller chromosome located more centrally than that of the larger one [4-6]. Most computational models are based on the assumption that the chromosomes are polymers in thermal equilibrium [7-12] and they are unable to predict the chromosome positioning schemes based on gene density and size.

Enzymatic activities associated with DNA repair, chromatin remodelling and transcription are ATP consuming processes and the theories of “active matter” [13-16] can be used to model such ATP consuming processes. It is

assumed that the inhomogeneous and stochastic forces acting on chromatin can be modelled via an effective temperature [17] which reflects the levels of activity locally. Each chromosome is described here in form of a polymer composed of monomers and each monomer represents an averaged section of chromatin. Therefore, it is obvious that the effective temperatures experienced by different monomers are different [18-21] and depend on the local activity levels.

MATERIALS AND METHODS

The model for chromosomes used here is the same as in Ref. [18].Numerical conventions and basic methodology of spherical chromatin domain (SCD) model proposed in Ref. [22] has been followed to model chromosomes in a spherical confinement. The cell nucleus is represented by a hollow spherical shell of radius R_0 where 23 pairs of chromosomes (human, female) in the form of linear polymer chains are confined. Each polymer chain is made of spherical monomers which represent 1 Mb domain of chromosome and they are connected by harmonic springs. Total 6098 monomers are present in the confining spherical shell. The interaction between neighbouring monomers along the same polymer chain is taken as

$$V_{nm}(r_i, r_{i+1}) = \frac{1}{2}k(r_i - r_{i+1})^2 \qquad (1)$$

where k represents spring constant, $r_i$and r_{i+1} are the position coordinates of $i^{th}$and $(i + 1)^{th}$monomer respectively. The interaction among monomers belonging to same or different polymer chains is chosen as a Gaussian interaction [23]

$$V_{m-m} = V_0 exp\left(-(r_i - r_j)^2/\sigma^2\right) \qquad (2)$$

with the value of monomer-monomer interaction at zero separation V_0 in order of $k_B T_{eq}$ where k_B is the Boltzmann constant. Confining sphere interact with each monomer only when the location of the monomer is outside the sphere otherwise it is zero. The interaction is of the form,

$$V_{wall} = \frac{V_{conf}}{a^5}(|r_i| - R_0)^5, \qquad |r_i| > R_0 \qquad (3)$$

where a is a scale factor and $V_{conf} = k_B T_{eq}$.

To include inhomogeneous activity, total number of genes present in each 1 Mb domain (monomer) on each chromosome is obtained using Gene Cards database [24]. Monomers having gene content below a preset cutoff are termed as "inactive" monomers and are assigned an effective temperature T_{inact} *which is* equal to thermodynamic temperature $T_{eq} \approx 310K$. Monomers with number of genes above the cutoff are termed as "active" monomers and are characterised by an effective temperature $T_{act} > T_{eq}$. The cutoff is chosen in such a way that only top 5% monomers in terms of their gene content are "active" and other monomers are "inactive". This accounts the fact that in a given cell type only a fraction of number of genes are transcribed.

It is proved experimentally [25] that the mean distance between any two monomers increases first as a function of their distance along the chain and then this distance is saturated which produces compact configuration of each chromosome. Random loop model [26-28] has been implemented here to produce such compactness of individual chromosomes by constructing a small number of permanent loops which connect pairs of randomly chosen monomers with low probability along each chromosome. This connection is implemented physically by placing springs with spring constant 10 times higher than the spring constant of the spring which connects neighbouring

monomers along the length of each chromosome. The value of looping probability is 3×10^{-3} which provides 1404 loops for the 6098 monomers which are considered here.

Overdamped Langevin equation with Euler discretisation is implemented for numerical simulation of the system of "active" and "inactive" monomers.

$$\gamma \frac{dr_i}{dt} = F_i + \eta_i \qquad (4)$$

where r_i is the position of i^{th} monomer,γ represents friction coefficient, F_i is the resultant of all non-stochastic forces experienced by i^{th} monomer and η_i accounts stochastic forces due to active and thermal fluctuations. The noise is taken Gaussian distributed satisfying the following relation,

$$< \eta_i(t)\eta_j(t') > = 2k_B T_i \gamma \delta_{ij} \delta(t - t') \qquad (5)$$

where T_i represents effective temperature of the monomer which depends on its local activity. Each component of $\eta_i/\sqrt{\gamma}$ is computed by multiplying Gaussian random number with zero mean and unit variance with $\sqrt{2k_B T_i/\gamma}$.

To standardise parameters, SCD model is followed assuming diameter of each domain $d \approx 500\,nm$, domain separation at equilibrium $l_0 \approx 600\,nm$ and the diameter of the nucleus $R_0 \approx 6.7\mu m$. Energies are calculated in units of $k_B T_{eq}$, taking $V_0 = 1.5\,k_B T_{eq}$. The spring constant is chosen as $k = 6\,k_B T_{eq}/l_0^2$. All physical lengths are measured in units of $a = 28\,nm$ ($0.168\,\sigma$). Unit of time is chosen in such a way that $\gamma = 1$.

Using population-based analysis, Kalhor *et al.* found in Ref. [29] that the centre of mass of chromosomes are positioning within the nucleus according to the size of the chromosomes though few chromosomes are not following this tendency and this size dependent positioning of chromosomes strongly agree with the results obtained from the independent FISH experiment [29] in lymphoblasts. The model discussed here is also capable of producing similar size dependent positioning. To do so, $T_{act} = 12.0\,T_{eq}$ is assigned to active monomers and $T_{inact} = 6.0\,T_{eq}$ is assigned to inactive monomers. Centre of mass of a chromosome is computed using the following relation.

$$r_{cm} = \frac{1}{N}\sum_{i=1}^{N} r_i \qquad (6)$$

where r_i is the position coordinate of i^{th} monomer of a chromosome, r_{cm} represents centre of mass of the chromosome and N is the total number of monomers present in the chromosome. Centre of mass of each chromosome is computed after averaging it over 2.0 million time steps and10 initial configurations where 0.4 million time steps have been given to the system for attaining steady state.

RESULTS AND DISCUSSION

The result is shown in Fig. [1] where mean radial position of centre of mass of each chromosome as a fraction of nuclear radius, obtained from computer simulation, is plotted as a function of chromosome size along with the experimental data of Kalhor *et al*. The figure reflects the fact that the theoretical and experimental data are within the range of error bars of each other which proves that the model discussed here can predict the size dependent positioning of chromosomes. The error bars of the experimental data have been extracted numerically from the Ref. [29] while error bars of simulation data represent the standard deviations of the probability distributions of centre of mass of chromosomes. Agrawal *et al.* showed similar result in Ref. [20] using a model which is more complex than the model used here. The main difference with the model used in Ref. [20] is random loop model and the assignment of activity which are implemented in this model in a very simpler way than in Ref. [20].

CONCLUSION

Here, it is shown that a simple model combing with inhomogeneous distribution of activity at monomeric level along with random loop model can predict radial segregation of chromosomes based on their size. The result obtained from the computer simulation provides a very close agreement with the experiment [29].The same model can also predict the other chromosome positioning scheme based on gene-density which is shown in Ref. [18].

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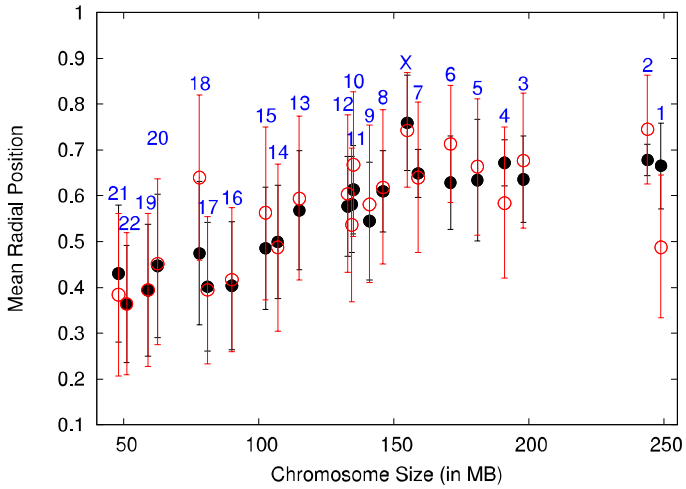

Figure 1: Average radial positions of centres of mass of all chromosomes as a fraction of nuclear radius are plotted as a function of their size with simulation (filled black circles) and experimental (open red circles) points. Experimental data are numerically extracted from Fig.6(b) of Ref. [29].

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RESEARCH ARTICLE

Isolation and Characterization of Effective Microbes for Biodegradation, Bioremediation and Plant Growth Promotion

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ABSTRACT

Effective microorganisms (EM) have a wide range of applications. A series of experiments were conducted to study the effectiveness of EM on biodegradation of organic wastes, bioremediation, and plant growth promotion. Controls were also kept along with each EM treated sample (Organic waste, Well water, Spinach, coir pith) to measure its rate of efficacy. Results showed that the EM treatment remarkably improved biodegradation, plant growth, and water quality as well. The inoculation of organic wastes with EM was effective and easily gets converted into a degraded product when compared to the control. It also showed a significant increase in plant height, the number of leaves, leaf length, and leaf width as compared to the plants that were allowed to grow without EM treatment. This study depicts the fact that EM can improve the quality of water. The effectiveness of composting the coir pith by EM technology was also studied and observed to have a great impact on its faster degradation.

Keywords: Effective Microorganisms, Biodegradation, MPN, Bioremediation, Coir pith degradation, Plant growth.

INTRODUCTION

Waste is considered the major cause of environmental degradation. Therefore, to reduce environmental pollution waste management is of utmost concern in this modern era. Due to the alarming rise in the population and industrialization, there is a hike in the amount of solid waste is being generated all over the world [1]. Accumulation of wastes at various places is a serious threat to living beings, so emergency action needs to be taken to eliminate such wastes. The bulk of organic kits comprise easily biodegradable components such as amino acids, carbohydrates,

volatile acids, peptides, proteins, fatty acids, and their esters. Here we are discussing certain biological measures to eradicate accumulating organic wastes in households. One of them is Effective Microorganism Technology which was utilized for the treatment of different types of waste to convert them into a useful by-product. Solutions of Effective microorganisms developed in Okinawa in 1970s which have been used for environment management [2]. For proper composting of organic materials by microorganisms different factors like C/N ratio, moisture influence the process [3].

Microorganisms present in soil have been classified mainly into two categories: beneficial and harmful, based on their function, how they affect the fertility of the soil, plant growth, health, and yield. Beneficial microbes are those which can decompose organic wastes, fix atmospheric nitrogen, secrete biocontrol agents, and enhance the nutrients in the soil by producing bioactive compounds like hormones, enzymes, and vitamins that stimulate plant growth [4]. While harmful microorganisms are capable of causing various diseases and they adversely affect the health and growth of the plants. Effective microorganisms (EM) are a more specific category of beneficial microbe that is a mixture of beneficial and naturally occurring microorganisms or they are also been explained as a combination of symbiotically coexisting aerobic and anaerobic beneficial microorganisms symbiotically in a liquid medium [5]. In Japan, over 40 years ago, Higa developed EM technology and described how they interact soil-plant environment to protect plants from pathogens and disease, to solubilize minerals in the soil, to conserve energy, to balance the ecology of soil microbes, etc [6]. EM was originally used in agriculture about fifteen years ago and has since been expanded to a variety of other fields. Studies show that EM has very vast applications including bioremediation, algal control, and household uses, composting, agriculture, gardening, and landscaping, cleaning septic tanks, live stocks [7].

EM consists of several species of microorganisms; they are Lactic Acid Bacteria, Yeasts, Fermentative fungi, Actinomycetes, Photosynthetic bacteria. These EM species of microorganisms secrete different organic acids, enzymes, antioxidants, and metallic chelates. These secreted compounds from EM species are found to neutralize or reduce odor during the process of degradation of waste materials [8]. When organic matter is putrefied by putrefactive microbes, it produces an odor. When EM is introduced to a local environment and begins to dominate it with its fermentation-type bacteria, the putrefaction process is halted and a fermentation process is initiated. They also produce an antioxidant environment that helps in the solid-liquid separation and this is the foundation of water treatment [9].

MATERIALS AND METHODS

Collection of materials

Soil samples were collected aseptically in plastic bags from near the households and later it was pooled. Similarly, Vegetable waste from the garbage of college hostels and households was gathered and pooled. 1kg of Sugar cane molasses/Jaggery water were collected from Thiruvanvandoor Panchayath's jaggery making unit, mud pots were collected from the Pongala trash disposal site, washed thoroughly, and kept in a hot oven for 180 min. Water sample for MPN analysis was collected in sterile containers under the aseptic condition from Ward 1 of Thiruvanvandoor Panchayath. Spinach seeds were collected from Vellayani Agriculture College Certified seeds were procured from Vellayani Agriculture University.

Methodology

EM preparation

Different strains of microorganisms were isolated from the collected soil sample. In this study, 1 gram of soil was taken for standard serial dilution procedure and they were spread plated and tested for enzymatic presence by amylase, protease, and cellulose tests. All the positive organisms were isolated and subcultured. The starter culture was prepared by soaking 200g of rice in 1L of water for 3 days. Filtered the solution and added 3 L of milk to the solvent (1:3). Spread plate method was carried out and the organisms were screened by enzymatic tests. Positive organisms were isolated and subcultured. The isolated strains were individually inoculated by single streaking on

selective media such as starch agar, skim milk agar, Congo red agar, czapek-mineral salt agar, and nutrient agar with 1% tributyrin to isolate amylase, protease, cellulase, and lipase producers respectively. Media for cultures were optimized at three different temperature 37ºC, 32 ºC and 28ºC at pH of 6.5 with salt concentration 0.5% of NaCl. The microbial consortium was prepared by mixing these two cultures with molasses. All biochemical tests were performed, including IMViC test, urease tests, nitrate tests, lactose and mannitol fermentation tests, and the findings were recorded for each organism's identification. The strains showing positive results were subjected to gram staining and their morphology was determined by light microscopy under oil immersion. EM bionoculam was named SAC EM bioinoculant .10ml of each bioinoculam was raised in large volume with help of Jaggary water 90ml.or rice water with jaggary 1:1 ratio. The prepared inoculam was bottled and saved kept at room temperature for two weeks or at 4◦C for 1 month.

Test for Biodegradation, Water treatment, and Plant growth promotion

Mixing consortia with organic waste

2ml of EM culture was mixed along with 250 ml of cane molasses. The mixture was later sprinkled on to 500 g of collected organic kitchen waste kept at a sterile mud pot and was closed with a wooden sheet. The pot was kept in open space for 1-5 days. The changes were observed and recorded every day.

Mixing of consortia with contaminated water

The water quality tests were done in ward no.1 of Eramallikkara, Thiruvanvandoor Panchayath of Alappuzha district. The MPN (Most Probable Number) of 26 household wastewater was examined using the Multiple Tube Fermentation technique. The water samples were collected from different sites which include Lakshamveed colonies, houses, churches, temples, schools, Public Health Centre, and major water bodies like a river. The results were recorded as the change in color from purple to yellow with the presence of gas in the inverted Durham tube. The index was measured to calculate the extent of microbial contamination.2ml of EM solution was added to the water sample that showed index number >1000 and kept for 24 hrs at 28ºC. After 24 hrs the sample was collected and MPN was carried out.

Coir pith degradation ability

Coconut coir pith is a biodegradable agricultural waste material that accumulates in large volumes. Coir fiber is used to make a wide variety of products. Almost 2 kg of coir pith waste is produced during the extraction of 1 kg of fiber [10]. In this work the ability of EM to degrade coir pith was also studied.2ml of EM consortia was mixed with 2 Kg of coir pith and kept in a tray for 1-2 weeks. After a week the tray was sprayed with molasses and kept at room temperature. Another tray with 2kg of coir pith uninoculated with EM was kept as control.

EM on Plant growth promotion

In agriculture, microbial inoculants (Effective Microorganisms) have been used to promote soil fertility and plant growth [11]. The spinach seeds collected from Vellayani Agriculture University were sprinkled with SAC-EM bioinoculants and allowed to grow in paper cups. After sprouting, EM treated water was the only source of irrigation, and usage of other known manure and biofertilizers was avoided. Control was also kept, irrigated with normal water.

RESULTS AND DISCUSSION

Screening for indigenous EM was conducted. Major bacterial and fungal colonies were isolated. These organisms were subjected to morphological, microscopical, and biochemical analysis. The morphological characteristics and the microscopic features of the four predominant bacterial strains isolated from the organic waste are shown in table 1. These colonies were identified as *Lactobacillus, Micrococcus, Pseudomonas fluorescence,* Actinomycetes. Gram staining and biochemical analysis were done to confirm the identity of the isolates. Five predominant fungal strains were

isolated; they are *Aspergillus niger, Aspergillus parasiticus, Aspergillus mari, Aspergillus clavatus, Saccharomycetes.* Enzymatic assay of fungal and bacterial colonies was carried for assessing the ability to produce extracellular enzymes. Since enzymes are the major contributory factors that will help in microbial degradation and bioremediation, the screening for enzyme producers is most important in the production of microbial consortia. The strains of microbes producing amylase, cellulase, protease, and lipase were characterized.

Biodegradation of organic waste

A pot experiment was conducted to study the biodegradation efficacy of EM. A set of control and EM treated bioorganic waste was kept in an open space for a week. The weight of reduction of organic waste without EM and organic waste were compared. The result was recorded periodically. The complete reduction of waste was observed on day 7 whereas control took around more than one week for the degradation to occur. It was observed that on day 7, the net weight of the sample was reduced to 25g from 250g on the addition of 2 ml of the prepared consortium. For the control, even after 10 days, the weight reduction was 90 g out of the total organic waste that is 250 g. The reactions were odorless and were less expensive and easy to undergo whereas from the control there was an emission of the foul smell of putrefaction. The figure indicates the reduction of biowaste from days 1-7. Application of EM for biodegradation of organic waste was reported by many workers (14-15), In this study also it was observed that EM technology has a great application in treatment of bioorganic waste.

Coir pith degradation by EM

EM was used to test for its coir pith degradation ability. The coir pith treated with EM was found to be completely degraded within 2 weeks. A reduction in the weight of coir pith was observed every day. From the seventh day onwards the rate of reduction was high whereas there was no change observed in the control. The coir pith degradation ability of EM isolates is shown in the figure 3. EM technology was found to be effective for the treatment of Coirpith by Lavanya and Padmaja 2018, it was observed that Lignin cellulose nad C:N, Phenol was reduced when coipith was treated for about 90 days in presence of effective microbes. The present study also corroborated with this findings.

Water treatment with EM

The effect of EM on treatment in contaminated well water with heavy coliform load was studied. The coliform count of well water at Thiruvanvandoor Panchayath was screened using the MPN technique. Highly contaminated 6 well water samples were selected and subjected to EM treatment. After treatment, the well water samples were again collected and subjected to MPN analysis. There was a considerable reduction in the total Coliform count was observed in each sample shown in the table. The findings of Szymanski et al 2003 imply that the EM has an effect on some parameters in on-site wastewater treatment systems (septic tanks), such as the formation of optimum conditions in septic tanks.. This finding of our study also suggests that EM has an impact on treatment of contaminated water sample with heavy total coliform forming bacteria

Effect of EM on plant growth

The current study was carried out to assess the effect of EM on plant growth promotion. The consortium with molasses was sprayed onto plants and was compared with that of control. Plant height, weight, strong root formation, number of leaves per plant, leaf width were significantly influenced by the treatment of EM. In leafy vegetables, leaf length is a major yield determining factor. In Figure 5 Effect of Plant growth on Microorganism percentage of growth against various parameters such as number of leaves, leaf length, leaf width, height of plant and weight of the plant were compared. Sharma et al 2017 reported the effectiveness of EM on plant growth promotions. The study observed that EM increases soil enzyme activities whereby increase in plant growth was observed when the EM based bio-compost were tried in our study bioformulation was made and was sprayed and treated the plant seeds before and after growth. Iriti et al 2019 reported that the effects of EM treatment on leaf in vivo chlorophyll a fluorescence of photosystem II (PSII), yield, and macronutrient content of bean plants grown on

different substrates (nutrient rich substrate vs. nutrient poor sandy soil) were investigated in bean plants grown on different substrates (nutrient rich substrate vs. nutrient poor sandy soil).Study showed that Plants that had been treated with EM remained healthy and was found to have improved plant growth when compared to that of control untreated plants. In this study also Amaranthus plants treated with EM formulation at seed stage, seedling stages were found to be healthy and high growth rate when compared to that of control plants.. As the availability of nitrogen to the plant increases the plant growth also increases. Our results showed that there is a significant difference between the EM treated plants and EM untreated plants. Effective microbes are proved to be efficiently used in chicken and manure composting as well as waste water treatment (14-15).The present study also showed that Effective microorganism used in our study can be effectively utilized in multipurpose applications microbial bioconversions.

CONCLUSION

The findings from our study reveal that kitchen wastes provided a better environment for the effective microorganisms (EM) to grow and produced a higher quality of compost. Organic matter helps in soil management for sustainable cultivation of any crop. The adaptation of effective microorganisms (EM) leads to detoxification of our landfills, decontamination of our environment and promotes highly sustainable, closed-cycle agricultural and organic waste treatment.

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Table 1: Treatment of Water

Sl. No	Sample No.	SAMPLE	DILUTIONS			INDEX Before EM Treatment	INDEX After EM Treatment
			10ml	1ml	0.1ml		
1		Suseelan	3	3	3	2400	93
2	7.	PHC	3	3	3	2400	28
3	11.	Anitha Raghu	3	3	2	1100	4
4	13.	Radhakrishnan V C	3	3	3	2400	20
5	21.	School	3	3	3	2400	20
6	23.	Church	3	3	2	1100	3

Table 2.Cultural characteristics and morphology of EM isolated from organic waste.

Colony morphology	Gram stain reaction	Organism	Isolate
Green fluorescent, Slimy colonies	Gram-negative, rod-shaped	*pseudomonas fluorescence*	Isolate-12
Cream white, Serrate margin, Powdery colonies	Gram-positive Elongated/branched	*Actinomycetes*	Isolate-5
White, mucoid, Round colonies	Gram-positive Bacilli	*Lactobacillus*	Isolate-14
Yellow, Pinpoint colonies	Gram-positive cocci	*Micrococcus*	Isolate-17

Figure 1: kitchen waste from day of collection to completely degraded form (day 7)

Figure 2: The percentage reduction of the mass of EM treated organic kitchen waste from day 1 to 7

Figure 3: Coir pith before and after treating with EM. Coir pith fibers visible after complete degradation

Figure 4: Comparison of control and EM treated spinach growth

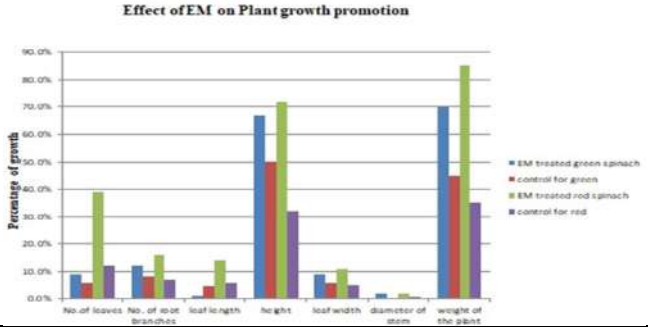

Figure 5: Effect of EM on Plant Growth Promotion

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REVIEW ARTICLE

Review of Marketed Anticancer Implants

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ABSTRACT

Cancer is the unconstrained growth of abnormal cells in a body. There are over 200 types of cancer. Various strategies have been implemented to treat this deadly dead-causing disease. Treatment with anticancer agents in implantable form shows better therapeutic response and increased life span in cancer patients. Implants are the medical devices that had constructed to restore a missing biological structure, aid a damaged biological structure, or intensify an existing biological structure. Most chemotherapeutic agents have low aqueous solubility and are cytotoxic. Targeted drug delivery is another concern in cancer treatment. Implantable dosage forms provide targeted and controlled drug delivery which helps to reduce drug toxicity. And also protect normal cells. Gliadel is an anticancer implant loaded with carmustine drug and provide better therapeutic activity. It acts by slowing the growth of cancer cells in body. Eligard is leuprolide loaded implant approved by FDA to treat prostate cancer, breast cancer, ovarian and endometrial cancer. Viadur is an implant loaded with leuprolide acetate which provides high efficacy in cancer treatment. Oncogel is an implant loaded with paclitaxel is a promising approach in cancer therapy that can reduce the systemic toxicity of the drug. The implant is capable of targeting drugs to tumor cells. This review deals with the information about marketed available anticancer implants.

Keywords: Cancer, Implant, Glioblastoma multiforme

INTRODUCTION

Cancer is a major public health problem and remains the second leading cause of death in worldwide [1]. Due to a steady increase in the population growth rate and aging, the global epidemic of cancer is eternally increasing. Cancer is distinguished as a disease associated with unsuppressed growth and speed of cells [2]. Chemotherapy aims to kill these cancerous cells [3]. But most of the chemotherapeutic agents have very poor aqueous solubility. Also, these drug molecules have poor physicochemical and biopharmaceutical properties, which results in formulation complications [4]. However controlled drug delivery systems have gained very much attention over the past thirty years and, implantable controlled release systems have shown dominance over conventional drug therapies [5]. Implant formulation provides an alternative means for delivering chemotherapeutic agents in cancer tissues. Drug loaded in implants results in sustained release of anticancer drugs results in minimizing systemic exposure [6]. Implantable devices containing either chemotherapeutic agents or radioactive elements have become an applicable treatment option [7].

Carmustine wafers; (fig 1)
Trade name: Gliadel® wafer

Drug: Carmustine is an anticancer drug. It is classified as an alkylating agent. This medication is used to treat certain types of brain tumors; glioblastoma, brainstem glioma, medulloblastoma, and metastatic brain tumors. There is no pill form of this medication and is administered as an infusion into a vein or as implantation [8]. Carmustine in implantable form is used for treating Glioblastoma multiform (GBM) which is a primary brain neoplasm. This is characterized by high proliferative activity [9]. This implant is a combination product of the drug Carmustine (7.7mg) and polymer Polifeprosan 20. The drug acts by slowing the growth of cancer cells. Polifeprosan 20 is a biodegradable copolymer that helps control the release of carmustine [10].

Manufacturing Company: This product is manufactured by Eisai Inc, for Arbor pharmaceuticals.LLC [11]. Arbor Pharmaceuticals is an Atlanta, Georgia-based pharmaceutical company that markets prescription products for the cardiovascular, hospital, neuroscience, and pediatric market [12].

FDA approval: In February 2003, the US FDA approved the Gliadel wafer for the treatment of brain tumors. This implant was approved as the first brain cancer treatment to deliver chemotherapy directly to the tumor site [13].

Pharmaceutical form: Carmustine implant is a sterile, off-white to pale yellow colour implant and is about 1.45 cm in diameter and 1mm thickness. The drug is homogeneously distributed in the copolymer matrix [14].

Administration: It is administrated intracranially. It is implanted and left the cavity after surgical removal of the brain tumor. Following maximal tumor resection, the maximum number of implants feasible with the cavity is placed [15]. Carmustine implant allows the drug to deliver directly to the site of the tumor. After removing the cancerous tissues from the brain about eight dime-sized implants were implanted in the space. In the following two to three weeks, the wafer dissolves slowly bathing the surrounding tissues with medication. The purpose of this implant is to kill the tumor cells left behind after surgery [16]

***In vivo* drug release:** This implant release drug in vivo over five days; when in continuous contact with interstitial fluid. The implant degrades in two separate phases. The first phase is the induction period and the second phase is the erosion period. There is some liberation of carmustine that occurs within the first 10 hours after implantation, during the induction phase. Hence the drug release is not purely managed by the erosion rate of the implant [17]. Clinical study shows that this implantation combined with radiotherapy significantly increases overall survival [18].

Warnings

Seizures: Seizures occurred in 37% of patients treated with Carmustin implants. 54% of treatment-emergent seizures occurred within the first five postoperative days.

Impaired Neurosurgical wound healing: This includes wound dehiscence, delayed wound healing, and subdural, sub-legal, or wound effusions. About 16% of Carmustin implant-treated patients experienced impaired intracranial wound healing and 5% of patients experienced cerebrospinal fluid leaks.

Intracranial Hypertension: About 23% of patients experienced brain edema. And also one Carmustin implant-treated patient experienced intracerebral mass effect which led to brain herniation.

Meningitis: Meningitis occurred in 40% of patients with recurrent glioma receiving Carmustin implants. Two reasons for meningitis were bacterial, and one patient required removal of implants and the other developed meningitis following reoperation for recurrent tumors.

Embryo-Fetal Toxicity: This implant can cause fetal harm when administered to pregnant women. The drug Carmustin is embryotoxic and teratogenic [19].

Leuprolide acetate implant; (Fig 2)

Generic name; Leuprolide acetate
Trade name; Eligard

Drug: The drug leuprolide is classified as a luteinizing hormone-releasing hormone (LHRH) agonist. The drug is used to treat prostate cancer, breast cancer, ovarian and endometrial cancer. This is hormone therapy [20].

Manufacturing company and FDA approval: The leuprolide acetate implant is sponsored by Atrix Laboratories, Fort Collins. This implant was first approved by FDA for palliative treatment of prostate cancer on January 24, 2002 [21].

Pharmaceutical form and dose: This is an injectable suspension administered via subcutaneous injection. 7.5 mg depot is administrated intramuscularly (IM) or subcutaneously once a month. 22.5 mg depot IM is administrated once every 3 months and 30 mg depot IM once every 4 months. Eligard implants are used to treat the symptoms of prostate cancer and not cancer itself [22].

Administration: This medicine is injected into an area with adequate subcutaneous tissues. The major components are leuprolide acetate, poly (DL-lactide-co-glycolide), and N-Methyl-2-pyrrolidone (NMP). Poly (DL-lactide-co-glycolide) is the water-insoluble biodegradable polymer and NMP is the biocompatible water-soluble solvent [23].

Drug release: This injection forms a solid drug delivery depot under the skin and the medicine is slowly released over time [24]. It consists of a biodegradable polymer and when it is injected into the body the water-soluble solvent diffuses from the site and water permeates into the polymer matrix. The polymer is water-insoluble. And it coagulates upon contact with aqueous body fluid to form a solid implant [24].

Side effects of Eligard

Common side effects of eligard include:

- Hot flashes
- Increased sweating
- Night sweat

- Tiredness
- Dizziness
- Chills
- Increased urination at night
- Mental/mood changes
- Insomnia
- Impotence
- Constipation
- Diarrhea
- Memory problems [25]

Leuprolide acetate implant; (Fig 3)

Generic name; Leuprolide acetate implant
Brand name; Viadur

Manufacturing company and FDA approval: The implant is manufactured by ALZA corporation crescendo pharmaceuticals [26]. It is approved by FDA in the year 2000 march 3.

Pharmaceutical form and dose: Leuprolide acetate implant is a sterile non-biodegradable, osmotically driven miniaturized implant. This implant is designed to deliver leuprolide acetate for 12 months of the period at a controlled rate. The implant contains a 65 mg drug [27]. The implant is a cylindrical titanium alloy drug reservoir. The cylinder measures 4mm by 45mm and houses a polyurethane rate-controlling membrane, an elastomeric piston, and a polyethylene diffusion moderator.

Administration: This implant is inserted subcutaneously in the inner aspect of the upper arm. A small incision is made in the upper arm and the implant is placed then the incision is closed with special surgical tape and covered with a bandage. And after 12 months the implant must be removed and another implant may be inserted to continue therapy [28].

Mechanism of action: The drug is a luteinizing hormone-releasing hormone (LH-RH) agonist and acts as a potent inhibitor of gonadotropin secretion. The continuous administration of this drug results in a reduced level of LH and follicle-stimulating hormone (FSH). In males, testosterone is reduced to castrate level [27]. Testosterone is appeared to be needed by the prostate cancer cells and these cancer cells shrink or stop growing when the body's supply of the hormone reduces. And by reducing the amount of testosterone, the implant helps to reduce pain, urinary problems, and other symptoms of prostate cancer [29].

Side effects

Common side effects include;

- Hot flashes
- Lack of energy
- Depression
- Sweating
- Headache
- Bruising
- Breast enlargement
- Impotence
- Pain and redness

The company stops the marketing of this product in April 2008 due to diminished market demand and increasing manufacturing costs [30].

Paclitaxel implant

Trade name; Oncogel

Drug: Paclitaxel is one of the most important anticancer agents, has been used for the treatment of different types of cancers. This drug arrests the cell cycle and induces cell death by stabilizing microtubules and interfering with microtubule disassembly in cell division [31].

Manufacturing company: Macromed, Inc, is a private drug development and manufacturing company that is the developer of oncogel implant [32].

Pharmaceutical form: The product consists of paclitaxel dissolved in water-soluble, biodegradable, and a thermosensitive polymer called poly DL-lactide co glycolide. Oncogel is a promising approach in cancer therapy that can reduce the systemic toxicity of the drug and deliver a high concentration of drug [33].

Administration: Paclitaxel has poor CNS penetration and dose-limiting toxicities when administered systematically. Oncogel (a controlled release depot formulation of Paclitaxel in ReGel form) provides controlled local drug release when placed into the CNS [34]. Regel is an example of an environmentally sensitive controlled release delivery system.

Oncogel has a high ability to target paclitaxel to the tumor site with a very small amount reaching the circulation. This results in an acceptable safety profile. Oncogel is a drug delivery technique that uses physical targeting of the drug to the site of action and provides controlled release of the drug. The drug is physically targeted into the tumor by intralesional injection or placement into the tumor cavity after tumor resection. This prolongs the release of drugs within the tumor [35]. And this gel will disappear in 4 to 6 weeks as it releases the drug [36]

Side effects

- Pain
- Injection site bruising
- Redness
- Irritation
- Muscle span

CONCLUSION

Cancer is a leading cause of death in worldwide. Cancer therapy using implantable drug formulation is an effective drug delivery platform. Treatment with an implantable formulation of anticancer agent shows increased life span in patients and good recovery. Most of the chemotherapeutic agents have limitations include low bioavailability, systemic toxicity, uncontrolled drug delivery, etc. Targeted drug delivery is another concern in chemotherapy. By the formulation of implantable dosage form, all these limitations can be overcome. Also, a high concentration of drugs can be delivered to the tumor site. Hence implantable drug formulation is a breakthrough in cancer therapy. An implantable drug delivery system containing either chemotherapeutic agents or radioactive agents is a better treatment option in conquering cancer disease.

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Fig 1: Carmustine wafers

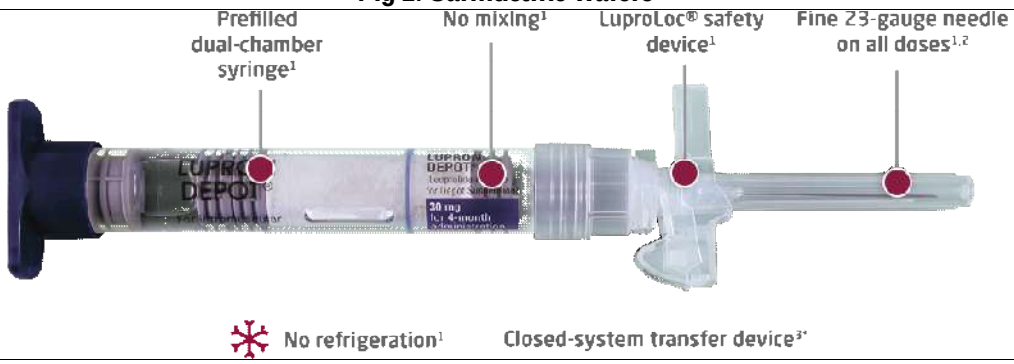

Fig 2: Leuprolide acetate implant

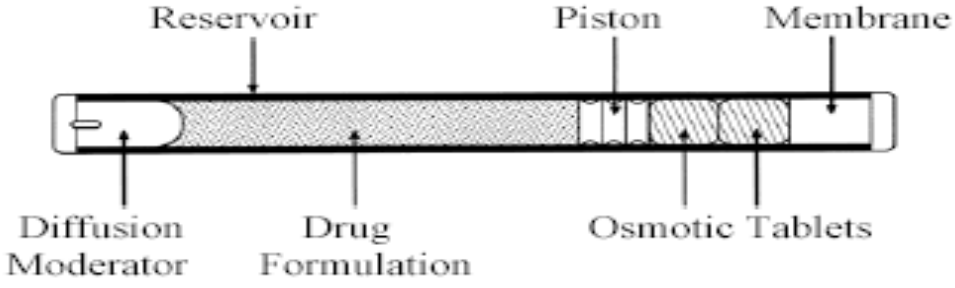

Fig 3: Leuprolide acetate implant

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RESEARCH ARTICLE

Health Status of Children with HIV/AIDS Living in Care Home

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ABSTRACT

Health status of children with HIV/AIDS can be variable depending on the stage of disease. Early identification of HIV infected children's can improve their health status. The study design was descriptive design with quantitative approach, 100 samples taken by purposive sampling technique. HIV children selected from the care home from Salem district in order to assess the health status of HIV children an observed checklist with face to face interview of participants has been conducted. Data was analyzed by using the statistical software named Spss 2.1 according to the findings of the study, the health status of HIV children of early detection can prevent the further opportunistic infection and improve the physical status.

Keywords: Health status, HIV children.

INTRODUCTION

The human immunodeficiency virus (HIV) targets the immune system and weakness people's defense against many infection. As the virus destroys and impair the function of immune cells , the child becomes gradually immune deficient .The adorable fact behind the scene is by right combination of drugs and loving support, kids with HIV can grow up to live long and fulfilling lives. Children get pretty much the same treatment as adults a combination of medication called ART. Adhering to ART kids with HIV and AIDS can safely go to school. So, awareness and education programe help to break down the stigma around HIV, so that children can have friends and feel normal growing up. The children orphaned due to HIV/AIDS are rehabilitated in terms of having shelter and provision of education and health care much needs to be done in terms of improving the all the aspects of children needs (1).

Statement of the Problem

A study to assess the health status of HIV children living in care home.

Objectives

To assess the health status of HIV children.

To determine the association between selected health status and demographic variable.

METHODOLOGY

The study design was descriptive design with quantitative approach, 100 samples were taken by using purposive sampling technique. HIV children selected from the care home of Salem district in order to assess the health status of HIV children. An observed checklist with face to face interview of participants has been conducted. Data was analyzed by using the statistical software named Spss 2.1.

RESULT AND DISCUSSION

The Percentage distribution of demographic variables of HIV children, 80% were in the age group of 13-18 years, All are males, 47% of children were in second birth order, 53% of HIV children are lost their both mother and father 100% of children all are immunized, all are in stage I of clinical stage, 80 % children were in high school education. 99% of children taking ART 1-4 years. Basically children with HIV /AIDS or having less immunity and easily affected by the infection Here the table shows that majority of the HIV children having problem 53% in skin 33% having blurred vision, 28% having unilateral or bilateral deafness 43% having nasal discharge, 28 % having oral thrush 65% having teeth decay, 44%having lymph node enlargement 41% rabbit heart beat 27% having abdominal pain, 73% having muscle pain 88% having frequent urination and 62% having restlessness. Here, HIV children having most of the problem in skin, mouth, musclo skeletal system and neurologic system. Need more attention in health status to avoid are reduce of the health problem(2). Table 3: the table shows that the demographic variable of Age, Birth order, parental living status, Education have shown statistically not significant association with the health problem of children living with HIV/AIDS (3). The association between selected demographic variables and the health status of HIV children of age (1.042 df=1 p=<0.05) Birth order (0.597 df=2 p=<0.05 NS) and education (1.042 df=1 P=<0.05 NS)

CONCLUSION

HIV children are more prone to get morbidities in the form of infections like ARI, ADD and other communicable disease due to weak immune system. All the HIV children are having physical and psychosocial problems. According to my study the health problems is reduced due to care given by care home. Health status of HIV children can be early detected in care home to prevent the further spreading of infections. So I conclude that, we need to increase the number of HIV care giving institutions in order to prevent HIV infections.

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Table 1. Frequency distribution of demographic profile of HIV children.

Demographic variables	Frequency	Percentage.
Age		
5-7 years	0	0 %
8 -12 years	20	20 %
13-18years	80	80 %
Gender		
Male	100	100%
Female	0	0%
Religion		
Hindu	97	97%
Christian	2	2%
Muslim	1	1%
Socio economic status		
Upper lower	1	1%
Lower lower	99	99%
Birth order		
First	29	29%
Second	47	47%
Third	24	24%
Parental living status		
Both alive	12	12%
Father death mother alive	17	17%
Mother death father alive	18	18%
Both dead	53	53%
Immunization status		
Completely immunized	100	100%
Partially immunized	0	0%
Not immunized	0	0%
Don't know	0	0%
WHO clinical stage		
Stage 1	100	100%
Stage 2	0	0%
Stage 3	0	0%
Stage 4	0	0%
Education qualification		
Primary education	0	0%
Middle school education	20	20%
High school education	80	80%
School drop out	0	0%
History of ART		
1-4 years	99	99%
5-8 years	1	1%
9-12years	0	0%

Table 2.Frequency and Distribution of Health Problems of HIV Children

Health Problems	Not Present		Present	
	F	%	F	%
General condition				
Active	60	60	40	40
Fever	73	73	27	27
Skin				
Dry skin	67	67	33	33
Night sweat	83	83	17	17
Lesions	86	86	14	14
Folliculitis	77	77	23	23
Dermatitis	84	84	16	16
Skin rash	88	88	12	12
Eye				
Eye discharge Flashes of	91	91	9	9
bright	90	90	10	10
vision Eye pain	100	100	0	0
Loss of sight	100	100	0	0
Blurred	67	67	33	33
Floaters	98	98	2	2
Ear				
Unilateral or bilateral deafness.	72	72	28	28
Discharge for the ear.	94	94	6	6
Nose				
Nasal discharge	57	57	43	43
Shortness of breath	64	64	36	36
Nasal congestion	62	62	38	38
Sneezing attack	65	65	35	35
Mouth				
Swollen lips	100	100	0	0
Oral thrush	72	72	28	28
Swollen tongue	91	91	9	9
Dry mouth	88	88	12	12
Canker sore Swollen salivary	100	100	0	0
gland	87	87	13	13
Swollen gums	93	93	7	7
Sore and cracks in the corners	76	76	24	24
of mouth Teeth decay	35	35	65	65
Neck				
Bilateral parotid enlargement	65	65	35	35
Lymph node enlargement	56	56	44	44
Chest				
Crackels / rhonchi Rapid heart	85	85	15	15
beat	59	59	41	41
Abdomen				
Abdominal pain Abdominal	73	73	27	27

Health Problems	Not Present		Present	
	F	%	F	%
tenderness	82	82	18	18
Vomiting	100	100	0	0
Nausea	100	100	0	0
Constipation	86	86	14	14
Dysphagia	94	94	6	6
Diarrhea	95	95	5	5
Musculo skeletal system				
Joint pain	44	44	56	56
Muscle pain	27	27	73	73
Numbness and Tingling	57	57	43	43
in the hand feet	78	78	22	22
Neurologic system				
Fatigue	85	85	15	15
Head ache	66	66	34	34
Insomnia	66	66	34	34
Irritable	94	94	6	6
Restlessness	38	38	62	62

Table 3: Association between selected demographic variables and health status of HIV children.

Demographic Variables	Total Health Problems		Chi – Square Value
	Lowest – 10 F	11 and Above	
Age			
5-7	0	0	1.042
8-12	6	34	df=1
13 -18	14	46	P=<0.05 NS
Birth order			
First	13	16	0.597
Second	17	30	df=2
Third	10	14	P=<0.05 NS
Parental living status.			
Both alive.	6	6	1.041
Father death	6	11	df=3
mother alive.	6	12	P=<0.05 NS
Mother death	22	31	
father alive.			
Both dead.			
Education			
Primary education	0	0	1.042
Middle school			df=1
education.	6	14	P=<0.05
High school			
education.	34	46	
School drop out.			

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RESEARCH ARTICLE

Advancements in Nanotechnological Approaches towards Sustainable Agriculture

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ABSTRACT

Sustainable agriculture is extremely critical for sustenance of all the life forms owing to the exceedingly demand of food products for an ever increasing population across the globe. Essentially, sustainable agriculture involves holistic management of livestock, crops and fisheries, so as to make the farming process self-sustaining for a longer period. Food and agriculture sector contributes heavily in meeting the human demands and thus sustainable agricultural practices would result in positive and long lasting consequences. Sustainable growth in the field of agriculture can be achievable by the intervention of advanced technologies such as nanotechnology. Conventional farming methods failed to fully utilize the available resources. To circumvent these limitations, nanotechnology can be effectively used to enhance the crop quality and productivity. Applications of nanotechnology in the field of agriculture will lead to improved plant growth, stabilization of soil and microbes, targeted usage of chemicals and most importantly nanotechnology contributes profoundly for waste management. Since the nanoparticles are extremely small in size and possess larger surface area, they display higher activity. Nanoparticles are used as seed priming agents resulting in enhanced seed germination rate, consequently favouring for overall growth of the plant. Nano-capsulated fertilizers and pesticides brought a revolutionary change encouraging for betterment of crop and animal health without affecting the environment. Nanotechnology bears tremendous potential to effectively integrate manifold activities of agriculture practices with sustainable productivity. Although the potential benefits of nanotechnology are countless, the environmental safety concerns needs to be cautiously examined.

Keywords: Sustainable agriculture, nanotechnology, nanoparticles, nano-capsulated fertilizers, productivity.

INTRODUCTION

Agriculture sector predominantly supplies the essential raw materials for varied industrial setups. Agricultural development relies on the competition between limit in natural resources and indefinite increase in population across the globe. Necessarily, development in the agricultural sector focuses on eco-friendly and sustainable strategies to combat the ever-increasing demand for the food produce. During recent years, this has been the primary goal and innumerable approaches have been implemented to achieve the said purpose (Johnston and Mellor, 1961). To a greater extent economic growth across various nations, preferably in the developing countries, determines the agricultural nutrient balances by impacting the soil fertility (Yunlong and Smit 1994; Campbell et al., 2014). Agricultural sector plays a vital role in restricting the ill effects of poverty and hunger, which would ultimately help the nation to rise (Mukhopadhyay, 2014). Unfortunately, a major part of the agriculture sector still has its foot prints in the rural areas, where extension of modern agricultural practices is limited. Advanced technologies and methodology in the field of agricultural sector plays an important role in determining the quality and quantity of the agricultural production (Yunlong and Smit, 1994). Overall development of agriculture depends on several factors such as climate change, natural resources; ecosystem processes (Thornhill et al., 2016). Innovative technologies like nanotechnology play a vital role in supplementing the cause for sustainable growth of agriculture. Nanotechnology in the field of agriculture has gained its attention since decades (Feynman, 1996; Bulovic et al., 2004). Since then, nanotechnology has been fine-tuned accordingly to contribute more efficiently and effectively (Bonnell and Huey, 2001).

Nanotechnology basically deals with materials at the nano scale (nanometers). The smaller size of the nanomaterial possesses larger surface area and thus displays more activity (Gibney, 2015). The altered magnetic property of the nanomaterials can be exploited in various applications including biological processes (Pokropivny et al., 2007). This ultimate technology which is reliable and to some extent cost effective posses the ability to bring a revolutionary change in the field of agriculture owing to several unique optical, electronic and plasmonic properties (Sun, 2007). Current situation demands fast and reliable technologies for monitoring and diagnosing biological processes in the agricultural sector (; (Aziz et al., 2015). Nanomaterials are primarily synthesized by two methods: chemical synthesis and green synthesis. Chemically synthesized nanomaterials are toxic to the environment whereas, the nanomaterials arising from the plants, referred to as green synthesis process is eco-friendly and cost-effective (Sekhon, 2014). Green nanotechnology is considered to be a safe process which preferably lessens the use of energy and emission of green house gases. Green nanotechnology has to be revolutionized in order to mitigate the existing limitations in the agricultural sector (Kandasamy and Prema, 2015). In modern agriculture, agrochemicals being predominantly used adversely affect the quality of food produce and the soil health. Pesticides and fertilizers severely impair the environment and ultimately elevate the health risk (Sertova, 2015; Kah, 2015). Nanotechnology can serve as an effective alternative to these chemical-based agro materials and can contribute in increasing the agricultural yields (Sekhon, 2014; Liu and Lal, 2015; Figure 1). Biosensors in the field of agriculture can also be exploited immensely supplemented with nanotools to enhance the sensitivity of the advanced techniques and will improve the outcomes of these applications (Sertova, 2015). Nanosensors can play a significant role in deciphering biochemical and molecular processes (Fraceto et al., 2016).

Nanoparticles and their applications

Carbon Nanotubes (CNTs) and Nanorods: CNTs represent two dimensional graphene sheet rolled into a tube. Essentially there are two distinct types of nanotubes; single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). CNTs bear a decent tensile strength (approximately 200 Gpa), making it an ideal material for systems including nanoelectro mechanics and reinforced composites. The diameter of the CNTs determine the properties, primarily the diameter of most SWNTs is about 1mm. Modulating the properties of CNTs will definitely widen up its applicability in various fields including agriculture (Raliya et al., 2013). Precision in agriculture systems may be facilitated by the use of CNTs. CNT nano-sponges preferentially adsorb more amounts (3 times) of toxic organic

solvents like dichlorobenzene from water as compared to the powder form of CNT (Camilli et al., 2014). This phenomenon may be widely exploited, particularly to remove water pollutants like oil, pesticides, fertilizers and pharmaceuticals (Porter et al., 2007).

Nanoemulsions: Though there are limited differences between micro and nanoemulsions, but the physical properties exhibited by both may differ widely. Due to the extreme small size of droplets, the surface area to volume ratio is higher; also the elastic modulus and laplace pressure are considerably higher as that of the ordinary emulsions. Majorly, nanoemulsions are optically transparent unlike general emulsions making them fit for incorporation into drinks (Gupta et al., 2016).

Agricultural Sustainability and Nanotechnology

The nanotechnology can play a vital role in enhancing the productivity and can also impart sustainable development through controlling several associated factors such as monitoring the water quality and reducing the use of pesticides (Gruère, 2012; Mukhopadhyay, 2014). Nanomaterials posses diverse attribute. Properties of nanoparticles greatly influence their efficiency which is essentially modulated by shape, chemical composition, surface charge, surface structure, and extent of particle aggregation or disaggregation (Ion et al., 2010). Nanotechnology has been proven to be a brilliant resource management strategy in the field of agriculture. Moreover this technology has gained wide attention in the food processing and packaging sector (Floros et al., 2010). Now-a-days, nanosensors have also been widely applied in the field of agriculture primarily to detect contamination and trace of heavy metal in the water and soils (Ion et al., 2010). Nanomaterials aid in improving the efficiency of microbes for degrading the waste materials and also get directly involved in catalyzing the waste from the environment (Dixit et al., 2015). Thus, nanomaterials play a key role in detoxifying the soil environment and facilitate the stress free growth of plants resulting in enhanced yields (Ion et al., 2010).

Nanofertilizers: Nanofertilizers are widely used to enhance the agricultural productivity. Mostly, nanofertilizers contain silica, titanium, iron, zinc, cadmium, selenium etc. which positively regulates the physiological processes. Control release of nanofertilizers is also a key component and is exhibited by several nanoparticles such as zinc core shell QDs and gold nanorods. Extensive studies on the biological fate, uptake and toxicity of several metal oxide nanoparticles like FeO, ZnO, CeO_2, TiO_2, Al_2O_3 have been reported and been applied in the agricultural sector (Dimkpa, 2014; Llop et al., 2014; Zhang et al., 2016). In alkaline soils, zinc plays a critical role as its deficiency leads to severe growth troubles in plants (Sadeghzadeh, 2013; Kandasamy and Prema, 2015; Marzbani et al., 2015). To accelerate the process of smart agriculture, nanofertilizers can contribute immensely and may minimize the productivity loss caused due to climate change (Helar and Chavan, 2015). In recent years, extensive studies related to expansion of nanomaterial based technology in the field of agriculture have been explored. Nanoparticles exhibit immense transduction property which can contribute in the analytical processes of agricultural products (Kandasamy and Prema, 2015). Moreover, these nanofertilizers possess low toxicity and thus highly relevant in biological systems.

Nanopesticides: Nanotechnology offer strategies to combat plant pathogens and act as a suitable alternative to conventional chemical pesticides. Nanopesticides can immensely contribute to plant defense response against incoming pathogens without displaying any harmful side-effects (Khota et al., 2012). Nanoencapsulated pesticide exhibits enhanced solubility, slow releasing properties, permeability, stability and specificity. These properties of nanopesticides are achieved primarily by shielding the encapsulated active ingredients from premature degradation or escalating their pest control efficiency for a longer period. Nanopesticides effectively reduce the use of conventional eco-hazardous pesticides and thus protect the human beings from severe ill effects and at the same time increases the crop productivity (de Oliveira et al., 2014; Kah and Hofmann, 2014; Grillo et al., 2016; Nuruzzaman et al., 2016). To expand the quality of products of desired chemicals to be precisely delivered to their target in biological processes; microencapsulation-like nanoencapsulation is used to the target biological process (Gouin, 2004).

Ecotoxicological Implications of the Nanoparticles: Though nanotechnology serves as a beneficial strategic tool in the agricultural sector, still it bears antagonistic effects and may possess threat to both human and plants. Thus, assessment of toxicity effects of nanoparticles is equally significant (Yang and Watts, 2005). The toxicity of nanoparticles depends on several factors including binding specificity, solubility and the charge at membrane surface. Nanoparticles such as Fullerenes, ZnO, TiO_2 and SiO_2 are photochemically active, thus excite electrons when exposed to sunlight which eventually results in formation of superoxide radicals mediated by oxygen (Hoffmann et al., 2007; Rana and Kalaichelvan, 2013). ROS generation is evident from TiO_2 and fullerenes whereas, silicon based nanoparticles protect against oxidative stress (Sayes et al., 2004; Daroczi et al., 2006; Tripathi et al., 2016b). Extensive studies would determine the deferred impacts of nanoparticles on the environment. It would be intriguing to reveal the effects of bioaccumulation of nanoparticles in the food chain and their adverse effect on the ecological system and how this affects the vital processes like respiration, photosynthesis, transpiration and translocation of food materials (Shweta et al., 2016).

Growth of Cultivated Plants and Its Ecotoxicological Sustainability: Across the food chain, the plants as producers hold an important position and greatly influence the overall characteristic of the ecosystem. Now-a-days plants are grown not only in soil but also in aqueous media. Nanoparticles of iron oxide and other metals can get deposited on plants like *Lepidium sativum* and *Pisum sativum*, pointing out the presence of nanoparticles in the natural ecosystem (Bystrzejewska-Piotrowska et al., 2012; Abbas et al., 2016). Use of nanoparticles synthesized by green technology process would be a superior choice over chemically synthesized nanoparticles (Chakravarthy et al., 2012; Perlatti et al., 2013). It is highly essential to determine the ecotoxicity level of nanomaterials present in the soil, since it would severely affect beneficial microorganisms (Mishra and Kumar, 2009). Nanocomposites devoid of toxicity would upgrade the agricultural produce. So, it is evidently critical to routinely examine the nanoparticles introduced in the agricultural field in order to sustain an eco-friendly environment.

Nanobiosensors

Nanoscale materials can be exploited in the field of development of biosensors. The performance and sensitivity of biosensors can be improvised by the use of nanomaterials (Sagadevan and Periasamy, 2014). Nanobiosensors have gained a wide attention owing to their sensitivity and effectiveness in health and agricultural sector. They are also used for genome analysis and in food and processing industries. Still advances in nanobiosensors are limiting and needs to be improvised (Fogel and Limson, 2016). Nanomaterials such as CNTs, QDs, gold, silver, cobalt nanoparticles, have been vigorously investigated for their applications in biosensors. Essentially, a biosensor is a device that combines a biological recognition element with physical or chemical principles. Due to the presence of the bioreceptor, which is combined with an appropriate transducer resulting in generation of signal, makes the biosensor highly sensitive. Biosensors in the form of dendrimers, thin films and enzymes have been implemented in the agricultural sector, preferably to record the biological processes in the form of electrical signal. In one hand, biomolecules including proteins, enzymes, nucleic acids and in the other hand, nanoparticles used in the sensors play a vital role in this mechanistic approach (Brolo, 2012; Rai et al., 2012).

Nanotechnologies in Food Industry

Nanotechnology has the capability to contribute bioactive ingredients in foodstuffs and can enhance the nutrient value (Martirosyan and Schneider, 2014). Texture modification of food can also be mediated by several nanotechnological tools. Nano biosensors can interact with food and attractive surfaces and eventually maintains the colours and glaziers of food. Intelligent packaging, nanoprinting, controlled release, nanocoding of paper and plastic materials are the outcomes of the interventions of nanotechnology in the food industrial sector (Siegrist et al., 2008; Bhushani and Anandharamakrishnan, 2014; Ghaani et al., 2016; Khond and Kriplani, 2016; Savina et al., 2016).

Food Process: Several food products including milk and meat contain nanoscale materials like casein and protein filaments respectively. Nutrients, mainly vitamins are delivered into the cellular process by encapsulation process which maintains higher efficiency in terms of target precision and its ultimate output. Fortifying nanoparticles to

foods and drinks do not affect the appearance or taste. Nanoparticle emulsions are used in several food products including ice creams resulting in a uniform texture throughout (Berekaa, 2015). Usually, the particles generated by capsulation technology are in nano- and microscale.

Food Packaging and Labeling: In the food industrial sector, maintaining safety, quality, taste and freshness are a challenge. Smart packaging may significantly minimizes these challenges. Development of smart packages is not yet fully explored. In recent years, nanosensors have been incorporated into packaging materials to sense any oxidation process occurring in the food, primarily by detecting change in the colour of the food (Bumbudsanpharoke and Ko, 2015). Packaging materials used in many drink products are made with nanocomposites, which increases shelf life of the product. Nanoparticles also slow down oxidation of food products and thus avoid degradation (Berekaa, 2015). Additionally, nanocoatings on the food contact surfaces contribute to antimicrobial activity as in case of silver nanoparticles which are entrenched in the plastic polymers. The antimicrobial properties of nanoparticles can thus be extensively used in the food packaging sector (Liu et al., 2009; Aziz et al., 2016). Silver nanoparticles neutralize the surface electric charge of the bacterial membrane, resulting in change in its permeability and finally lead to cell death. Nanosensors tremendously assist in food labeling process and along with nanoparticle-based inks can contribute towards smart recognition of the food products (Li et al., 2016). Nanobarcodes can also be introduced in the food packaging sector. It has been shown that nanoparticles display higher adsorption process and thus can remove contaminants from any surface (Raliya et al., 2013; Hajirostamlo et al., 2015; Li et al., 2016).

CONCLUSION AND FUTURE PERSPECTIVES

Sustainable agriculture practices are a key requirement. This is achievable by incorporating eco-friendly technologies such as nanotechnology which offers a balance between environmental pollution and agricultural yield. Since conventional technologies are well known to exaggerate pollution, it would be pivotal to have alternate strategies to overcome these limitations. Nanotechnology can offer a better opportunity to enhance the agricultural produce in a sustainable approach. Even, nanoparticles displays toxicity attribute both for plants and animals, thus future research in this field may reveal strategies to exploit nanoparticles to the fullest. Comprehensive database development related to nanotechnological interventions in the field of agriculture across the globe may be beneficial for utilization of this technology in an effective manner. Nanotechnology can offer varied utilities in the agricultural and food sector. Nanobiosensors possess multifaceted benefits as they can be integrated with nanopesticides and nanofertilizers for contributing towards precision agriculture. Nanotechnology holds innumerable benefits and thus needs to be explored heavily so that the agricultural sector can be economically developed and consequently the nutritional demand can be fulfilled across the globe.

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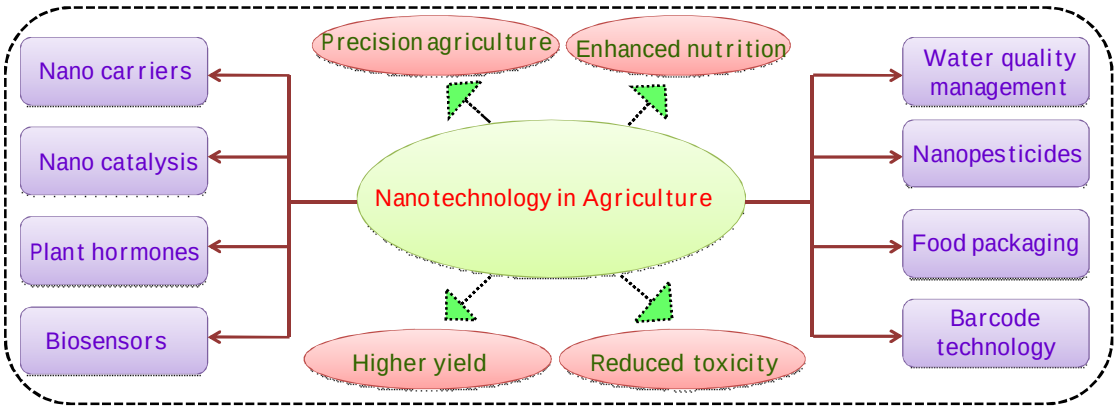

Figure 1: Multifaceted applications of nanotechnology in the agricultural sector.

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RESEARCH ARTICLE

Synthesis and Study of Photocatalytic Activity of Cadmium Sulphide Nanoparticles

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ABSTRACT

A novel chemical synthesis approach has been adopted for synthesis of cadmium sulphide nanoparticles at low temperature and characaterized by different characterization techniques. The phase analysis by X-ray crystallographic study shows that the thus synthesized nanoparticles are in hexagonal phase with increasing crystallite size increases with rise in experimental temperature. SEM images shows particle type of morphology with slight increase in size with higher temperatures, and well defined particles are formed at 90 ºC. The growth mechanism has been explained. Moreover, the photocatalytic activities of thus synthesized nanoparticles have been determined and compared.

INTRODUCTION

A substantial rise in industrialization from the last few decades there have been substantial rise in Industrialization which is good for development of the mankind. However at the same time it has some negative impact on environment in terms of the discharge of toxic chemicals, mostly dyes in to water resources resulting in their contamination. Such contaminated water, reportedly has, carcinogenic impact on living organisms [1]. A possible account for such an observation is the invention of a process for effective disinfection and destruction disinfection byproducts (DBPs). Commonly used chemical disinfectants by water industries are free chlorine, choramines and ozone which react with various constitutents in natural water to form many carcinogenic disinfection byproducts. It has been prediced that DBPs will be formed at any time during chemical oxidants are used in water treatment [2.3]. Added to this, the resistance of some pathogens, such as Cryptosporidium and Giardia to conventional chemical disinfectants require extremely high disinfectant does leading to excessive DBP formation. Therefore it is urgently needed an innovative approach to enhance the reliability and effectiveness of disinfection at the same time avoiding the DBP formation [4]. To combat the present situation, maximum interest has been focused on photocatalytic semiconductors. In this regard, maximum interest has been focused on semiconductor photocatalysts, which are capable of acting as sensitizer for light induced photocatalysis. They are supposed to involve advanced oxidation process, that lead to degradation of the organic pollutants forming ecofriendly by products. In this regard, CdS is a n-type wide band gap semiconductor (bandgap: 2.42 eV) belonging to II-VI chalcogenides. Its field of applications is

vast such as light diodes, transistors, photoelectric and emission devices [5], different types of biological sensors [6-10], optoelectronic fields, solar cells, luminescence devices [11-17] and as a photocatalyst [18-23].

Experimental

All the reagents were of analytical grade and used without further purification. Cadmium chloride ($CdCl_2$), Ethylenediamine ($C_2H_4(NH_2)_2$), Carbon disulfide(CS_2), Hydrazine (N_2H_4), Acetone($CH_3)_2CO$, $CdCl_2$ were and Acetone were purchased from PALLAV company Hydrazine was procured from CDH company, Ethylenediamine was from MERCK company and Carbon disulfide was purchased from RAINBOW.INC company. Methyl blue ($C_{37}H_{27}N_3Na_2O_9S_3$) was bought from SIGMA-ALDRICH(MERCK) company and used prepared CdS Nps in lab. Distilled water used as a solvent from CESIUM company. Distilled water used as a solvent for all synthesis purpose.

Synthesis

In the typical procedure, 0. 55 g of $CdCl_2$ was dissolved with 55 mL. distilled water taken in a 250 mL. round bottom flask. We noticed it completely soluble in water. Next a magnetic bead was put into the flask with the setup was placed on a hot plate with a perticular RPM, temperature value and after that 3mL of Carbon disulfide(CS_2) was added to the solution & It was seen that oil droplets formed flowing on the surface of solution due to the CS_2 is insoluble in water. It is try to dissolved by constant stirring with the help of the magnetic bead. By using gloves and dropper, 3.5 mL of ethylediamine was added above the solution. A light white milky solution was formed due to the forming a hydrated compound upon contact with atmospheric H_2O. A reflux condenser is then attached to the round bottom flask and the setup was placed on a hot plate provided with magnetic stirrer. After 1 ½ hr it looked light yellowish color. The reaction was carried out for 3 h with temperature 50 ºC at RPM 500- 700. On vigorous stirring a yellow colored solution was formed which was filtered with the help of Buchner funnel and Whatman filter paper. The residue was taken out and put it into the oven for 1 hr. The same procedure was repeated for synthesis at 70 and 90 ºC.

Photocatalytic activity of CdS

50 mL of 5.5 x 10^{-4} M aqueous solution of methylene blue (MB) was placed in a 100 mL beaker. To this, 25 mg of catalyst was added and the entire mixture was stirred in dark for about 1 h to so as to attain equilibrium of adsorbed methylene blue species with the desorbed amount present in the solution on the catalyst. The above beaker was placed under sun light with constant stirring. The amount of the dye decomposed, was analyzed from time to time by taking a small amount from the mixture and determining its absorbance with the help of colorimeter at 664 nm.

Characterization techniques

The powder X-ray diffraction patterns of the products were recorded on X'Pert Pro PANalytical Instrument using Cu K_α radiation in the range of 2θ = 10-70º with scanning rate of 3º per minute. The morphology of the samples was studied by scanning electron microscope (SEM) on Carl Zeiss instrument. The absorbance of the sample was noted with colorimeter of systonic company.

RESULT AND DISCUSSION

Figure 1 shows the X-ray diffraction (XRD) patterns of the products inferred that the different crystal reflection planes could be indexed on the basis of hexagonal lattice of CdS (a= 4.136 and c= 6.713) [JCPDS 77-2306]. The absence of any additional peak in the corresponding diffractograms also suggests the formation of high purity CdS. From the XRD patterns it can be seen that at 50 ºC the sample is mostly amorphous with a very small percentage of crystallinity. However with rise in temperature of 70 and 90 ºC there is a substantial increase in crystallinity with rise in temperature. The crystallite size was also determined using Scherrer's formula as,

$D=K\lambda/(\beta \cos \theta)$

Where K, K, λ, β, and θ are Scherrer constant, wavelength of X-ray radiation target used, maximum peak width in half height and angle of diffraction respectively and the data is as follows.

Temperature in degree celcius	50	70	90
Crystallite size (nm)	34	46	63

Figure 2 shows SEM images of the products obtained at reaction temperatures 50, 70 and 90 ºC. It clearly demonstrates that the formation of CdS nanoparticles (size: 100-200 nm) takes place at these temperature range. The rise in crystallinity is also reflected in the SEM patterns at higher temperatures. Moreover the particle size was found to be controlled at higher temperature and optimum particles of size around 120 nm was obtained at 90 ^{0}C. Based on the results, the below mechanism was postulated. Ethylene diamine (EDA) which was taken as a templating agent also act as a complexing agent, binds with Cd^{2+} and forms a complex [24]. In this process the release and availability of Cd^{2+} was reduced for which the reaction is slowed down favouring crystallisation and separating the growth step from the nucleation step [25]. So, Cd^{2+} ions from Cd-EDA complex are released slowly and reacts with S^{2-} from thus evolved H_2S gas from the combination of ethylene diamine and carbon disulphide.

At lower temperatures (50 and 70 ^{0}C), there is slow releasing of Cd^{2+} ions from the complex which reduces the speed of reaction. Hence, the formation of CdS become slow and the growth on the nucleating center is less, so that the size of the particle is lesser. When the reaction temperature increased to 90 ^{0}C., the complex becomes unstable and nearly breaks to generate higher concentration of Cd^{2+} ions in the solution leading to faster growth of CdS nanocrystals to bigger particles. So, the observed particle size of CdS nanoparticles are 80, 90 and 120 nm at temperatures of 50^0, 70^0 and 90 ^{0}C. respectively. The photocatalytic decomposition of aqueous MB solution has been carried out in absence/presence of CdS, acting as catalysts, under dark/UV radiation. These studies show no appreciable degradation of MB either in absence (or presence) of catalysts in absence sunlight even after 4 h. On the contrary, MB dye degraded very fast in presence of catalysts and sunlight. These data have been used to calculate the fraction of MB left undegraded at different interval of time, from which % degradation of MB has been calculated using the relationship: $D = (C_0-C_t)/C_0 \times 100$. The observations have been shown in Figure 3. These observations suggest that ~ 74% degradation of MB in 40 minute take place in presence of CdS. The same catalyst has also been used for studying the reusability and has been found that the reusability of the CdS drastically decreases after 2nd use. This is possibly because of the fact that CdS is vulnerable to photocorrosion. The further exposure to UV radiation up to 180 min showed no further sign of any appreciable degradation.

CONCLUSIONS

In summary, different morphology of CdS nanostructures were successfully prepared by a simple way using wet chemical route using copper chloride, sodium sulphide and water as a solvent using EDA as templating agent. CdS nanostructures are obtained with different temperatures 50-90 ^{0}C with reaction time period 4 h and the result show that uniform and well self assemble structures could be obtained. These particles were characterized structurally and optically. XRD results indicated the presence of pure crystalline phase CdS. SEM analyse the morphology of the sample prepared. Further the photocatalytic study indicated 74% of dye degradation using the sample synthesized at 90 ^{0}C under sunlight and within a time period of 4 hours. These observations paves new pathway for trial with other photocatalytic compounds under the similar experimental conditions exploration their effectivity as catalyst.

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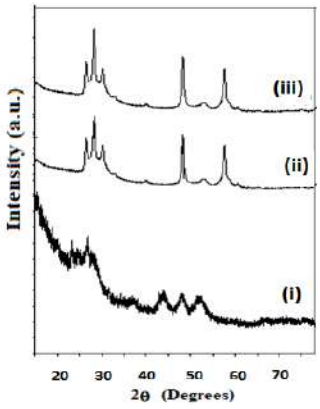

Figure 1: XRD patterns of the sample synthesized at temperatures (i) 50^{0}C, (iii) 70^{0}C and (iii) 90^{0}C.

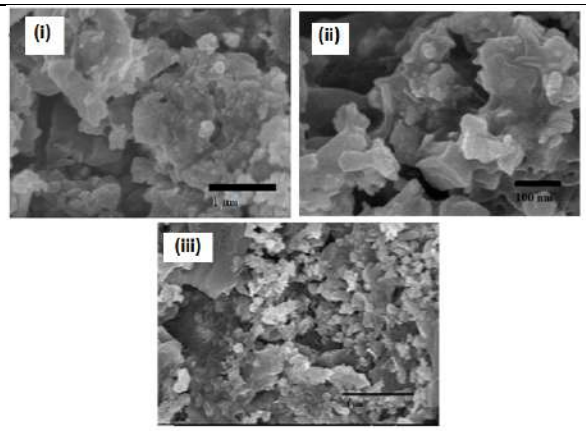

Figure 2: SEM images of samples synthesized at temperatures (a) 50 ^{0}C, (b) 70 ^{0}C and (c) 90 ^{0}C.

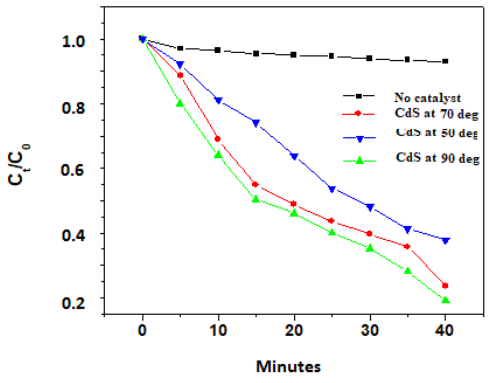

Figure 3: Percentage of photo degradation of MB vs solar irradiation time

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RESEARCH ARTICLE

Impact of Fly Ash on the Growth Parameters of *Vigna radiata.* L

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ABSTRACT

The present study has been carried for the potential use of industrial waste, fly-ash. Which has a great ability in agriculture due to its efficacy in modification of soil health and crop performance. The high concentration of elements (K, Na, Zn, Ca, Mg and Fe) in fly-ash increases the yield of many agricultural crops. But compared to other sectors, the use of fly-ash in agriculture is limited. In 2003 Central Fuel Research Institute has developed FASAT. FASAT technique is cost effective & eco friendly in disposing the waste. Through this technology, Fly ash has a vast potential for use in agriculture & is possible to use FA in bulk quantities. In this work FASAT technique was used by taking pot culture with different proportion of FA & soil in the ratio of 1:1, 2:1, 3:1 & 4:1. The experiment was carried out at the research field of CUTM, BBSR. Experimental pots were prepared as control1 i.e C1 (no FA), control 2 i.e C2 (no Soil) and FA: Soil in the ratio of T1=1:1, T2=2:1, T3=3:1 and T4=4:1. Effect of FA on root and shoot length were observed maximum in T1 (FA: Soil=1:1) as recorded on 7, 14 and 21 days of experiment. The highest root length (4.7cm) was observed in T1 followed by C1(4.2cm),C2(1.8cm), T2(3.8cm), T3(3.3cm) & T4(2.5cm) at 21 DAS. The chlorophyll is one of the important biochemical content which is used as a capability of the plant growth. Chlorophyll content in green gram leaves at 21 DAS showed highest in T2 (0.468 mg g–1) as compared with others. The highest leaf area (6.47cm2) was recorded in T1 (FA: Soil=1:1) and lowest leaf area (2.72cm2) was recorded in C 2(no Soil) at 21 DAS.

Keywords: FASAT, Fly Ash, DAS, Control, *Vigna radiata*

INTRODUCTION

Mostly Coal is used in thermal power stations for power generation and fly ash is the waste product of the above said process. The fly ash possess macronutrient and micro such as Mn, Mo and Hg. These nutrients may bio

accumulates and affects the living being and the environment. However, these nutrients can be used in a limited way for the growth and yield of crop. Fly ash can be used in its limited potential in both monocotyledons and dicotyledons plant [1,2]. It has been already proved by workers that fly ash can be used in the agriculture. Further its alkaline character and presence of macro and micro nutrients in it can be fully exploited in agriculture. In 2003 Central Fuel Research Institute has developed FASAT using the above said characters. FASAT technique uses the fly ash in a sustainable manner in agriculture. Kishore et al, have documented that fly ash improves the soil fertility and yield of crop plants [2,3,4]. All the facts and figures gave the impetus to design the experiment to study the effect of different concertation's of fly ash on the growth and yield of a leguminous crop, *Vigina radiate*[7,8]

MATERIALS AND METHODS

Seed of green gram (*Vigna radiata*). Black poly bags, Soil and Fly ash. The expt. was carried out at the research field of CUTM, BBSR. Experimental pots were prepared as control i.e C1 (no FA),control i.e, C2(no soil) and FA:soil in the combination of T1=1:1, T2=2:1, T3=3:1, T4=4:1 . After pot preparation, seeds were evenly sown in the prepare treatment set, kept under natural condition & watered daily. After 3-4 days of sowing there are about 5-8 plants per pot. Then at a regular interval the periodic growth was recorded.[5,10,11]

Growth parameters

Growth parameter i.e root length, shoot length & leaf area was measured at a regular interval of 7, 14 & 21 days.

RESULTS AND DISCUSSIONS

Germination percentage of green gram decrease gradually with increase of fly ash concentration in soil. The maximum (90%) germination percentage was recorded in T3(FA:Soil=1:1) and the minimum percentage of germination was recorded in T2 (100% fly ash) in a 7 days(DAS) study.

Effect of FA on root and shoot length were observed maximum in T1 (FA:Soil=1:1) as recorded on 7, 14 and 21 days of experiment(Figure 2 & Figure3). The highest root length (4.7cm) was observed in T_1 followed by T_2(4.2cm),T3(4cm),and T_4(3.8cm), at 21 DAS. The maximum shoot length (20.9cm) was observed in T_1 (FA:Soil = 1:1) and minimum shoot length (10.1cm) was observed in C_2 (Fly ash) at 21 DAS. Effect of fly ash amendment was studied with respect to area of leaves is represented in table 6. From the result it is revealed that the highest leaf area (6.47cm^2) was recorded in T_1(FA:Soil=1:1) and lowest leaf area (2.72cm^2)was recorded in C_2 (Fly ash) at 21 DAS.

CONCLUSION

In the present investigation it can be concluded that the growth parameters such as root length, shoot length and area of leaf increases significantly in T1 (FA:Soil=1:1) than other treatments. It also clearly proves that fly ash can be sustainable used in the agriculture in its limited potential. The poor growth of plant in C2 (Fly Ash) further enhances the facts that fly ash alone cannot improve the growth and yield of crop plants.

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Plate - 1 Seeds of *Vigina* sown

Plate-2 Germination of Seeds

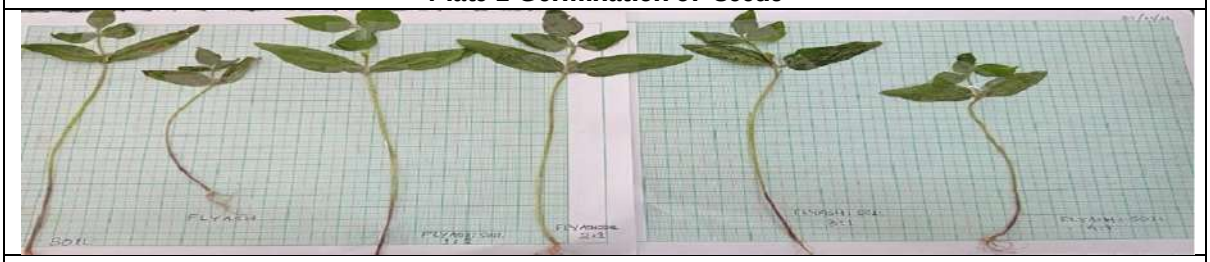

Plate-3 Measurement of Shoot Length

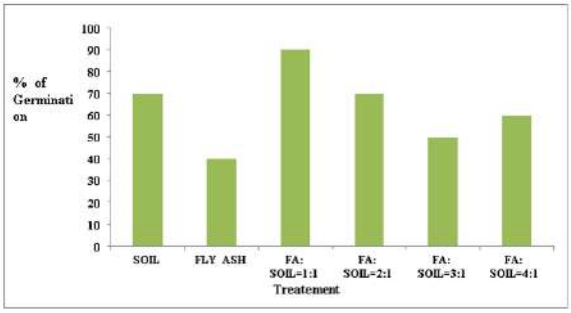

Figure-1 Showing % of germination

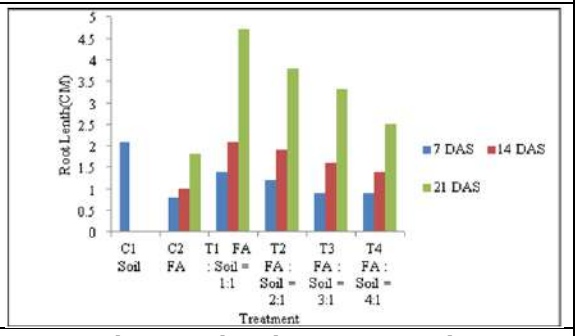

Figure-2 Showing Root Length

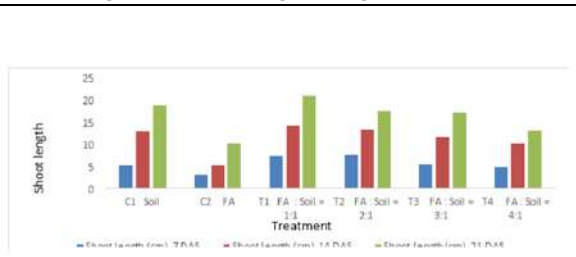

Figure -3 Showing shoot Length

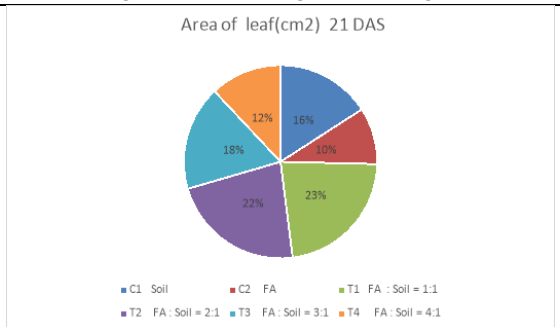

Figure-4 Showing Area of leaf after 21 days

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REVIEW ARTICLE

Is Post-COVID-19 Cognitive Impairment a Reality? A Review

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ABSTRACT

The global pandemic COVID 19 has facilitated various kinds of research and studies all over the world. Cognitive impairment in the aftermath of COVID 19 is a field of research worth pursuing. This paper is a review of studies made in this direction. Collection of data was undertaken in the months of May-June 2021. Out of 2903 studies identified on neurological manifestations of Covid. 19, 2811 studies were excluded, as not relevant. The 92 selected studies were then carefully assessed for eligibility for post-Covid cognitive involvement. This scrutiny filtered the search down to 40 full text articles related to the topic. Some studies focus on the factors responsible for cognitive dysfunction such as encephalopathy, dysexecutive syndrome, decrease in Nitric Oxide concentration and hypoxemia. Studies on different age groups are also classified. A considerable percentage of young adults showed cognitive deficits post COVID. Among the elderly, memory impairment and acceleration of neurodegenerative disorders such as Alzheimer's disease are probable after the infection, even in those who have no history of cognitive decline. Those with pre-existing cognitive illness like Alzheimer's, exhibited a decline in cognitive function, specifically with regard to memory, behaviour and language functions. Cognitive impairment in children is also a possibility, since the unfavourable experiences during the pandemic may be classified as Adverse Childhood Experiences (ACEs) which make a negative impact on the development of the child's brain. Observations from the literature search suggest that the long-term consequences of

the effect of coronavirus infection on the brain and the cognitive deficits caused by it still remain to be studied in detail.

Keywords: Covid 19, cognitive impairment, encephalopathy, dysexecutive syndrome, hypoxemia, neurodegenerative disorders, adverse childhood experiences.

INTRODUCTION

COVID – 19 is an ongoing global pandemic resulting from the SARS-CoV-2) infection. Although most of those affected by COVID 19 recover from it, the after-effects continue to manifest in some people for months after recovery. A lot of research has been undertaken to investigate the long-term impact of the disease on the quality of life of the survivors. Though respiratory symptoms are predominant for patients with COVID-19, it has been noted that they also suffer from neurologic and neuropsychiatric complications. Histopathologic examinations of brains from deceased COVID-19 patients show that SARS-CoV-2 may infiltrate the central nervous system [1]. Researches have not yet proved how exactly the SARS-CoV-2 infiltrates the central nervous system (CNS).However, there is the possibility of transmission of SARS-CoV-2 from systemic circulation to cerebral circulation [2]. It is also possible that the virus is disseminated through the cribriform plate and olfactory bulb [3]. Direct damage to cortex and adjacent subcortical structures and indirect effects due to systemic impairment and psychological trauma are factors which contribute to cognitive impairment [4]. This paper makes a review of literature concerning the factors responsible for cognitive impairments in subjects recovered from COVID 19, and neurological and neuropsychiatric manifestations in young adults, elderly without signs of prior cognitive decline, elderly with pre-existing cognitive illness, and children.

METHODS

This review was conducted with the intention of gaining an insight into the cognitive involvement among Covid 19 survivors. Collection of data was undertaken in the months of May-June 2021. PubMed database was searched, and 2903 studies were identified as relevant to neurological manifestations of Covid 19. An analysis of the summary of these papers led to the exclusion of 2811 studies. The 92 selected studies were then carefully assessed for eligibility for post-Covid cognitive involvement. This scrutiny filtered the search down to 40 full text articles related to the topic.

DISCUSSION

General Neurological Manifestations of Covid 19

As a result of the pandemic of SARS-CoV-2 infection, various neurological signs and symptoms are found to be manifested [5]. SARS-CoV-2 virus, the main causative agent forCOVID-19, has the potential to assail the brain. It may enter the brain either through blood circulation or through nasal cavities. Viruses possibly enter through the Angiotensin-converting enzyme 2 receptors, found on the endothelial cells of cerebral vessels. In his review of the neurological manifestations of Covid 19, Ravindra K. Garg has pointed out that COVID-19 affects both the central and the peripheral nervous systems [6]. When the central nervous system is affected, the signs and symptoms manifested are stroke, headache, sensory alterations, seizures, dizziness and ataxia encephalitis. When the peripheral nervous system is affected, hypogeusia, hyposmia and skeletal muscle injury are manifested. At times even before typical respiratory symptoms appear, neurological features may take place. However, not much attention has yet been paid to neuro-cognitive syndromes among the consequences of COVID-19 [7].

Factors Influencing Cognitive Impairment Post Covid

Encephalopathy: Quite a number of researchers have found encephalopathy as one of the complications of COVID-19 [8,9,10]. Encephalopathy usually results in cognitive impairments like executive dysfunction [11,12].

Dysexecutive Syndrome: A report from COVID-19 literature refers to a dysexecutive syndrome found in 14 out of 39 patients (36%) [13]. Many papers have noted that the confusion and attention difficulties exhibited by the patients affected by Covid 19 suggest a dysexecutive syndrome [13,14,15,16]. One study concluded that one in three subjects with COVID-19 had dysexecutive syndrome after discharge from hospital [17]. All these suggest that executive dysfunction syndrome may be a consequence of COVID-19 in a significant number of cases. Helms and his team of researchers studied the neurological manifestations in 58 Covid 19 patients of median age 63 years. They found agitation to be the most frequent neurological complication (69%). 39 (67%) patients exhibited diffuse pyramidal signs. Nearly one-third of the subjects expressed a dysexecutive syndrome, through signs of inattention, disorientation, or poorly organized movements [13].

Decrease in Nitric Oxide (NO) Concentration: An experiment conducted on animals revealed that the reduction in the Nitric Oxide levels in the brain induced cognitive and behavioural disorders. Inversely, when agents known to increase NO production were administered to the animals, thereby increasing NO concentrations in their brains, their memory showed improvement [18]. Annweiler et al. suggest that SARS-CoV-2 virus induces decrease in NO levels in the brain causing neurological signs in patients with COVID-19 [19].The Nitric Oxide production is associated with the renin-angiotensin system (RAS), which is targeted by SARS-CoV-2. The interaction between the virus and the angiotensin-converting enzyme 2(ACE2) receptor, through its spike (S) glycoprotein, results in over activation of the RAS [20]. This is found to have an effect on cerebral neurons and other such cell types [21].

Effect of Hypoxaemia: Cognitive disorders have been observed as late sequelae in patients who had acute respiratory failure (ARF) and had been under invasive mechanical ventilation (IMV) alone or along with extracorporeal membrane oxygenation (ECMO) [22]. Hypoxaemia, which occurs when a patient suffers from severe ARF, may cause long-term cognitive impairment [23]. Holzgraefe et al. performed a systematic literature search on this aspect [24]. They included studies on hypoxaemia and the effect of ECMO on cognitive functioning in patients treated for acute respiratory failure. From two studies they concluded that cognitive impairment is caused by arterial oxygen saturation falling below 90%. One study was made at discharge and another was made at 1-year follow-up. The results were conflicting. A knowledge gap is evident from their observations.

Cognitive Impairments in Different Age-Groups

Young Adults: A study in the UK has pointed out many neuropsychiatric manifestations like alterations in mental status and cerebrovascular events in the post COVID-19 scenario [25]. In another similar study, 19.9% of the subjects showed impaired concentration and attention and 18.9% exhibited impaired memory [17]. However, it is not clear whether neurocognitive deficits occur in young patients after an attack of mild COVID-19. Woo, Malsy et al. selected young adults who visited their outpatient clinic for post-COVID-19 care and used a screening approach to detect their neurocognitive post-COVID-19 manifestations. They were 18 subjects 20–105 days (median, 85 days) recovered after mild/moderate disease. A screening test (Modified Telephone Interview for Cognitive Status) was conducted on these subjects.78% revealed mild cognitive deficits. The impairments exhibited were mainly in their attention levels and short-term memory. However, the results could not be correlated with the management, length of hospital stay and viremia. Moreover, the test findings did not have any correlation with fatigue and mood disturbances. From these results they concluded that, whatever clinical course is followed, young adults recovered from COVID-19 commonly expressed sustained sub-clinical cognitive complications [1]. De Lorenzo R. et al took up a study to investigate whether residual dysfunction is found after COVID-19.18-year-old patients were selected and assessed after nearly 23 days from being discharged from hospital. Among the patients included 25.4% had new-onset cognitive impairment [26].

Older Adults

Elderly with No Pre-existing Cognitive Illness: The impact of SARS-CoV-2 on the central nervous system (CNS) having been established, the question arises whether it becomes a cause for decrease in cognitive function,

and the development of Alzheimer's disease (AD), and dementia in later life. An international consortium has been organized by the Alzheimer's Association, comprising of representatives from more than 30 countries. The intention of this consortium is to study the short-term and long-term sequelae of SARS-CoV-2 on cognition, and functioning of the brain. at 6, 9, and 18 months [27]. Increase in the proinflammatory cytokine levels, hypoxia, and acute respiratory distress resulting from COVID-19 may lead to decline in cognitive function in healthy individuals as well as predisposed elderly [28]. According to a study one in five subjects recovered from the pandemics in 2002 and 2012, reported memory impairment [17, 27]. Patients recovered from COVID -19 may suffer from poor occupational and functional abilities which may lead to mental health concerns which in turn may lead to cognitive dysfunction [29]. The long-term effects of Covid 19 are predicted to be neurovascular coupling, expediting brain aging and other neurodegenerative disorders associated with old age [27].

Thus, SARS-CoV-2 which infects endothelial cells expressing ACE2 can cause demyelination, and neuro degeneration. This may also result in a hypoperfusion restricting the energy substrates which are necessary to maintain neuronal networks. This in turn accelerates decrease in cognitive functions in the elderly. The damage in the limbic and cortical regions may lead to amnesia. SARS-CoV-2 infection in the elderly results in excessive secretion of pro-inflammatory cytokines which are responsible for the increase in oxidative stress that leads to cellular membrane damage. It also leads to reduction of the surface expression of excitatory amino acid transporters which are essential for terminating glutamatergic signalling. When the glutamate levels are elevated, an excitotoxic environment will be caused, which precipitates neuronal loss and causes further damage to the surrounding parenchyma. The cytotoxic insult created by viral entry into neurons initiates an apoptotic pathway. This pathway leads to the development of Alzheimer's disease and Parkinson's disease. Pathologies related to neurodegenerative disorders may be precipitated in the elderly months after acute viral infection, due to its slow infiltration all through the central nervous system. Worsening of the motor and cognitive deficits occurs due to the viral aggravation of underlying neuropathology [30]. Among the after-effects of the infection, memory impairment and acceleration of neurodegenerative disorders such as Alzheimer's disease appear to be probable, since the hippocampus is particularly vulnerable to coronavirus infections. Studies on the impact of COVID-19 are necessary for screening and treatment programmes in the future to minimize the long-term cognitive consequences of COVID-19 [4].

Elderly with Pre-existing Cognitive Impairment: One of the indirect effects of the COVID 19 pandemic is that long-term care centres have suffered a setback, resulting in high rates of infection and mortality. Due to the threat of spreading infection, patients with dementia have been denied face-to-face appointments. Clinical trials in Alzheimer's disease have been particularly affected by the pandemic. As a result, the elderly with pre-existing cognitive impairment have faced a worsening of their condition in several countries [31]. A study on 40 subjects with neurodegenerative diseases like mild dementia, Alzheimer's and mild cognitive impairment (MCI) was taken up by a cognitive disorders' unit in Spain. The study revealed that after 5 weeks of lockdown there was a significant worsening of the neuropsychiatric symptoms like aberrant motor behaviour, apathy, agitation and anxiety [32]. A Dementia Centre in Rome took up a study on subjects with MCI, dementia, and subjective cognitive decline after one month of lockdown. 54.7% exhibited an aggravation of behavioural disturbances [33]. Similarly, in another sample of patients with Alzheimer's disease, worsening of neuropsychiatric symptoms was observed [34]. A decline in well-being, increased anxiety and lack of sleep were observed in another sample of older adults with mild dementia or MCI [35]. Also, a dementia care centre in Tricase (Italy) has reported that an interview was conducted by telephone on caregivers of 32 elderly with frontotemporal lobar dementia. They found that, compared to their previous visit, 53% of patients exhibited significant decline in memory, as well as deterioration in behaviour and language function during the Covid 19 lockdown [36].

Cognitive Impairment in Children

Adverse Childhood Experiences (ACEs) are said to have a negative influence in the construction and structuring of the child's brain architecture [37]. The social and economic consequences of COVID-19 such as the overwhelming fear of catching the contagion and disease, loss of family members to the disease, social isolation, the lack of support

networks, augmented drunkenness, drug use and mental illness in the families and domestic violence can be classified as ACEs and can result in toxic stress [38]. This toxic stress can cause cognition disorders, impaired mental and physical health, and decline in the working capacity and quality of life of future adults [39]. The pandemic has imposed social isolation on the children, resulting in disturbances in the daily routines of activities and sleep. Lack of physical and outdoor activities and the excessive use of electronic gadgets are blocks to child development and prevent the child from reaching his/her full potential [40]. The lockdown meant to contain the pandemic has pushed children into a sedentary lifestyle devoid of free air and salubrious sunlight which is harmful to the developing brain. Lack of physical exercise, along with intake of foods preserved in cans and rich in sodium have a negative impact on the growth of children and adolescents. From the literature it is also seen that symptoms of anxiety and depression of pregnant women during the pandemic affect children's neurodevelopment and influence adversely their behavioural patterns [41].

CONCLUSION

A review of the COVID-19 literature reveals that many interacting factors cause signs and symptoms of nervous system disorders and sub-clinical cognitive dysfunction as after-effects of COVID-19 infection. COVID-19 causes a reduction in the NO concentration level in the brain, and this in turn induces cognitive and behavioural disorders. Another part of neurological consequences of this viral infection is the executive function disturbance (dysexecutive syndrome) associated with frontal lobe pathology. Abstracting, lack of attention and behaviour control, difficulty in planning, and disorientation are features of dysexecutive syndrome. Encephalopathy too has been frequently associated with cases of COVID-19 infections, and encephalopathy is usually connected with general cognitive disorders, including executive function disturbances. Mechanical ventilation for acute respiratory distress syndrome (ARDS), can lead to long-term cognitive impairments, as hypoxia may lead to cerebral atrophy and ventricular enlargement. In the long run, neurocognitive symptoms may manifest in patients who have undergone artificial ventilation for ARDS. It has been reported that even those who had a mild COVID-19 infection, continue to suffer from persisting symptoms, such as palpitations, chest pain and shortness of breath. They also suffer from headaches, as well as muscle and joint aches. Neuropathy, cognitive impairment, and fatigue are also evident.

COVID-19 has also been a cause of risk to child development because of children's anxiety regarding the onset of illness. The confinement imposed on them as a protective measure, isolation from their peers, and the increased stress level of parents and care givers, also add to this risk, leading to later impairment in their mental and physical health. The impairment of cognition might affect the working capacity and quality of life of future adults. So studies on the impact of COVID-19 on children and methods to prevent harm to children's growth and to promote their development in the positive direction become necessary. Anxiety about complications caused by the infection, keeping oneself aloof from human contact, and feeling of insecurity that one may not be accepted in society, may have a negative impact on mental well-being, which in turn affects the quality of life. Psychological trauma due to loss of income, fear, death of friends and relatives, and the effect of COVID-19 on those with pre-existing cognitive issues like dementia and Alzheimer's disease could also have an effect on cognitive impairment post COVID. Observations from this literature search suggest that post-COVID-19 subjects may recover from respiratory tract symptoms, but the long-term consequences of the effect of coronavirus infection on the brain and the cognitive deficits caused by it, still remain to be studied in detail.

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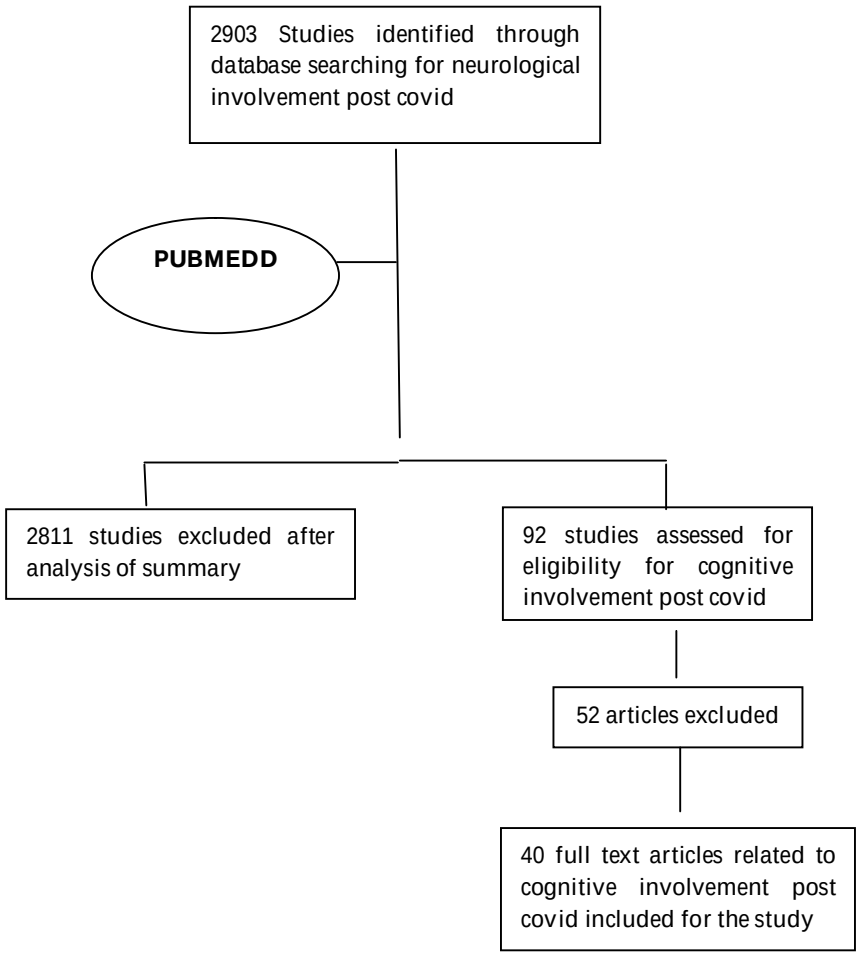

Fig 1: Review flow chart

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RESEARCH ARTICLE

RNA Interference: An Efficient Gene Editing Tool

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ABSTRACT

RNA interference (RNAi) is one of the several post-transcriptional gene-silencing strategies which suppress the expression of any specific gene. Essentially the double-stranded RNA- mediated RNAi process is extensively used as a knockdown tool to reveal the function of gene(s). In plants, primarily the transgenes producing hairpin RNA facilitate the RNAi process. RNAi technology is aimed to develop plant traits by manipulating the expression of endogenous genes and also to empower the plant to defend against invading pathogens by silencing specific gene(s) of the infecting agent. RNAi as a natural process can be exploited immensely to engineer plants, which are capable of defending themselves from biotic and abiotic stress without compromising the productivity. RNAi can showcase the path leading to integrated pest management technology and ultimately contribute to sustainable agricultural process. RNAi has been widely used to generate disease resistant plants and also to modulate the metabolic pathways which eventually lead to enhanced production of secondary metabolites having multifaceted benefits. RNAi strategy is considered to be advantageous over reverse genetics, co-suppression and antisense-mediated gene silencing, in terms of its stability and efficiency. Revealing the underlying molecular phenomena of RNAi would augment our ability to engineer disease resistant plants.

Keywords: RNA interference, gene-silencing, hairpin RNA, pest management, sustainable agriculture.

INTRODUCTION

In eukaryotes, the highly conserved RNA interference (RNAi) is essentially a double-stranded RNA (dsRNA)-mediated "gene-silencing" process, which displays high precision and efficiency. Majorly, the RNAi is exploited to analyze and reveal function of any specific gene primarily by knocking out of that gene. RNAi technology has already been performed for genome-wide analysis in flies and worms (Fraser et al. 2000; Gonczy et al. 2000; Kiger et al. 2003). Automated RNAi screening technology using microarray and assay plates have been developed for high-throughput analysis of cultured cells (Kiger et al. 2003; Mousses et al. 2003). Recent studies have exposed the multifaceted roles of RNAi which includes epigenomic regulation of chromatin, regulation of gene expression,

suppression of transposon activity and inhibiting viral infection (Hannon 2002; Grewal and Moazed 2003). RNAi holds extensive potential to be practically used in improvising the crop improvement strategies. RNAi as a gene-silencing phenomenon was firstly discovered in worms (Waterhouse et al. 2001; Hannon 2002). Initial studies revealed the role of sense and antisense RNA specific to a gene resulting in repression of gene expression (Guo et al. 2003). Subsequent reports highlighted that the presence of dsRNA along with the sense or antisense RNA essentially mediates the gene silencing (Abdelgany et al. 2003). This phenomenon was then termed as RNAi (Hannon 2002). Strikingly, RNAi did not display its effect on the introns, hence believed to be a post-transcriptional regulation mechanism. RNAi related phenomena such as co-suppression was believed to be a critical mechanism occurring in plants. During the co-suppression, both the endogenous gene and transgene expressions are silenced. Coat protein mediated protection (CPMP) is also considered to be a type of RNAi-related phenomenon, where a transgene coding for a sense coat protein contributing towards viral resistance. Initially, the viral resistance was believed to be due to the coat protein but eventually it was reported that transgenes derived from untranslatable coat protein could also act against the invading plant virus. CPMP largely shares similar mechanisms as post-transcriptional gene silencing (PTGS) process. Analogous molecule was recognized in an *in vitro* RNAi system of *Drosophila melanogaster* and was referred to as small interfering RNA (siRNA; Hannon 2002). These similarities stoutly suggested that both the RNAi and PTGS shared the same repression mechanism and raised the prospect that dsRNA is essentially the strand of the siRNA which eventually hybridizes to the messenger RNA (mRNA) as a guide, and finally the RNA induced silencing complex (RISC) cleaves the mRNA in the vicinity of the center of the siRNA. The reaction being very specific, mismatches between the target mRNA and siRNA significantly reduces the efficiency (Elbashir et al. 2001; Abdelgany et al. 2003; Tang et al. 2003). Essentially here we emphasize RNAi technology and its application as an effective strategy to engineer stress resistant plants.

RNA interference in plants

RNA interference and RNA silencing

Essentially, the RNA silencing mechanism is a homology-based event that is initiated by dsRNA, which consequently represses the gene expression (Denli et al. 2003). Initially RNAi was believed to be a viral defense mechanism and subsequently it was of the view that it is a well conserved silencing mechanism present across simpler to higher form of eukaryotes (Hannon 2002). It was reported that both of the sense and antisense RNA have the ability to suppress the expression of genes and eventually was fixed that the dsRNA contributes for gene silencing (Fire 1998). In fact, dsRNA is considered to be a competent elicitor of RNA silencing as compared to the sense or antisense RNA. Earlier reports in plants highlight that the RNA silencing functions at three different levels. Firstly, at the cytoplasm level, which involves the dsRNA-mediated silencing resulting in cleavage of specific mRNA, considered as PTGS (Hamilton and Baulcombe 1999). Secondly, micro-RNAs (miRNAs)-mediated silencing of native mRNA, eventually leading to either mRNA cleavage or restriction of translation. Thirdly, sequence-specific methylation of DNA leads to the repression of transcription process, referred to as the transcriptional gene silencing (TGS). The basic RNA silencing mechanism relies on the dsRNA which acts as the trigger and is recognized by a specific processing enzyme, DICER that is capable of cleaving mRNA into short fragments ranging from 20-25 nucleotides (Kusaba 2004; Figure 1). These short RNA fragments are eventually incorporated into a specific complex known as the 'RNA-induced silencing complex' (RISC). Consequently, RISC is targeted for degradation through a sequence-specific manner. RNAi systems in the Drosophila have exposed the comprehensive molecular mechanism of RNA silencing (Hannon 2002; Schwarz et al. 2003). Initially DICER, a member of the RNase III family recognizes a long dsRNA and digests it into 20~25 nucleotide siRNA duplexes. Subsequently, each of the duplex gets unwrapped and largely one of the two strands is integrated to the RISC. Then the antisense strand of the siRNA associates with the mRNA as a guide sequence. Finally the RISC cleaves the mRNA. Any mismatch between the siRNA and the intended mRNA significantly condense the effectiveness of the mRNA cleavage process, predominantly when these are positioned in the vicinity of the center of the siRNA (Elbashir et al. 2001; Abdelgany et al. 2003). RNAi technology is an area of severe exploration that is crucial for new discoveries, specifically in the fields of plant defense, development and most importantly regulation of gene expression.

RNAi-mediated analysis of gene function

RNAi being a knockdown technology is considered to be highly efficient and its easy applicability makes it a significant tool for analysis of gene function across the genome in plants. Essentially RNAi in case of plants is achieved by a transgene encoding for a hairpin RNA. Typically, antisense-mediated gene silencing was widely used prior to the RNAi technology, specifically for analyzing gene function in plants. Hairpin RNA mediated RNAi is believed to be more efficient and effective strategy as compared to that of antisense-mediated gene silencing (Chuang et al. 2000; Waterhouse and Helliwell 2003). The target gene is primarily cloned as an inverted repeat and spaced with any unrelated sequence and is tagged into a strong expressing promoter sequence, such as maize ubiquitin 1 and 35S CaMV promoter (Serio et al. 2001). The efficiency of this RNAi system can be enhanced by inserting an intron as the spacer since an introns sequence is quite essential for stability of the inverted repeat and finally the targeted gene can be silenced completely (Smith et al. 2000; Wesley et al. 2001). Conversely, the underlying molecular mechanism by which the intron enhances the silencing is not fully revealed (Helliwell and Waterhouse 2003). RNAi strategy can be implemented against different targets including 5' and 3' untranslated region (UTR). Derived from an inverted repeat of a heterologous 3' UTR, a high-throughput RNAi vector is generated for genome wide functional analysis (Brummell et al. 2003). In plants, RNAi is essentially induced by particle bombardment of a transgene producing hpRNA or through direct introduction of dsRNA (Schweizer et al. 2000; Klahre et al. 2002; Guo et al. 2003). This technology is handy for the study of gene functions in plants particularly where transgenic approaches requiring stable transformation are challenging. Virus-induced gene silencing (VIGS) is another such approach which is still used to analyze function of specific gene/s in plants (Waterhouse and Helliwell 2003). In the VIGS, RNA viruses usually generate dsRNA all through their life cycle primarily by the virus-encoded RNA-dependent RNA polymerase (RdRP). Similarly, amplicon is a known technology associated to VIGS (Waterhouse and Helliwell 2003).

Hairpin structures mediate targeted RNAi in plants

Numerous strategies have been used to generate dsRNA. At the outset, dsRNA was generated by transforming individual plants infiltrated with constructs carrying sense and antisense RNA respectively. Consequently, the plants are crossed to produce plants harbouring ds RNA against any specific mRNA. The affectivity and efficiency of this system is relatively higher than either sense or antisense approaches (Waterhouse et al. 1998). An advanced strategy makes use of a construct having both the sense and antisense sequences, intervened by an introns sequence, altogether controlled by a common ubiquitous promoter (Figure 2; Mansoor et al. 2006). When the construct is transcribed, the inbuilt sequences result in the synthesis of a hairpin RNA that participates in the gene silencing process (Smith et al. 2000). The hairpin strategy has been reported to be an efficient as it is capable of silencing any specific gene by 95-100% and has been used more frequently in plants for manipulating the plant traits (Wesley et al. 2001; Miki and Shimamoto 2004; Helliwell et al. 2005). For transient gene silencing, phytopathogenic viruses have been extensively used, generally referred to as the VIGS. Nevertheless, studies have revealed that VIGS-induced promoter sequence silencing is inherited to the next generation; specifically the methylated sequences are heritable in nature (Jones et al. 2001). The major limitation of VIGS is the range of host plants to which these phytopathogenic viruses are effective. Also, bacterial-mediated siRNAs expression elicits gene silencing and strikingly these bacterial extracts can be sprayed externally on the plants (Tenllado et al. 2003). For instance, by spraying virus-specific siRNA through the crude bacterial extract confers resistance to Plum Pox Virus (PPV) and Pepper Mild Mottle Virus (PMMoV). This strategy has gained advantages over others, since here RNAi induction is possible even without producing transgenic plants.

Genetic improvement of crop plants

RNAi-mediated metabolic engineering of plants using

Plants are imperatively the natural resource which provides abundant products, such as wood, fibre, food, dyes, oils and pharmaceuticals. A lot of precious secondary metabolites are synthesized in only trace amounts and, thus, are tricky to acquire in adequate quantities. RNAi-mediated metabolic engineering provides a promising way of overcoming the limitations of isolating small amount of secondary metabolites. For instance low glutelin content 1

(Lgc-1) has been reportedly silenced in rice plants. Glutelin being one of the important seed storage proteins possess adverse effects for patients with kidney disorders (Kusaba et al. 2003). The dsRNA-induced Lgc-1 silencing is highly advantageous. The plant trait generated by this method was confirmed by the presence of siRNAs specific against the targeted gene (Kusaba et al. 2003). Similarly, this approach was also employed to silence two vital enzymes (ghFAD2-1 and ghSAD-1) involved in the biosynthesis of fatty acid in cotton, as a outcome of which there was an increase in the oleic acid and decrease in stearic acid content respectively (Liu et al. 2002). Similarly, hay fever, commonly known as seasonal allergic asthma affects a majority of population across the globe. The pollen proteins, Lolp1 and Lolp2 are the major contributors as allergens. Using RNAi technology, the expression of these pollen proteins can be down-regulated, primarily by expressing complementary DNA sequences (Petrovska et al. 2005). Another instance of the application of RNAi technology is over-expression of the CaMXMT1 gene which significantly upregulates caffeine level (Ogita et al. 2004). This approach also showed the involvement of theobromine during the synthesis of coffee. Predominantly, this species results in an extremely bright-yellow covering of flowers that usually absorbs around 50% of photosynthetically active radiation, the consequence being a significant decrease in the yield. In order to counter this situation, a hairpin construct-mediated genetic modification results in plant with reduced petals (Byzova et al. 2004). One of the breakthrough finding in the *Papaver somniferum* is the silencing of enzymes associated with codeine reductase (COR) (Allen et al. 2004). A hairpin RNA construct facilitated this process of generating transgenic plants which predominantly accumulated non-narcotic alkaloid (S)-reticuline at the expense of codeine, thebaine, morphine and opium. This was one of the contributions of RNAi technology which modulated multiple steps of a complex metabolic process in any plant. Similarly, in tomato plants, RNAi-mediated repression of DET1, a photomorphogenesis regulatory gene raised the quality of the fruit produce (Davuluri et al. 2005). The transgenic potato plants favoured the degradation of the DET1and resulted in accumulation of higher content of carotenoids and flavonoids. This way the quality of the yield was upheld without compromising the quantity and was devoid of any detrimental effects (Davuluri et al. 2005). Likewise, RNAi-mediated manipulation of *Arabidopsis* ACR2 gene responsible for synthesizing arsenic reductases facilitates bioremediation of heavy metals like Arsenic (Dhankher et al. 2006).

RNAi-mediated disease resistance

Predominantly, the major application of RNAi technology was in the field of plant immunity, by generating disease resistant varieties. This approach was fruitful particularly for virus-induced plant diseases, though the detailed mechanism is not yet revealed. The strategy of pathogen-derived resistance (PDR) is primarily achieved by genes derived from the pathogen, resulting in the inhibition of pathogenic life cycle (Voinnet 2001; Goldbach et al. 2003; Jung et al. 2020). Interstingly, it was established that once the silencing process is triggered then it confers a systemic resistance whereby, the entire plant receives this gene silencing signal (Voinnet et al. 1998). Plant viruses are also equipped with suppressor proteins to counter the plant defense mechanism. Strategies employed by plant viruses may vary depending on the host type. In order to offset simultaneously the multiple strategies of plant viruses, RNAi technology can be found to be most effective. For instance, a hairpin construct, harbouring intergenic region of the Mung Bean Yellow Mosaic Virus (MBYMV) significantly reduces the infection rate of phytopathogenic DNA viruses in Vigna mungo plants. For conferring pathogen-mediated resistance, the coding sequences of the viral proteins including rolling-circle initiator protein (Rep), a vital protein for viral replication have been used (Bendahmane and Gronenborn 1997; Sangare´ 1999; Pooggin et al. 2003; Vanitharani 2003). Protoplast transfusions with the RNAi strategy result in a decline of about 90% of the Rep transcript and 65% of the viral DNA (Vanitharani 2003). The same strategy to target the viral Rep sequence proved to be ineffective against Tomato yellow leaf curl Sardinia virus (Noris et al. 2004), suggesting that the RNAi-mediated may not be effective against all geminiviruses (Andika et al. 2005). Recently, it was reported that the geminiviruses can overcome RNA silencing by following specific evasion strategies, but the underlying mechanisms are still to be revealed (Bisaro 2006). In similar fashion RNAi technology proves to be an effective alternative against phytopathogenic bacteria (Depicker et al. 1978; Lichtenstein et al. 1984). Crown gall disease primarily caused by oncogenes is silenced by using RNAi process, thus conferring resistance from *Agrobacterium tumefaciens* (Ooms et al. 1981; Dunoyer et al. 2006). Likewise, for effectively counteracting on

nematode genes, RNAi technique holds to be successful and contributes towards plant immunity (Bakhetia et al. 2005).

RNAi and male sterility

Modulating crop fertility is one of the significant approaches, where the plant developmental process is primarily focused (Goldberg et al. 1993). Studies on male gametogenesis in plants are an area of extreme exploration (Gorman and McCormick 1997). For the production of F1 hybrid seeds, pollen-manipulation is critical. There are several genes identified across numerous plant species displaying anther-specific expression. In order to interfere with the male fertility process, manoeuvring of genes using RNAi technology is proven to be beneficial (Kapoor et al. 2002). RNAi strategy effectively contributes to male sterility. For instance, silencing TAZ1: an anther-specific protein results in extensive abortion of microspores initiated quickly after their discharge from pollen tetrads, ultimately resulting in declined germination (Kapoor et al. 2002). Strikingly, the function of genes like OsGEN-L has been identified by RNAi technique. It was observed that the plants with OsGEN-LRNAi displays male sterility and low fertility traits (Moritoh et al. 2005). Likewise, RNAi-mediated spatial expression of a maize gene MS45 resulted in a male-sterile phenotype. A high incidence of male-sterile plants was obtained by continuous expression of a transgene having inverted repeat fragments of the MS45 promoter. MS45 gene is expressed under a heterologous promoter for restoring the male fertility (Mariani et al. 1990; Cigan et al. 2005). A substitute strategy can be employed to design an inducible promoter in order to coerce the expression of the silencing construct (Greenland et al. 1998).

Limitations of RNAi technology

The fundamental process deployed in the RNAi technology relies on the similarity of the siRNA with the target gene. A possible limitation of this RNAi strategy is the off-target consequences, eventually leading to the repression of non-target sequences (Malik et al. 2006). The occurrence of off-target effects in plants is strongly limited as evident from transcriptome profiling. No off-target effects were found during the process of silencing the salicylic acid-binding protein 2 (SABP2) (Bartel 2004; Kumar et al. 2006). Few reports demonstrate translational repression of non-target genes in case of animals owing to partial similarity of siRNA to that of the 3' un-translated regions (Lin et al. 2005; Birmingham et al. 2006). In order to minimize the off-target effects, chemical modifications at 2-O-methyl ribosyl in the guide strand is done and has been reported to reduce and negative consequences of RNAi-mediated gene silencing (Jackson et al. 2006; Elmen et al. 2005). Conversely, in plants the possibility of off-target effects shouldn't be ruled out and for that reason desires vigilant attention. Necessary concern in interpreting the resulting genotype and phenotype because of RNAi technology is warranted. Validation of the RNAi data using the existing theoretical and practical implements to envisage and distinguish the imminent off-target effects of siRNAs in plants is intriguing.

CONCLUSION AND FUTURE PERSPECTIVES

RNAi technology being an efficient knockdown technology is considered to be one of the suitable alternatives to generate genetically improved crop plants, mostly for those plants having low transformation rates (Liu et al. 2002; Ogita et al. 2003). Since a decade, RNAi technology has brought a revolutionary approach among plant biologists owing to the ease of manipulating genes and their functions, resulting in plants with novel traits. RNAi-engineered plants display stability in their traits throughout a number of generations (Kusaba et al. 2003). Most importantly, in order to minimize the off-target effects, ability to improve tissue-specific promoters to repress gene expression could be prioritized. Also, it would be intriguing to develop edible crops from non-transgenic tissues primarily to avoid general unacceptability towards transgenic crops. Strikingly, the advantageous aspect of the RNAi technology relies on the targeting efficiency and the ability to suppress the expression of multiple genes simultaneously. This would significantly reduce the time required to silence individual genes and can modulate multiple steps of biochemical pathways, thus enabling the plant to respond differently to varied stimulus. The underlying molecular mechanism of RNAi technology still needs to be fully revealed, essentially in terms of the relationship between RNAi and the plant development process. Revealing the concealed potential of RNAi technology would contribute towards crop improvement and productivity.

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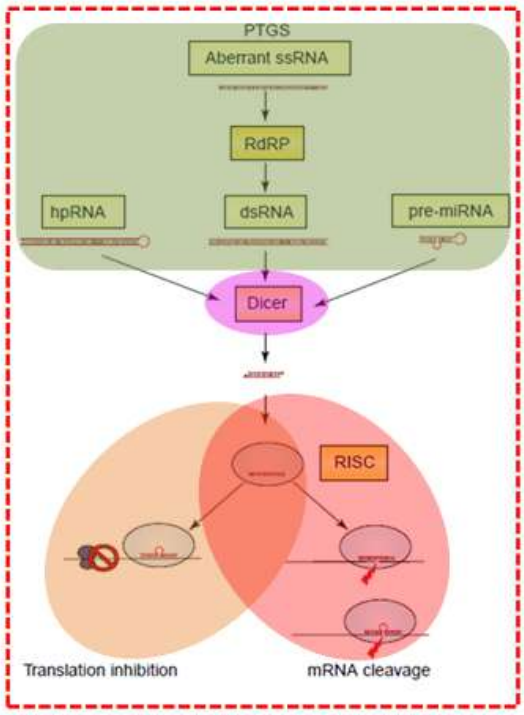

Figure 1: The basic "molecular mechanism of RNAi" in plants.

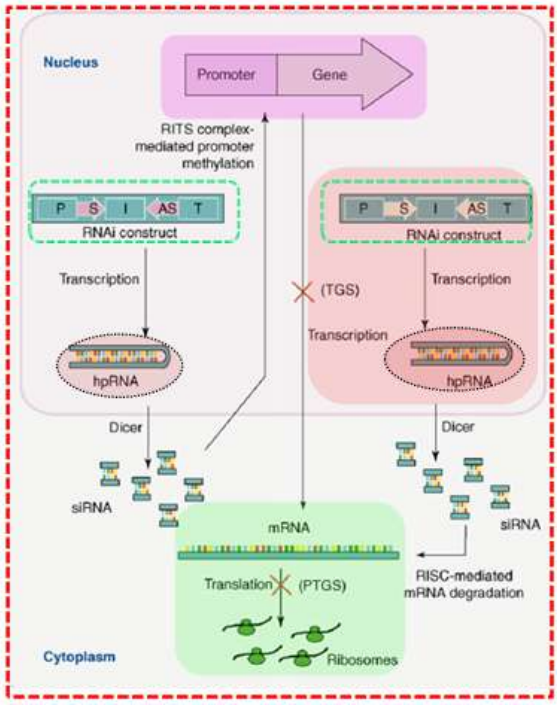

Figure 2: Hairpin structure-mediated induction of transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS).

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RESEARCH ARTICLE

Antimicrobial Activity of Leaves of *Alstonia scholaris* (Linn.) R.Br.

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ABSTRACT

The purpose of this study was to observe the consequences of both antibacterial and antifungal action of methanol and chloroform extracts of *Alstonia scholaris* Linn. R.Br against the following microbes (*Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*) by Agar well diffusion method. Ampicillin and Fluconazole are taken as standard. The trial for susceptibility testing of the above human pathogen was carried out by Agar well diffusion method. A well(100µl) was punctured with a cork borer(6mm) in the petri plate contain solid agar media(MHA/SDA) and added different concentration of extract [60,70,80,90,100mg/ml(100µl/well)] into individual well. Incubated the bacterial plates at 37^{0}C for 24 hours and fungal plates at 28^{0}C for 72 hours in upright position. The positive gram bacteria i.e. *Staphylococcus aureus* (MTCC902) showed maximum and highest inhibition zones (23.66mm and 21.33mm) by taking both chloroform and methanol extracts at a conc. of 100mg/ml as comparable to other bacterial and fungal strain, while *E. coli* (MTCC723) did not displayed any remarkable results (>7mm) against chloroform extracts. The fungal strain i.e. *C.albicans* (MTCC4748) and *A. niger* (MTCC478) is showed moderately higher and lower zone of inhibition towards both the extract of *A. scholaris*. We found 100mg/ml of given conc. of plant extracts manifested potential result against all the targeted pathogens except *Escherichia coli*. In near future it will be beneficial for pharmaceutical companies for potent drug designing against the recommended human pathogens.

Keywords: *Alstonia scholaris*, Leaves, Antimicrobial activity, Agar well diffusion, Methanol, Chloroform, Zone of inhibition

INTRODUCTION

Various international and national pharmaceutical companies used formulated plant chemicals for the treatment of different diseases in worldwide [1] [2] [3] [4]. Among the Indian plant species about 105 species were tested to know about antimicrobial activity. Among them antifungal property showed by 20 species and antibacterial activity

showed by 30 species. Now the current investigation is to gain knowledge about the antimicrobial activity of *Alstonia scholaris*. Under the family Apocyanaceae the genus *A. scholaris* comes. Frequently people in English called it devils tree or blackboard tree. *A. scholaris* is an evergreen tropical and sub-tropical tree native to Australasia, Southern China and tropical Asia. As we know *A. scholaris* is a common medicinal plant because almost all of its parts were used in medicine preparation. Plant parts of *A. scholaris*, especially bark was used to treat chronicdiarrhea, dysentery and bowel movements and also it is an antiperiodic agent and also cure anthelminthic and astringent like disorder [5]. Leaves of *A. scholaris* were used as medicine for treatment of many diseases i.e. dropsy, ulcers beriberi, congestion of liver etc.,. Also the plant secrets latex has been applicable in treatment of rheumatoid pain, tumors, sores and ulcers [6]. Methanolic extracts of flowers and roots have been show a potentially active antimicrobial activity [7][8]. So here the present investigation is carried out to study the antimicrobial property of Methanolic and chloroform activity of leave extract of *A.scholaris.*

MATERIALS AND METHODS

Collection and Processing of Plant Materials

The insect free healthy leaves of *A. scholaris* were collected in the month of July 2019 from the locality of Jatani, Khordha (Odisha). The collected specimen was authenticated by Professor Kalpita Bhattta (Department of Botany), School of Applied Science, Centurion University of Technology and Management, Jatani, Khordha (Odisha). The collected specimen washed under running tap to remove all the dusts from the leaf surface and deposited in the Department of Botany. The leaves were dried in room temperature under fan by shed drying for 2-3 weeks in the laboratory.

Extraction of Plant Materials

The dried leaves transformed into fine powder via electric blender. Single polar and non-polar solvents were taken here for individual extract preparation of the crushed sample. Around 32 gm of pulverized sample extracted with adding 250 ml of the following solvents; methanol and chloroform separately by using soxhlet apparatus [9]. In the end of the process the obtained extract of each solvent was percolated through Whatman filter paper No. 1 and the remaining solvents were vaporized at 40^{0}C via heating mantle and the prepared extract stored refrigerator for later usage.

Test Microbes

Two human pathogenic bacteria specifically Gram-positive strain i.e. *Staphylococcus aureus* (MTCC902) and Gram-negative strain i.e. *Escherichia coli* (MTCC723), likewise two pathogenic fungi such as *Candida albicans* (MTCC4748) and *Aspergillus niger* (MTCC478) were tested individually for their susceptibility towards antimicrobial assay. The test microbes were obtained from Institute of Microbial Technology, Chandigarh, India. Both bacterial and fungal strains were kept in nutrient agar and potato dextrose agar respectively [Hi-media Laboratory Pvt., Mumbai, India] and were repeatedly sub cultured in order to obtain the purity [10]. A sterile loop filled with test microbe was inoculated on nutrient broth and incubated at 24hours at 37^{0}C for bacteria and 72 hours at 28^{0}Cfor fungi to continued sterility.

Screening of Antibacterial Activities

Crude extracts were experimented by agar well diffusion method to know their antibacterial activity against Gram-positive and Gram-negative bacteria [11] [12]. Mueller Hinton Agar (MHA) was a selected test medium developed by a standard procedure [13]. Sterile and fresh culture plates were taken and poured Mueller Hinton Agar (MHA) equally in each petri plates. Sterile cotton swab was used as inoculum to spread the bacteria uniformly on agar plate. A sterile borer (6mm) was used to bore wells in every plate for crude extracts. Labeled all plates properly and 100µl (50, 60, 70, 80, 90,100mg/ml) of each extract was put in the well [14] [15]. All petri plates were incubated for a period of 24 hours at 37^{0}C. The evidence of bacterial inhibition zones surrounding the well were found, accounted in mm.

Screening of Antifungal Activity

The screening of antifungal activity was carried out by incorporating crude extracts of different solvents in SDA medium poured before the sterile plates. The SDA medium autoclaved at 15 lb pressure, 121^{0}C for 15mins; then kept few seconds for cooling and poured the media into all the petri plates equally and left for 30mins to solidify. The target pathogen swabbed uniformly throughout the surface of SDA and added 100µl(50,60,70,80,90,100mg/ml) of plant extracts by micropipette aseptically, into the well which was punctured in the middle of the petri plates by a sterile cork borer(6mm) [16]. Then the plates were incubated for 72 hours at 28^{0}C and the growing condition accounted.

Statistical Analysis

Antimicrobial activity studies were carried out by triplicate method. Statistical data were demonstrated as mean ± standard deviation [16].

RESULTS AND DISCUSSISONS

By taking two different solvents i.e. methanol and chloroform, leave extracts of *Alstonia scholaris* Linn. R.Br. prepared and investigated against the human pathogenic bacteria and fungi by Agar well diffusion method to find the antimicrobial activities of plant. The following table and figure are displayed the result of antibacterial and antifungal activity of methanol and chloroform extracts of *Alstonia scholaris* against *E. coli*, *S. aureus*, *C. albicans* & *A. niger*. Among the human pathogen *Staphylococcus aureus* (Gram positive) displayed maximum and larger inhibitions zones such as 23.66±2.51mm & 21.33±0.57mm against chloroform extract (100mg/ml) and methanol extract (100mg/ml) respectively in compared with other tested bacterial and fungal strain. Whereas *Escherichia coli* (Gram negative) showed no result (Inhibition zone >7mm was not taken in consideration) and complete resistance to chloroform extract of *A. scholaris* and at the same time *E. coli* showed promising zone of inhibition (21±4.58mm) against methanol crude extract. However Gram-positive bacteria were more susceptible towards both polar and non-polar solvent extracts than Gram-negative bacterial strain.

In case of fungal strains, *Aspergillus niger* the pathogenic fungus showed moderately higher zone of inhibition 18.66±1.15mm against chloroform extracts and 14.66±1.52mm against methanol crude extracts. In compare to *A. niger*, *Candida albicans* displayed lower zone of inhibitions i.e. 12.33±1.15mm and 8.33±0.57mm against methanol and chloroform extracts of *A. scholaris* respectively. Antimicrobial activities of both the extracts were compared with standards (ampicillin as antibacterial agent & fluconazole as antifungal agent) and controls (methanol and chloroform as –ve controls). Control plates did not show any effective results (Inhibition zone >7mm) so the values are excluded. All the strains showed maximum, moderate, lower inhibition zones against chloroform and methanol crude extracts, which was also a good sign for effectual drug (antibacterial and antifungal) designing and cure against tested human pathogens.

CONCLUSION

The current investigation of solvent extract of *Alstonia scholaris* Linn. R.Br specify that targeted microorganisms more susceptible at the concentration of 100mg/ml(100µl/Well) except E. coli could manifests effective zone of inhibitions which is a most promising result that concluded in this study and which will be very potential for drug designing and cure the disease related to those particular microorganisms in near future.

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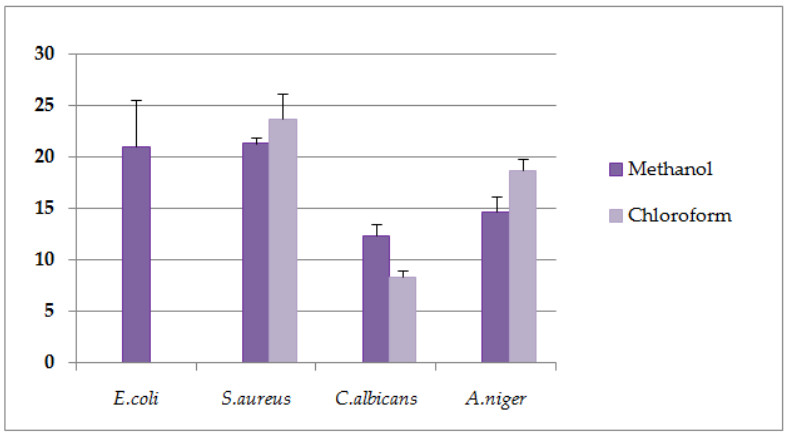
Table1: Showing the value of Zone of Inhibition in different plant extract

Test microbes		**Methanol extract**	**Chloroform extract**	**Standard**
Bacterial strain				**Ampicillin**
Escherichia coli	**Z.I**	21±4.58	NIL	17.16
Staphylococcus aureus	**Z.I**	21.33±0.57	23.66±2.51	23.44
Fungal strain				**Fluconazole**
Candida albicans	**Z.I**	12.33±1.15	8.33±0.57	21.22
Aspergillus niger	**Z.I**	14.66±1.52	18.66±1.15	17.51

[Z.I = Zone of inhibitions in mm] [NIL = Zone of inhibitions less than 7mm] [All values = mean ± standard deviation]



[Figure showed the antimicrobial activity of methanol and chloroform extracts of leaves of *A.scholaris* against different microbes]

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RESEARCH ARTICLE

Top-down Approach towards Exfoliated Graphene Sheets

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ABSTRACT

A synthesis approach towards the defect free graphene, which produce some excellent properties like high intrinsic mobility, good thermal conductivity along with excellent elastic properties has been discussed. It also shows good catalytic properties, owing to its large specific surface area it widens up the area of applications in catalytic and environmental fields.. As the surface morphology , size, shape and properties of the graphene are highly influenced by the synthesis approach, therefore, present article focuses mainly on the different top-down approaches of synthesis of graphene and explains the available existing techniques both fundamental as well as advanced approaches with broad classification (in terms of top down and bottom up point of views). This will impart the reader accurate idea regarding their usefulness in appropriate fields.

INTRODUCTION

Graphene has undoubtedly has lots good properties, due to which lots of research has been focused on it. In order to exfoliatae and separate the layers, the van der Waals interactions needs to be overcome [1-13].Among different reported methods the synthesis are broadly of two types (1) the "top-down" approach and (2) "bottom-up" approach. In "top-down" approach involves tediousness in synthesis as well as low yield. On the other hand the bottom up method is verily suitable for different forms of graphene, with high purity,such as "nano-ribbons" and "graphene dots (so-called nanoflakes)" [14-18]. Many classical ways like chemical bath deposition, mechanical grinding etc has been adopted from last few decades. Regarding this another route called exfoliation, which is a alternative route to this micromechanical route for graphite and as well as its derivatives (for example graphite oxide), is highly in use for its high yield of production and cost effectiveness [19]. The above discussion provide examples of the top-down approaches where the precursor of graphen, for example organic compounds or graphite etc, are stripped down to a few layers of graphites, through different methods of chemical, physical, mechanical and thermal approaches [20-24].

Mechanical approaches

Such methods are of high applicability owing to their capabilities of forming high quality graphene films of size lower than tens micrometers from the precursors. The "Mechanical exfoliation" method is one of the important

techniques for obtaining high quality graphene however, for mass production, this method is not suitable owing to the fact that the thickness of the film thus obtained, is not uniform throughout the material. Also this method is of very high production cost owing to low yield of graphene. The "the scotch tape" or "peel off" method is one of such method as reported by some of the scientific groups where graphite is micromechanically alleviated repeatedly micromechanically to split it up and hence producing thin pieces of graphene. Subsequently they has to be dissolved in acetone and are studied under electron microscopes. The optically transparent flakes are then dissolved in acetone. The "flakes" which contains both the mono as well as multi layers of graphene were studied using electron microscopy so as to obtained the details of the surface morphology. In addition to the electron microscopic techniques, the surface morophology are sometimes being determined by the use of atomic force microscopy. It it important to note here that, the ordinary light microscopes using visible light can not be use for such procedures, as the dimension of the graphene (mainly its thickness) is so small that visible light wavelength is very much large in comparison to that and it can not be visualized by ordinary microscopes of glass and/quartz length. Using the electron microscopy a magnification as high as one lakh times can be obtained. This technique has later been modified by research groups to avoid the stage where flakes are produced with sizes larger than 1 mm [21]. Before that it was supposed that the "free-standing atomic planes" does not exist [15] as it was thought that such structures will be highly unstable if scalled down to nanometer scale [22, 23].

"Ultrasonic oscillation" method is also used for "mechanical exfoliation" in which a wedge of diamond is used to "scrape off" graphene sheets from graphene. This, approach is similar the method of removal of graphene from crude carbon. The important priviledge of this method is due to control of frequency of oscillation and contact pressure, which results in uniform properties of graphene sheets. Jayasena et al. has successfully prepared nanohorns-like structure around the edges using this method. However this method is not suitable for scaling up purpose. By a different research group, different stabilizing liquids such as N-methyl-2-pyrrolidone, sodium cholate, etc has been used where a local sheer rate of around 103 s-1 is used. This method could successfully replace the sonication method and hence could utilize less power consumption along with formation of defect-free and pure graphene. The properties of graphene obtained using this procedure is similar to that achieved by sonication method in different types of solvents as well as surfacting agents, which make it suitable for manufacture of graphene in industrial scale. Such methods yield graphene which is similar to that obtained by sonication of graphite using different types of solvents as well as surfacting agents, which makes it suitable for industrial scale preparation. Different synthetic approaches has been reported using "graphene oxide" as a "precursor" where the the precursor starts getting reduced at high temperatures (as high as up to 1500 degrees) and pressure. Such a treatment also addressed as "solvothermal" or "hydrothermal" and helps to form graphene of high crystalline order, as high temperature and pressure reduces the graphene oxide in to the formation of "graphene sheets" of few layered dimensions. The use of high pressure further helps in fabrication in terms of reduction as well as improvement in surface properties [30].

Pyrolytic method

For scalling up process, pyrolysis is widely used in which an appropriate percentage of mixture of Na and ethanol are mixed and heated in a airtight vessel at above two hundred degree calcium for 3 days to obtain a precursor [25-29]. This when pyrolysed rapidly and purified and dried produces graphene sheets [30-36].This method can produced graphene of 0.1 g per mililitre of ethanol and yielding 0.5 g of graphene in each reaction. [37] This method can also be used to obtain high energy density suppercapacitor application which are derived from biomass resembling in properties of graphene [38]. Carbon nanotubes can also be used as a precursor which is suitable for synthesis of good quality graphene nanoribbons of few microns in size [25]. For this, there had been lots of methods such as plasma etching [34] or placing the nanotubes in a oxidizing solution such as acidified potassium permanganate [35]. Moreover, good quality of the product is achievable from such slicing of nanotubes [36]. Under a constant load of 0.06 N/cm^2 carbon nanotubes can be abraded between ground-glass surface which results in great friction as as to slice these substrates in to sheets of graphenes. Indeed this method is very much suitable for

preparation of good quality of the product as here no chemical modifaction is used and purity of the product is maintained.

Reduction

Graphite nanoflakes can easily be obtained by reducing chemically graphene oxide. Thus obtained graphene can suitably used in electrodes sensors, supercapacitors, fillers in polymers, paints etc. In this method a lower degree of exfoliation can be achieved [37–41]. High yields of graphene can also be produced by rapid heating [42]. It has been reported that using the paper making techniques, monolayer flakes of carbon with strong bondings can be achieved which are termed as graphene oxide paper with very high tensile module [43]. The membranes of graphene or its bases are impermeable to fluids excepting water [44]. Graphene oxide has been successfully be reduced under mild condition in acidic medium at ordinary conditions [45].

Electrochemical Approaches

Some of the interesting chemical methods has been used in past to exfoliate graphene for example one of the research groups used 0.1 M sulphuric solution as electrolyte and graphite flakes as anode along with platinum wire as cathode. Under the volatage of 10 V, the graphite flakes start to dissolve and after two minutes exfoliated graphite can be obtained by filtration. Thus obtained powder was dispersed in an appropriate solvent such as dimethyl formamide. One-pot method has also been used for synthesis of graphene by this exfoliation method. The experiment was conducted with incorporation of [BMIm][BF4] in aqueous medium under static potential of below 15 V. After this the neutral pH of the media is achieved by washing with water and alcohol.

Sonication

Different types of solvents like TEA can be used to separate graphene from graphite [49-51].Graphene thus obtained in this method has a yield of more than 70% and of thick ness 1 nm whose conductivity is as high 5000 $S \cdot m^{-1}$ [52]. One of the great drawbacks of the solvent aided methods is that the graphene sheets thus obtained are tend to restack together after the sonication method as there are van der Waals forces of attraction. Regarding this, surfactants and dispersing agents are highly in use before sonication so as to avoid such restacking. Here graphene sheets obtained are devoid of any chemical modifications and are of high purity and a yield of as high as 5.3 miligrams per mililitres also because of harmless ionic liquids are used, it is a green method and is stable [51]. Different stabilizing agents such as sodium dodecyl benzene sulphonate, sodium dodecyl sulphate, triethanol amine, cetryl trimethy ammonium bromides are effectly been used for effective stabilization of the graphene layers and do not allow them for aggregation [53].

After this the solution was cooled filter and washed with de ionized water and taken for characterization [54]. The pi pi interactions can be utilized for destacking and stabilization effect to the graphene sheets. In this regard, aromatic compounds can be used to preven the graphene sheets from restacking [55–59]. In some of the cases the high energy interfacial tension between two immiscible liquids, for example heptanes and water or hexane and water, to prevent restacking, and graphene obtained in this procedure are almost above 90% transparent and good conductive capabilities. In this process graphene can be coated on glassy surfaces with the help of chlorosilanes with three graphene layers [60]. Such methods are highly useful in solar panel formations, optolectronics, electronics etc. In this regard sono chemical treatments are also useful to reduce graphite oxide to graphene. Here the pH of the solution is modified by adding appropriate amount of a proper base such as sodium hydroxide and/or hydrazine, followed by sonication for atleast two hours, at a self generated temperature as high as up to 70 degrees [12, 61–64]. Hydrazine is found to react with graphene oxide with sonic radiation which caused cavitation with more active sites for removal of epoxide and hydroxyl group. Here the self generated media temperature reduces the graphene oxide to graphene [12].

In typical procedure, water dispersion of graphene oxide and isopropyl alcohol were put in a sealed bag and radiation of electron beams provided for ten minutes with voltage of 2 mega electron volt and with 10 mili ampere [65-71]. The graphene sheets which has been reduced, are then separated by centrifugation, washed dried in vacuum [70]. In some cases laser scribing is used whose concept is similar to that of pyrolysis with a difference here is laser beam. Such a technology has the potential applications in outfitted with piezoelectric patches for harvesting energy from movement of body parts. It has a good application in camoulflage uniforms as a power source [72]. Instead of lasers, other light sources such as Xenon flash, UV ligh and other type of lasers such as pulsed laser, femtosecond laser are used [73–79].

CONCLUSION

In the present article various top-down synthetic approaches have been discussed for fabrication of highly exfoliated and high quality graphene. These methods have extensively been discussed along with their limitations and improved properties of graphene sheets thus produced. Thus, the article is expected to provide the readers sound knowledge to adopt a technique as per the requirement.

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REVIEW ARTICLE

A Review on Diagnosis and Pharmacological Treatment of Jaundice

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ABSTRACT

Jaundice is a complex disease. Jaundice is actually the high bilirubin level in the body. Yellowing of skin, mucous membranes and skin are common presentations of jaundice. Jaundice has various variants including pre-hepatic jaundice (due to hemolysis of red blood cells), hepatic jaundice (due to defect in capture, conjugation and excretion of bilirubin by liver) and post hepatic jaundice (due to the obstruction of extra hepatobiliary system). The causes of various variants of Jaundice is either acquired or congenital. High plasma bilirubin level can cause various manifestations involving satiety, gastrointestinal bleeding, diarrhea, anemia, edema, weight-loss and can be fatal because it can cause psychosis, lethargy, seizures, coma or even death. High bilirubin level can help in the diagnosis of Jaundice. Differential diagnosis of various variants of Jaundice can be carried out on the basis of bilirubin level (conjugated and unconjugated), ultrasonography and other radiological techniques. The proper management of Jaundice is high water intake and low fat diet. The primary effective treatment for pre-hepatic jaundice and neonatal physiological jaundice is phototherapy. Infusion of immunoglobulins is also used for treatment of pre-hepatic jaundice. Proper nutrition, steroids and immunosuppressant are used for treatment of hepatic jaundice. The treatment for post hepatic jaundice is decompression and surgery.

Keywords: Jaundice, Hyperbilirubinemia, Hemolysis, Hepatobiliary, Obstruction

INTRODUCTION

Jaundice is a yellowing of the skin, whites of the eyes, and body fluids. It is caused by an increase in the amount of bilirubin in the blood. Bilirubin is a yellowish pigment that is produced from the breakdown of heme primarily from haemoglobin and red blood cells (RBCs). Bilirubin is transported by the blood to the liver, where the liver processes it, allowing it to be excreted in bile. Bile is a thick, yellow-green-brown fluid that is secreted into the upper small

intestine (duodenum) to get rid of waste products (such as bilirubin and excess cholesterol) and to aid in the digestion of fats [2]. Jaundice may arise from increased breakdown of red blood cells, inherited changes in bilirubin metabolism, liver disease or damage, and whenever there is interference with bile excretion. Normally, about 1% of our red blood cells retire every day, to be replaced by fresh red blood cells. The old ones are processed in the liver and disposed of. Much of the resulting bilirubin leaves the body in the stool. If there are too many red blood cells retiring for the liver to handle, yellow pigment builds up in the body. When there is enough to be visible, jaundice results. Jaundice can be caused by too many red blood cells retiring, by the liver being overloaded or damaged, or by the inability to move processed bilirubin from the liver through the biliary tract to the gut.

Most babies have some jaundice during the first week of life. The ordeal of birth can send many red blood cells to an early retirement (especially if a vacuum is used!), and babies' livers are often unprepared for the load. Before mom's milk comes in and stooling begins in earnest, bilirubin accumulates more easily [3-6]. Jaundice is even more common in premature babies. Physiologic jaundice is the name for normal jaundice commonly seen in healthy babies. Pathologic jaundice is the name given when jaundice presents a health risk, either because of its degree or its cause. Pathologic jaundice can occur in children or adults. It arises for many reasons, including blood incompatibilities, blood diseases, genetic syndromes, hepatitis, cirrhosis, bile duct blockage, other liver diseases, infections, or medications [7]. The term also applies to physiologic jaundice exaggerated by dehydration, prematurity, difficult delivery, or other reason. Another condition called Gilbert syndrome is a benign, hereditary condition in which mild jaundice develops. It is caused by low levels of some bilirubin-processing enzymes in the liver. This condition, once recognized, requires no further treatment or evaluation [8]. There are other more rare hereditary causes of elevated bilirubin levels. A yellow to orange colour may be imparted to the skin by consuming too much beta carotene, the orange pigment seen in carrots [9].

In this condition, the whites of the eyes remain white, while people with true jaundice often have a yellowish tinge to the eyes. In order to understand jaundice, it is useful to know about the role of the liver in producing bile. The most important function of the liver is the processing of chemical waste products like cholesterol and excreting them into the intestines as bile. The liver is the premier chemical factory in the body—most incoming and outgoing chemicals pass through it. It is the first stop for all nutrients, toxins, and drugs absorbed by the digestive tract. The liver also collects chemicals from the blood for processing. Many of these outward-bound chemicals are excreted into the bile [10-13]. One particular substance, bilirubin, is yellow. Bilirubin is a product of the breakdown of hemoglobin which is the protein inside red blood cells. If bilirubin cannot leave the body, it accumulates and discolours other tissues. The normal total level of bilirubin in blood serum is between 0.2 mg/dL and 1.2 mg/dL. When it rises to 3 mg/dL or higher, the person's skin and the whites of the eyes become noticeably yellow.

Symptoms of Jaundice [14]

Following are the major jaundice symptoms:

1. Extreme weakness
2. Headache and fever
3. Loss of appetite
4. Severe constipation
5. Nausea
6. Yellow discoloration of the eyes, tongue, skin and urine
7. Dull pain in the liver region

Types of Jaundice [15-17]

Neonatal jaundice: Jaundice is common in newborn babies. It occurs as a result of the liver being underdeveloped and not fully functional. In most cases, neonatal jaundice is nothing to worry about. It requires no treatment and usually disappears after a week.

Jaundice in adults and older children: Jaundice that occurs in adults and older children is usually a sign of an underlying health problem. There are three types of jaundice.

Hepatocellular jaundice: Hepatocellular jaundice is the most common type of jaundice. It occurs when bilirubin is unable to leave the liver cells and cannot be removed from the body by the kidneys. Hepatocellular jaundice is usually caused by liver failure, liver disease (cirrhosis), hepatitis (inflammation of the liver) or by taking certain types of medication.

Haemolytic jaundice: Haemolytic jaundice is when too much bilirubin is produced as a result of a large number of red blood cells being broken down. This can be due to a number of conditions, such as anaemia or a problem with the metabolism (the way that the body produces and uses energy).

Obstructive jaundice [18]: Obstructive jaundice occurs when there is an obstruction (blockage) in the bile duct, which prevents bilirubin from leaving the liver. This type of jaundice is usually caused by a gallstone, a tumour or a cyst in the bile duct or pancreas

Causes of Jaundice [19-20]

Increased production of bilirubin

There are several uncommon conditions that give rise to over-production of bilirubin. The bilirubin in the blood in these conditions usually is only mildly elevated, and the resultant jaundice usually is mild and difficult to detect. These conditions include: 1) rapid destruction of red blood cells (referred to as hemolysis), 2) a defect in the formation of red blood cells that leads to the over-production of hemoglobin in the bone marrow (called ineffective erythropoiesis), or 3) absorption of large amounts of hemoglobin when there has been much bleeding into tissues (e.g., from hematomas, collections of blood in the tissues).

Acute inflammation of the liver: Any condition in which the liver becomes inflamed can reduce the ability of the liver to conjugate (attach glucuronic acid to) and secrete bilirubin. Common examples include acute viral hepatitis, alcoholic hepatitis, and Tylenol-induced liver toxicity.

Chronic liver diseases: Chronic inflammation of the liver can lead to scarring and cirrhosis, and can ultimately result in jaundice. Common examples include chronic hepatitis B and C, alcoholic liver disease with cirrhosis, and autoimmune hepatitis.

Infiltrative diseases of the liver: Infiltrative diseases of the liver refer to diseases in which the liver is filled with cells or substances that don't belong there. The most common example would be metastatic cancer to the liver, usually from cancers within the abdomen. Uncommon causes include a few diseases in which substances accumulate within the liver cells, for example, iron (hemochromatosis), alpha-one antitrypsin (alpha-one antitrypsin deficiency), and copper (Wilson's disease).

Inflammation of the bile ducts: Diseases causing inflammation of the bile ducts, for example, primary biliary cirrhosis orsclerosing cholangitis and some drugs can stop the flow of bile and elimination of bilirubin and lead to jaundice.

Blockage of the bile ducts: The most common causes of blockage of the bile ducts are gallstones and pancreatic cancer. Less common causes include cancers of the liver and bile ducts.

Drugs

Many drugs can cause jaundice and/or cholestasis. Some drugs can cause liver inflammation (hepatitis) similar to viral hepatitis. Other drugs can cause inflammation of the bile ducts, resulting in cholestasis and/or jaundice. Drugs also may interfere directly with the chemical processes within the cells of the liver and bile ducts that are responsible

for the formation and secretion of bile to the intestine. As a result, the constituents of bile, including bilirubin, are retained in the body. The best example of a drug that causes this latter type of cholestasis and jaundice is estrogen. The primary treatment for jaundice caused by drugs is discontinuation of the drug. Almost always the bilirubin levels will return to normal within a few weeks, though in a few cases it may take several months.

Genetic disorders: There are several rare genetic disorders present from birth that give rise to jaundice. Crigler Najjar syndrome is caused by a defect in the conjugation of bilirubin in the liver due to a reduction or absence of the enzyme responsible for conjugating the glucuronic acid to bilirubin. Dubin-Johnson and Rotor's syndromes are caused by abnormal secretion of bilirubin into bile. The only common genetic disorder that may cause jaundice is Gilbert's syndrome which affects approximately 7% of the population. Gilbert's syndrome is caused by a mild reduction in the activity of the enzyme responsible for conjugating the glucuronic acid to bilirubin. The increase in bilirubin in the blood usually is mild and infrequently reaches levels that cause jaundice. Gilbert's syndrome is a benign condition that does not cause health problems.

Developmental abnormalities of bile ducts: There are rare instances in which the bile ducts do not develop normally and the flow of bile is interrupted. Jaundice frequently occurs. These diseases usually are present from birth though some of them may first be recognized in childhood or even adulthood. Cysts of the bile duct (choledochal cysts) are an example of such a developmental abnormality. Another example is Caroli's disease.

Jaundice of pregnancy: Most of the diseases discussed previously can affect women during pregnancy, but there are some additional causes of jaundice that are unique to pregnancy.

Cholestasis of pregnancy: Cholestasis of pregnancy is an uncommon condition that occurs in pregnant women during the third trimester. The cholestasis is often accompanied by itching but infrequently causes jaundice. The itching can be severe, but there is treatment (ursodeoxycholic acid or ursodiol). Pregnant women with cholestasis usually do well although they may be at greater risk for developing gallstones. More importantly, there appears to be an increased risk to the fetus for developmental abnormalities. Cholestasis of pregnancy is more common in certain groups, particularly in Scandinavia and Chile, and tends to occur with each additional pregnancy. There also is an association between cholestasis of pregnancy and cholestasis caused by oralestrogens, and it has been hypothesized that it is the increased estrogens during pregnancy that are responsible for the cholestasis of pregnancy.

Pre-eclampsia: Pre-eclampsia, previously called toxemia of pregnancy is a disease that occurs during the second half of pregnancy and involves several systems within the body, including the liver. It may result in high blood pressure, fluid retention, and damage to the kidneys as well as anemia and reduced numbers of platelets due to destruction of red blood cells and platelets. It often causes problems for the fetus. Although the bilirubin level in the blood is elevated in pre-eclampsia, it usually is mildly elevated, and jaundice is uncommon. Treatment of preeclampsia usually involves delivery of the fetus as soon as possible if the fetus is mature.

Acute fatty: Liver of pregnancy Acute fatty liver of pregnancy (AFLP) is a very serious complication of pregnancy of unclear cause that often is associated with preeclampsia. It occurs late in pregnancy and results in failure of the liver. It can almost always be reversed by immediate delivery of the fetus. There is an increased risk of infant death. Jaundice is common, but not always present in AFLP. Treatment usually involves delivery of the fetus as soon as possible.

Blood tests: These may initially include a complete blood count (CBC), liver function tests (including a bilirubin level), lipase/amylase level to detect inflammation of the pancreas (pancreatitis), and an electrolytes panel. In women, a pregnancy test may be obtained. Additional blood tests may be required depending upon the initial results and the history provided to the practitioner.

Urinalysis: Urinalysis is an analysis of the urine and is a very useful test in the diagnosis of screening many diseases.

Imaging Studies

- Ultrasound: This is a safe, painless imaging study that uses sound waves to examine the liver, gallbladder, and pancreas. It is very useful for detecting gallstones and dilated bile ducts. It can also detect abnormalities of the liver and the pancreas.
- Computerized tomography (CT) scan: A CT scan is imaging study similar to an X-ray that provides more details of all the abdominal organs. Though not as good as ultrasound at detecting gallstones, it can identify various other abnormalities of the liver, pancreas, and other abdominal organs as well.
- Cholescintigraphy (HIDA scan): A HIDA scan is an imaging study that uses a radioactive substance to evaluate the gallbladder and the bile ducts. ϖ Magnetic resonance imaging (MRI): MRI is an imaging study that uses a magnetic field to examine the organs of the abdomen. It can be useful for detailed imaging of the bile ducts.
- Endoscopic Retrograde Cholangio Pancreatography (ERCP): ERCP is a procedure that involves the introduction of an endoscope (a tube with a camera at the end) through the mouth and into the small intestine. A dye is then injected into the bile ducts while X-rays are taken. It can be useful for identifying stones, tumours, or narrowing of the bile ducts.

Liver Biopsy

In this procedure, a needle is inserted into the liver after a local aesthetic has been administered. Often ultrasound will be used to guide placement of the needle. The small sample of liver tissue which is obtained is sent to a laboratory for examination by a pathologist (a physician who specializes in diagnosis of tissue samples). Among other things, a liver biopsy can be useful for diagnosing inflammation of the liver, cirrhosis, and cancer.

Jaundice Treatment: Treatment depends on the cause of the underlying condition leading to jaundice and any potential complications related to it. Once a diagnosis is made, treatment can then be directed to address that particular condition, and it may or may not require hospitalization.

- Treatment may consist of expectant management (watchful waiting) at home with rest.
- Medical treatment with intravenous fluids, medications, antibiotics, or blood transfusions may be required.
- If a drug/toxin is the cause, these must be discontinued.
- In certain cases of newborn jaundice, exposing the baby to special colored lights (phototherapy) or exchange blood transfusions may be required to decrease elevated bilirubin levels.
- Surgical treatment may be required.

Medical Treatment: Treatment varies based on the medical condition responsible for causing jaundice, and the associated symptoms and complications. Treatments may include the following:

- supportive care,
- IV fluids in cases of dehydration
- medications for nausea/vomiting and pain,
- antibiotics, ϖ antiviral medications,
- blood transfusions,
- steroids, ϖ chemotherapy/radiation therapy, and
- phototherapy (newborns).

Medications: Medications may or may not be necessary. After diagnosing the cause of the patient's jaundice, the health care practitioner will direct the patient's treatment and prescribe medications if they are necessary. As outlined above, various medication options exist depending on the underlying cause of the jaundice.

Surgery: Surgical treatment may be necessary in certain cases of cancer, congenital malformations, conditions that obstruct the bile ducts, gallstones, and abnormalities of the spleen. Sometimes, a liver transplant may be necessary. Many healthy babies have some jaundice during the first week of life. It usually goes away. However, jaundice can happen at any age and may be a sign of a problem. Jaundice can happen for many reasons, such as:

- Blood diseases
- Genetic syndromes
- Liver diseases, such as hepatitis or cirrhosis
- Blockage of bile ducts
- Infections
- Medicines

CONCLUSION

Jaundice is very common disease. Yellowing of skin, sclera and mucous membranes are common manifestations of jaundice due to defect in production metabolism and excretion of bilirubin. The causes of jaundice are either congenital or acquired. Serum bilirubin level and ultrasonography are used for differential diagnosis. High water intake and low fat diet are best proper managements of jaundice. The treatment of jaundice varies with the type of jaundice.

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RESEARCH ARTICLE

Graphene Composite of Cadmium Sulphide Nanoparticles and Study of Effect on Polluted Water

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ABSTRACT

A novel chemical synthesis approach has been adopted for synthesis of cadmium sulphide nanoparticles at low temperature and characaterized by different characterization techniques. The phase analysis by X-ray crystallographic study shows that the thus synthesized nanoparticles are in hexagonal phase with increasing crystallite size increases with rise in experimental temperature. SEM images shows particle type of morphology with slight increase in size with higher temperatures, and well defined particles are formed at 90 ºC. The growth mechanism has been explained. Moreover, the photocatalytic activities of thus synthesized nanoparticles have been determined and compared. Subsequently graphene composite of these nanoparticles were also synthesized and photocatalytic activity of the particles as well as their nanocomposites were also studied. Experimental results indicated that the graphene composite shows enhancaed photocatalytic activity which is ascribed due to synergistic activity of the composite. Moreover, the photocatalytic activities of thus synthesized nanoparticles have been determined and compared.

Keywords: Nanomaterials, semiconducting materials, Cadmium Sulphide, Graphene composite, Photocatalytic activity.

INTRODUCTION

A substantial rise in industrialization from the last few decades there have been substantial rise in Industrialization which is good for development of the mankind. However at the same time it has some negative impact on environment in terms of the discharge of toxic chemicals, mostly dyes in to water resources resulting in their contamination. Such contaminated water, reportedly has, carcinogenic impact on living organisms [1]. A possible account for such an observation is the invention of a process for effective disinfection and destruction disinfection byproducts (DBPs). Commonly used chemical disinfectants by water industries are free chlorine, choramines and ozone which react with various constitutents in natural water to form many carcinogenic disinfection byproducts. It has been prediced that DBPs will be formed at any time during chemical oxidants are used in water treatment [2.3].

Added to this, the resistance of some pathogens, such as Cryptosporidium and Giardia to conventional chemical disinfectants require extremely high disinfectant does leading to excessive DBP formation. Therefore it is urgently needed an innovative approach to enhance the reliability and effectiveness of disinfection at the same time avoiding the DBP formation [4].

To combat the present situation, maximum interest has been focused on photocatalytic semiconductors. In this regard, maximum interest has been focused on semiconductor photocatalysts, which are capable of acting as sensitizer for light induced photocatalysis. They are supposed to involve advanced oxidation process, that lead to degradation of the organic pollutants forming ecofriendly by products. In this regard, CdS is a n-type narrow band gap semiconductor (bandgap: 2.42 eV) belonging to II-VI chalcogenides. Its field of applications is vast such as light diodes, transistors, photoelectric and emission devices [5], different types of biological sensors [6-10], optoelectronic fields, solar cells, luminescence devices [11-17] and as a photocatalyst [18-23]. In last few years graphene has attracted the attention of many researchers due to its excellent electronic, thermal and mechanical properties. Because of this it is used in synthesis of high efficient nanocomposites, super capacitors, sensors, for hydrogen storage devices, batateries. It also acts as an excellent adsorbent for heavy metals ions for water purification purposes. Among different types of synthetic methods, the Hummer's method has been widely used due to its cost effectivity and simplicity. This method is followed by a wide range of researchers for achieving highly exfoliated graphene sheets. Thus in the present study, we aim at fabrication of CdS nanoparticles and subsequently its graphene composite, by simple wet chemical method. Such a composite is expected to prove as an efficient photocatalyst for removal of harmful dyes present in industrial waste water by advanced oxidation process.

Experimental

All the reagents were of analytical grade and used without further purification. Cadmium chloride ($CdCl_2$), Ethylenediamine ($C_2H_4(NH_2)_2$), Carbon disulfide (CS_2), Hydrazine (N_2H_4), Acetone($CH_3)_2CO$, $CdCl_2$ were and Acetone were purchased from PALLAV company Hydrazine was procured from CDH company, Ethylenediamine was from MERCK company and Carbon disulfide was purchased from RAINBOW.INC company. Methyl blue ($C_{37}H_{27}N_3Na_2O_9S_3$) was bought from SIGMA-ALDRICH (MERCK) company and used prepared CdS Nps in lab. Distilled water used as a solvent from CESIUM company. Sulphuric acid (H_2SO_4) and Hydrogen peroxide (H_2O_2) were purchased from PALLAV company, Graphene powder ($C_{140}H_{42}O_{20}$) was purchased from TECH INSTRO company and potassium permanganate ($KMnO_4$) from SIGMA-ALDRICH(MERCK) company. Distilled water used as a solvent for all synthesis purpose.

Synthesis

In the typical procedure, 0.55 g of $CdCl_2$ was dissolved with 50 mL. distilled water taken in a 250 mL. round bottom flask. We noticed it completely soluble in water. Next a magnetic bead was put into the flask with the setup was placed on a hot plate with a perticular RPM, temperature value and after that 3 mL of Carbon disulfide (CS_2) was added to the solution & It was seen that oil droplets formed flowing on the surface of solution due to the CS_2 is insoluble in water. It is try to dissolved by constant stirring with the help of the magnetic bead. By using gloves and droppers, 3.5 mL of ethylediamine was added above the solution. A light white milky solution was formed due to the forming a hydrated compound upon contact with atmospheric H_2O. A reflux condenser is then then attached to the round bottom flask and the setup was placed on a hot plate provided with magnetic stirrer. After 1 ½ h it looked light yellowish color. The reaction was carried out for 3 h with temperature 50 ºC at RPM 500- 700. On vigorous stirring a yellow colored solution was formed which was filtered with the help of Buchner funnel and Whatman filter paper. The residue was taken out and put it into the oven for 2 hr. The same procedure was repeated for synthesis at 70 and 90 ºC. The above set of steps were also repeated in the absence of ethylene diamine.

Synthesis of graphene composite

Aqueous suspension of GO was synthesised by modified Hummers method. In a typical procedure, 2.5 g of Graphite powder and 1g of Sodium nitrate was slowly added into 50 mL of sulphuric acid under stirring in an ice bath at

below 10°C. After 30min 6.2 g of potassium permanganate was added slowly, after 2 h of continuous stirring the resulting dark green suspension was removed from ice bath to maintain normal temperature then 100mL of water was slowly added to it under stirring which results increase in temperature up to 90 °C. after that 8 mL of 30% hydrogen peroxide was added, after addition of hydrogen peroxide a yellowish brown solution was obtained and it allowed to settle for hours, then the final mixture was separated and washed with water for several times to remove excess of sulphuric acid along with soluble impurities. After that the resulting black pasty mass was allowed to dry vacuum oven at 60 °C. For preparation of the CdS-graphene composite, 0.45 g of graphene was mixed with 50 mL of water. To which 0.25 g of $CdCl_2$ was added followed by 3 mL of carbon disulphide, 3.5 mL of ethylene diamine and 3 mL of hydrazine hydrate. The mixture was heated to 90 ºC with constant stirring for 3.5 hours.

Photocatalytic activity of CdS and its composite

50 mL of 5 x 10^{-4} M aqueous solution of methylene blue (MB) was placed in a 100 mL beaker. To this, 25 mg of catalyst was added and the entire mixture was stirred in dark for about 1 h to so as to attain equilibrium of adsorbed methylene blue species with the desorbed amount present in the solution on the catalyst. The above beaker was placed under sun light with constant stirring. The amount of the dye decomposed, was analyzed from time to time by taking a small amount from the mixture and determining its absorbance with the help of colorimeter nearly at 664 nm. The same experiment was also repeated with the equal amount of graphene composite.

Characterization techniques

The powder X-ray diffraction patterns of the products were recorded on X'Pert Pro PANalytical Instrument using Cu K_α radiation in the range of 2θ = 10-70º with scanning rate of 3º per minute. The morphology of the samples was studied by scanning electron microscope (SEM) on Carl Zeiss instrument. The absorbance of the sample was noted with colorimeter of systonic company.

RESULT AND DISCUSSION

Figure 1 shows the X-ray diffraction (XRD) patterns of the products inferred that the different crystal reflection planes could be indexed on the basis of hexagonal lattice of CdS (a= 4.136 ´ and c= 6.713 ´) [JCPDS 77-2306]. The absence of any additional peak in the corresponding diffractograms also suggests the formation of high purity CdS. From the XRD patterns it can be seen that at 50 ºC the sample is mostly amorphous with a very small percentage of crystallinity. However with rise in temperature ot 70 and 90 °C there is a substantial increase in crystallinity with rise in temperature. The crystallite size was also determined using Scherrer's formula as,

$$D=K\lambda/(\beta \cos \theta)$$

Where K, λ, β, and θ are Scherrer constant, wavelength of X-ray radiation target used, maximum peak width in half height and angle of diffraction respectively and the data is as follows.

Temperature (in degree Celsius)	50	70	90
Grain	34 nm	46 nm	63 nm

Figure 2 shows SEM images of the products obtained at reaction temperatures 50, 70 and 90 ºC. It clearly demonstrates that the formation of CdS nanoparticles (size: 100-200 nm) takes place at these temperature range. The rise in crystallinity is also reflected in the SEM patterns at higher temperatures. Moreover the particle size was found to be controlled at higher temperature and optimum particles of size around 120 nm was obtained at 90 °C. Based on the results, the below mechanism was postulated. Ethylene diamine (EDA) which was taken as a templating agent also act as a complexing agent, binds with Cd^{2+} and forms a complex [24]. In this process the release and availability of Cd^{2+} was reduced for which the reaction is slowed down favouring crystallisation and separating the growth step from the nucleation step [25]. So, Cd^{2+} ions from Cd-EDA complex are released slowly and reacts with S^{2-} from thus evolved H_2S gas from the combination of ethylene diamine and carbon disulphide. At lower temperatures (50 and 70 °C), there is slow releasing of Cd^{2+} ions from the complex which reduces the speed of reaction. Hence, the formation

of CdS become slow and the growth on the nucleating center is less, so that the size of the particle is lesser. When the reaction temperature increased to 90 ^{0}C., the complex becomes unstable and nearly breaks to generate higher concentration of Cd^{2+} ions in the solution leading to faster growth of CdS nanocrystals to bigger particles. So, the observed particle size of CdS nanoparticles are 80, 90 and 120 nm at temperatures of 50^0, 70^0 and 90 ^{0}C. respectively. The photocatalytic decomposition of aqueous MB solution has been carried out in absence/presence of CdS, acting as catalysts, under dark/UV radiation. These studies show no appreciable degradation of MB either in absence (or presence) of catalysts in absence sunlilght even after 4 h. On the contrary, MB dye degraded very fast in presence of catalysts and sunlight.

These data have been used to calculate the fraction of MB left undegraded at different interval of time, from which % degradation of MB has been calculated using the relationship: $D = (C_0-C_t)/C_0 \times 100$. The observations have been shown in Figure 3. These observations suggest that ~ 74% degradation of MB in 40 minute take place in presence of CdS. The same catalyst has also been used for studying the reusability and has been found that the reusability of the CdS drastically decreases after 2nd use. This is possibly because of the fact that CdS is vulnerable to photocorrosion. The further exposure to UV radiation up to 180 min showed no further sign of any appreciable degradation. Figure 3 also suggests that the graphene composite do have greater photocatalytic activity in comparison to pure CdS. The higher catalytic activity of composite in comparison to the CdS nanoparticles is because of greater mobility of charge carriers of graphene nanosheets (Hu et al. 2011). Reportedly Graphene is a good electron acceptor, which which resistes the recombination of the electron-hole pair in CdS due to enhancement of the electron transfer at the interface [26-30]. Moreover, the two dimensional nanosheets of graphene having very high surface-to-volume ratio and very high specific surface area, disperses the CdS nanoparticles easily and allows enhanced light absorption on the surface of the catalyst [31]. For reusability accessment, the same photocatalytic study was carried with the used CdS-graphene composite and the observations indicate that the dye absorption on the surface of the catalyst decreases gradually. The percent degradation of the dye decreases by 7 and 15% with subsequent runs, in comparison to the initial run. This also indicates the reusable efficiency is not that much low, due to which the composite can be effective up to 3rd run for a photocatalysis.

CONCLUSIONS

In summary, different morphology of CdS nanostructures were successfully prepared by a simple way using wet chemical route in presence of templating agents at three different temperatures. These particles were characterized structurally and optically. XRD results indicated the presence of pure crystalline phase CdS. SEM analyse the morphology of the sample prepared. Further the photocatalytic study indicated 74% of dye degradation using the sample synthesized at 90 ^{0}C under sunlight and within a time period of 4 hours. However greater photodegradation efficiency, up to 85%, was noticed when graphene composite was used. However due to photoerrosion of the CdS surface its photocatalytic activity is not suitable for subsequent re-uses. These observations paves new pathway for trial with other photocatalytic compounds as well as their composites, under the similar experimental conditions and exploration for their effectivity as catalyst.

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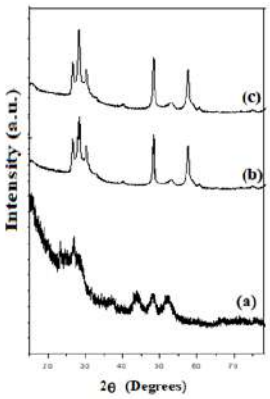

Figure 1: XRD patterns of the sample synthesized at temperatures (a) 50⁰C, (b) 70⁰C and (c) 90⁰C

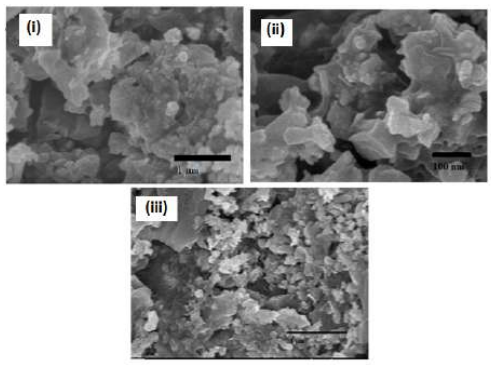

Figure 2: SEM images of samples synthesized at temperatures (i) 50 ⁰C, (ii) 70 ⁰C and (iii) 90 ⁰C

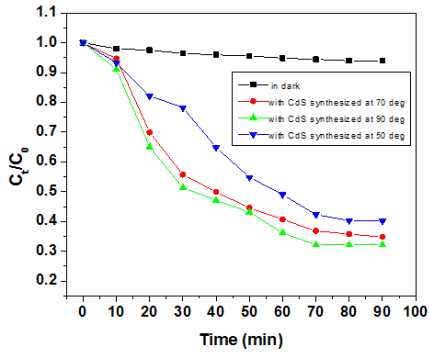

Figure 3: Percentage of photo degradation of MB vs solar irradiation time in presence of CdS nanoparticles synthesized at different temperatures.

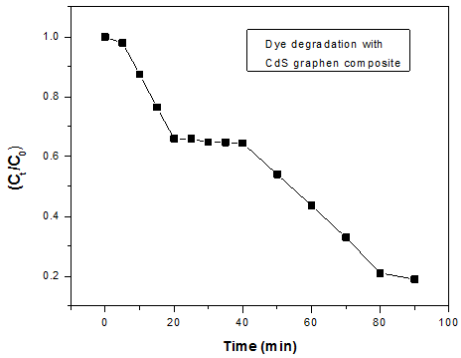

Figure 4: Percentage of photo degradation of MB vs solar irradiation time in presence of graphene compsite of CdS nanoparticles synthesized at 90 ⁰C.

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RESEARCH ARTICLE

Disease Management Strategies Leading to Enhancement of Self-life of Horticultural Crops

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ABSTRACT

Effective post-harvest techniques allows agricultural sector to fulfil the global demands by retaining the required nutritional quality of horticultural produce. To enhance post-harvest quality of harvested produce, which are in a state of ripening, undergoes senescence and metabolically active must be taken care of by practicing efficient methods, otherwise it would result into significant financial loss. Effective post-harvest techniques primarily focus in limiting the rate of metabolic process resulting in delay of senescence, maturation and minimizing the risk of microbial contamination. A variety of management practices including physical, chemical and gaseous treatments have been introduced. Physical treatments include irradiation, edible coatings and heat. Chemical treatments comprise of antioxidants, applying suitable antimicrobials. Temperature management has been a classical practice. This study focus on the prevailing status of post-harvest techniques including the use of ozone and plasma, resulting in maintaining quality and reducing loss of fresh produce.

Keywords: Post-harvest, nutritional quality, senescence, irradiation, edible coating, antioxidants.

INTRODUCTION

Horticultural produce are rich in nutrients, however during post-harvest storage they are prone to various metabolic reactions resulting in their decay. To overcome this situation, well coordination from the level of farmers up to the consumers needs to be maintained. The level of this coordination varies, usually slack at local level and highly complex at global level depending on the ease of adopting efficient post- harvesting strategies. According to the Food and Agriculture Organization (FAO), 33% of global food was wasted on the weight basis during 2009 (Lipinski et al., 2013). Essentially, reducing food losses is vital. Quality of fresh produce is highlighted from its flavour, texture, appearance and nutritional value. But, for safety of the consumers it is equally important to make sure that the produce is devoid of any microbial infection or chemical contamination. Essentially contamination of fresh produce

with pathogens or microorganisms can lead to serious illness (Warriner et al., 2009). Maximum uncertainties lead to spoilage of the fresh produce, thus the role of post-harvest treatments becomes critical (Olaimat et al., 2012). Several effective techniques including physical, chemical and gaseous techniques are introduced in order to safeguard the horticultural produce, keeping an eye on maintaining safety and nutritional standards. Maintaining appropriate temperature is equally vital and traditionally being practiced.

Physical treatments

Heat treatment: Heat treatment has been widely used technique which leads to decrease in chilling injury, delay in ripening primarily by inactivation of degradative enzymes (Schirra et al., 2000), avoidance of contaminations and fungal infections (Figure 1). This treatment includes hot water rinse, hot water dip and saturated water vapour heat. Duration of heat treatment can be short up to 1 hour or long ranging to 4 days. Heat treatment adds to longevity of horticultural produce. For instance, *Penicillium* sp. Causes blue mould on grape fruit and it can be restricted by simple dipping of fruit in hot water (50°C) for 2 minutes (Lurie, 1998). A mixture of HF-01, sodium bicarbonate and *Bacillus amyloliquefaciens* and hot water is one of the promising techniques used to maintain quality of fruits (Hong et al., 2014).

Edible coating: Edible coatings are essentially layers of surface coatings applied on the surface of fresh produce, primarily to enhance the safety of the fresh produce especially where the cuticle is lost (Gol et al., 2013; Dhall, 2013). This act as a barrier by not allowing the movement of moisture on the surface, resulting in slowing down of respiratory rate, decreased oxidation, and protects from mechanical damages, leading to increase in quality and safety of fresh produce (Dhall, 2013; Mohebbi et al., 2012; Ghasemnezhad et al., 2013). Edible coatings usually contain hydrophobic groups, protein based compounds chiefly to increase the functionality of edible coating (Mohebbi et al., 2012). Also, edible coating layers add to antimicrobial activity (Ghasemnezhad et al., 2013).

Irradiation: The basis of this technique is to desensitize the nucleic acids and proteins so as to restrict further metabolic reactions, eventually delaying the senescence (Farkas, 2014). Preferably, high energy electron beams, γ radiations are projected onto the surface of fresh produce, resulting in breaking of essential molecular bonds, nucleic acids including DNA and RNA. By slowing down the metabolic processes, self life of fresh produce is enhanced. Though, the extent of radiation is critical as the desired effect primarily depends on the doses. Doses are measured in kilograys (kGy), low doses less than 1 kGy can only lead to the disruption of cellular activity, inhibiting sprouting of tubers, roots and bulbs. Medium doses range from 1-10 kGy effect the microbial loads and high doses ranging above 10 kGy can be an effective tool against various bacterial and fungal species (Ferrier, 2010). But, exposure of fresh produce to medium and high level doses may cause defects in flavour, texture and visual. Irradiation is widely used post-harvest management technique to restrict the growth of microbes and thus bring about food safety (Mahto et al., 2013). For instance, irradiation dose of 1 kGy is considered to be highly effective for enhancing self life in litchi fruit (Pandey et al., 2013). It is also reported that irradiated fresh produce is safe for consumption (Artés et al., 2009).

Chemical treatments

Antimicrobial agents: In order to maintain safety of the fresh produce, various antimicrobial and anti-browning agents are used, which can be categorized into chemical and natural agents (Carrasco et al., 2010). Chlorine based agents, organic acids, peroxyacetic acid (PAA), electrolysed water are a few widely used agents. Again, the level of chlorine is critical to bring about required changes. NaClO is one of the commonly used oxidizing agents. But, chlorine may not be the best alternative as chlorinated compounds may form carcinogenic compounds (Carrasco G. et al., 2010). PAA is also a strong oxidizing agent (Rodgers et al., 2004). PAA is efficient in detoriating the growth of *Listeria. monocytogenes* and *Escherichia. coli* present on many kinds of fresh produce (Cengiz et al., 2013). Hydrogen peroxide (H_2O_2), an oxidant possesses antibacterial, sporicidal activity (Aguayo et al., 2003). Application of H_2O_2 extends self life and facilitates in decreasing microbial populations (Wendehenne et al., 2004). Organic acids, ascorbates largely slow down enzymatic reactions and restrict the growth of microbes (Singh et al., 2013).

Nitric oxide: Nitric oxide (NO) is one of the reactive gases, primarily involved in various signalling pathways in plants (Palou et al., 2010). With the maturation of fresh produce, NO level decreases. External exposure of the horticultural produce with NO will help to compensate the endogenous loss of NO and facilitates in maintaining the self life and longevity (Sivakumar et al., 2010). This also favours the fresh produce in preventing those to ripen by restricting the concentration of ethylene hormone, which would otherwise fasten the ripening phase (Cantín et al., 2012). NO exposure results in decline rate of respiration and water loss.

Sulfur dioxide: Application of sulphur dioxide (SO_2) prevents decay of many fresh produce. Primarily fumigation is done from the horticultural field followed with weekly fumigation of the storage chambers (Hassenberg et al., 2012). SO_2 specifically can reduce fungal contamination over the fresh produce, though it can also initiate injuries on the surface, and also possesses health risk. Despite of this, SO_2 treatment is widely used as a post-harvest method to increase the longevity.

Gaseous treatments: Several gaseous agents are used in order to restrict the fresh produce from decay and to increase the sustainability, longevity and quality by removing the hazardous agents. An overview on gaseous treatments is listed in Table 1.

Plasma

Plasma is considered to be one of the best methods specifically in decontaminating horticultural produce (Figure 2). Plasma mainly comprises of ionized gas molecules, dissociated by an input source of energy. Based on the activation energy, high or low temperatures commonly referred to as thermal or cold plasma is generated (Janssen et al., 2014). Cold plasma is generated by transforming argon gas to plasma by using radio frequency energy waves (Lluís et al., 2003). In order to achieve effective decontamination, microorganisms need to be eliminated at DNA level, which is achievable by the optimum usage of cold plasma (Silverman et al., 2004). More detailed studies are required to improve the efficacy of this technique.

CONCLUSION AND FUTURE PERSPECTIVES

Various strategies are employed to extend the self life and quality of the horticultural produce. Primarily, specific techniques are applicable to certain kinds of products, thus efficiency of post-harvest techniques needs to be closely monitored in order to be effective against broad spectrum of hazardous agents. Appropriate application of mixed techniques can be effective in restoring the nutritional and physical attributes, mostly by delaying the decay processes. Advanced technologies based on application of ethylene inhibitors, chemical modulators for delay in ripening are emerging. Use of nanotechnology in the field of post-harvest management is also promising. Developing nanocomposites with an aim to control microbial growth and/or to release specific agents into the fresh produce would be of great use. Studies on various aspects of devising and delivery systems will help to elevate the efficiency of postharvest technologies.

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Table 1: List of various gaseous agents for post-harvest treatment of fresh produce

Treatments	Benefits	Example of application
Ethylene	Initiates ripening thus enhances fruit colouration and quality	Tomato, banana, persimmon, avocado, kiwifruit, mango and citrus fruits (Wills, 2005; Blankenship et al., 2003; Sisler et al., 2003; Watkins, 2006)
Ozone	Easy incorporation into cold storages, washing system, better efficiency as compared to chlorine	Apples, cherries, carrots, garlic, kiwi, onions, table grapes (Serek et al., 2006; Beaudry et al., 1999; Saltveit, 2003; Kader, 2004)
1-Methylcyclopropene	Maintains integrity of the cell wall and colouration, develops aroma and flavour	Broccoli, cucumber, date, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum (Rodriguez-Lafuente et al., 2010; Mahajan et al., 2007; Sousa-Gallagher et al., 2013; Niemira, 2012)
Modified atmosphere packaging	Delay in senescence by slowing down respiration and rate of decay	Cherries, carrots, fresh-cut fruits, leafy green vegetables (Baier et al., 2013; Fernández et al., 2013; Wills et al., 2007; Singh et al., 2012)
Controlled atmospheric storage	Retards senescence and metabolic reactions, resulting in delay in severity	Avocado, strawberry, cherry, cabbages, kiwifruit, avocados, persimmon, pomegranate, asparagus, banana (Saltveit et al., 1999; Garner et al., 2001; Watkins et al., 2002)

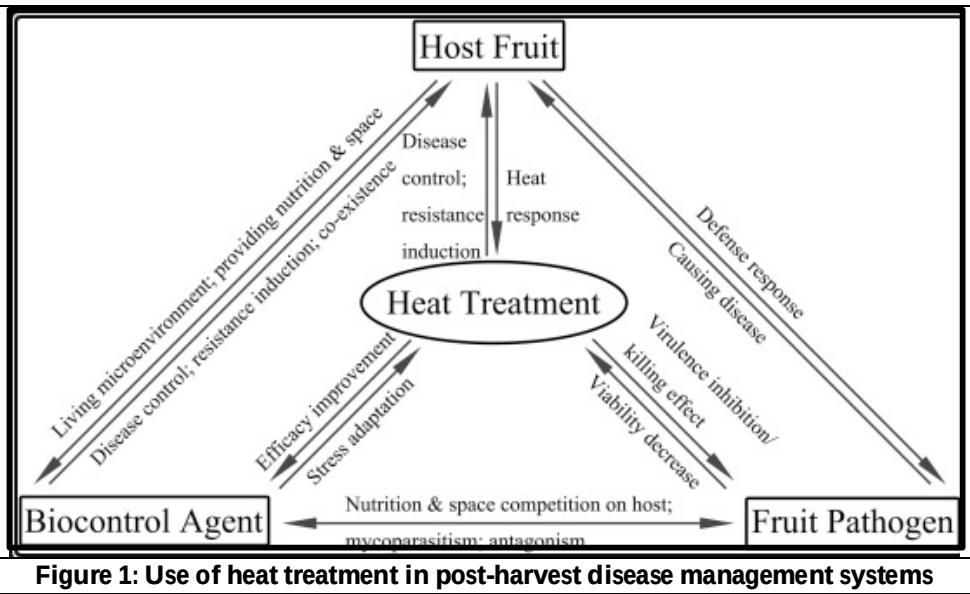

Figure 1: Use of heat treatment in post-harvest disease management systems

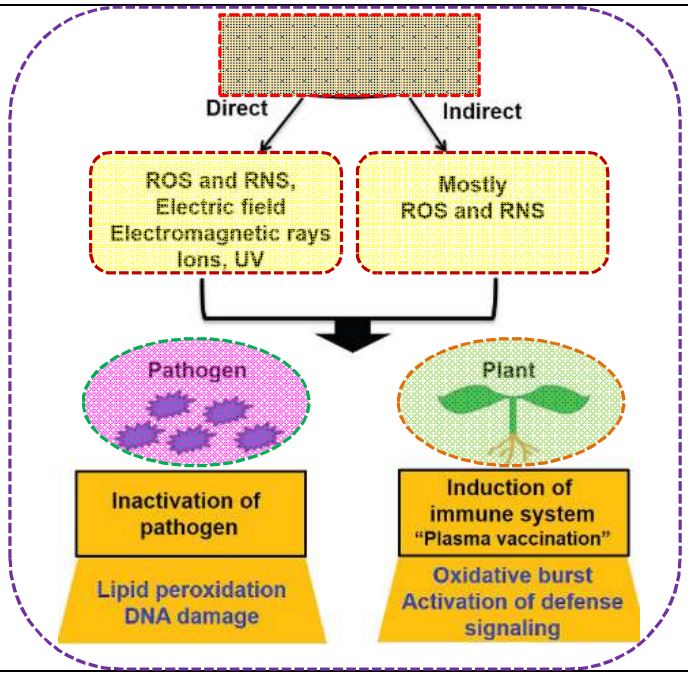

Figure 2: Non-thermal atmospheric pressure plasma-mediated post-harvest management.

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RESEARCH ARTICLE

Precision Nutrient Management for Enhancement of Nutrient use Efficiency

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ABSTRACT

Precision nutrient management is an important aspect of agriculture that accurately defines key factors for productivity improvement, sustainability, profitability and climate change. Improved grain production but do not improve the efficiency of certain nutritious foods. Assessment of plant nutrient needs much very effective for plant growth and development at different stage of crops which help to supply nutrient from different sources and a measure of the reliability of their availability. Inexpensive and nutrient decision tools are gaining popularity. Precision nutrient management designed to maximize long-term performance, particularly aimed increase crop production and profit with judicious use and proper nutrient balance and minimizing impacts on wildlife and the environment. The systematic implementation of good practices in a site-specific management system provides a good opportunity to build a sound and sustainable agricultural system. Resource management right time, right method and right place are the best practice. Various technologies are available to make decisions making tools related to nutrient management.

Keywords: Precision nutrient management, tools used, site specific nutrient management, nutrient use efficiency

INTRODUCTION

The modern and intensive agriculture has led into use of fossil-fuel based high energy inputs and if these are not used judiciously, they cause harm to the environment (Maitra *et al*., 2018; Maitra *et al*., 2021; Shankar *et al*., 2021a). The nutrient management uses advanced, innovative and descriptive technologies that help to manage the natural and temporal variability of nutrients from the soil to increase productivity and profitability for agricultural sustainability (Jinn and Jiang, 2002). During the present days, climatic aberration is also creating problem in efficient crop management (Hossain *et al*., 2021). Studies have shown the benefits of proper nutrient management in reducing nutrient losses. It has been reported that the ways to accurately to control nitrogen loss can effectively reduce by

comparing the traditional nitrogen management (Praharaj*et al*., 2021; Mondal *et al*., 2021; Shankar *et al*., 2021b). In general, this approach emphasizes feeding crops with precise quantity of nutrients as and when required (Bhattacharyay *et al*., 2020).The 5R nutrient stewardship concept enables SSNM to improve the crop yield. These 5Rs are, namely, right source of nutrient, right rate, right time, right place and right method (Gebbers and Adamchuk, 2010, Rao *et al*., 2011). A complete supply of water and nutrients other than nitrogen should be ensured when using a device such as a SPAD and LCC meter or optical sensors for accurate nitrogen handling. This precise management of device-based nitrogen can also lead to the effects of certain stress conditions such as excess salt in the soil or water, premature rainfall. Also, as LCC and SPAD radiation can lead during nitrogen foundation administration or under adverse conditions and it is expected that deficiency of Phosphorus (P) and Potassium (K) may interfere with leaf greenness and affect SPAD and LCC radiation. There is a need to define stressful situations in which nitrogen demand management is not possible. It appears that the amount of sunlight received in the region will affect the SPAD or LCC price range and information needs to be collected to solve this problem (Shankar *et al*., 2020; Mohanta *et al*., 2021).

Efficient nutrient management is synonymous to reduction of environmental pollution as caused due to non-judicious use of nutrients. Further, in the process of chemical N fertilizer production, carbon dioxide is generated. To meet the present need of chemical N fertilizers, annually 300 Tg of carbon dioxide is released to the atmosphere (Maitra et al., 2021). In 2015, the UN has announced Sustainable Development Goals (SDGs) to be achieved by 2030(UN, 2021). Among the SDGs, "SDG 2 (end hunger, achieve food security and improved nutrition and promote sustainable agriculture), SDG 13 (take urgent action to combat climate change and its impacts)" are related to efficient plant nutrient management as it has potential to check global warming and climate change, enhance of crop productivity towards ending the hunger and create favorable environment for achieving agricultural sustainability.

NUTRIENT MANAGEMENT TOOLS

Chlorophyll meters

Chlorophyll meters are very reliable in standard tissue analysis as nitrogen filling tools. Chlorophyll Meter is mainly used for hand-held Minolta SPAD 502(Zhuhai, 2016). The SPAD 502 chlorophyll meter is a fast and portable device developed by Minolta, Osaka of Japan. It quickly delivers high levels of nitrogen content of chlorophyll content (Boggs *et al*., 2003) by cutting the leaf blades using two LED lights. Red infrared radiation waves are used to pass through the leaves. A certain amount of light is emitted and everything is transferred to the leaf. The silicon photodiode identifier converts the electrical signal. The amount of light reaching the detector is equal to the amount of chlorophyll in light.

Optical sensors

Visual nerves are visible and close to infrared spectral responses from the plant to detect levels of nitrogen stress. The chlorophyll contained in the upper layer of the leaf regulates the intense spectral light (400-720 nm) reflected although the light of the NIR electromagnetic spectrum (720- 1300 nm) depends on the formation of mesophyll body tissues. Indicative vegetation indicators such as the differentiated vegetable index calculated where FNIR and Fred are found, respectively, fragments released by the NIR and red rays returned to the target area, providing details on photosynthetic efficiency and potential product (Zhang*et al*., 2021).

Leaf color chart

Leaf color chart is a strip with high-quality plastic with a variety of shades of green ranging from light green to yellow to dark green. The leaf colour chart (LCC) was the first time introduced in the agricultural sector of the world by scientists of Japan (Furuya, 1987). The upgraded version of the six LCC panels was developed in conjunction with the International Rice Research Institute (IRRI) and the agricultural research programs of several Asian countries in 1996. to 20 after re-planting or sowing until flowering and the recommended amount of nitrogen fertilizer if the color of the rice leaves falls into critical stages. The LCC shade has been found to be the limit for the range of re-grown rice

varieties spread across the Indo-Gangetic plains (Hussain *et al*., 2003). The number of LCCs was reports that Gangetic plain in Bangladesh be 3.5 in the lower. A critical number of LCCs 2 and 3.5 were found to be suitable for hybrid rice flavored and fragrant, respectively. Singh *et al*. (2007) 350 farm experiments performed by Indian Punjab using a six-panel, IRRI-LCC fertilizer N rice fertilizer, less than 9.4-54.2 kg of N ha-1 fertilizer were used without yield reduction compared to farmers' practice. to use the garment N from time to time.

Omission plot technique

The extrusion structure is applied to the estimated fertilizer requirements for the target stone. All the nutrients used are used without the nutrient of what is left over. Provides a reasonable amount of supply for native soil nutrients. If all the nutrients such as nitrogen potassium other than phosphorus are used in a phosphorus-free environment, limited yield to the traditional supply of phosphorus. The high yield gap between the probable test and the yield in the construction plan is even used to calculate the fertilizer requirement in the bypass camp.

Green seekers

Using Green Seeker's visual sensor, Singh *et al*. (2011) conducted a study in Karnal, Modi Puram and Ludhiana also observed a strong relationship between the Green Seeker optical sensor-based seasonal yield in Feke's 5-6 and7-8 growth stages and actual wheat by older. The amount of additional N fertilizer that needs to be determined by taking the difference in the assaying ratio between the feasibility of a non-fertilizer feed supply and the application of the fertilizer and the efficiency factor. wheat, and whether the N-optical Ensor-directed N dose was applied to Feekes 5-6 or Feekes 7-8stage, had an impact on the N of fertilizer rate to be applied according to the N fertilizer algorithm. The Green seeker handheld crop sensor (GS) was developed by Trimble agriculture as an active light source optical sensor used to measure plant biomass and displayed as NDVI (normalized difference vegetation index), which is used for N prescription recommendation (Ali *et al*., 2018).

Nutrient expert

Nutritional expertise is widely used in computer-based programs for direct management of nutrients in crop production (Sairam *et al*., 2020). These types are designed to take into account the diversity of space and time in the supply of nutrients and to ensure nutritional management. rice. By using Nutrient Expert®(NE) for Rice–South Asia (India) as software tool develops by IPNI specific recommendations for farmers based on 3 to 5 years of yield, organic fertilizer and organic matter in the field, achievable yield, soil fertility indicators, residual content (Ghosh *et al*., 2020; Mohanta *et al*., 2021). It takes care of the availability of resources to calculate their yield goal. The standard algorithm for fertilizer requirement in Nutrient Expert is developed from a set of farm test data.

Site specific nutrient management (SSNM)

To feed the growing population, India will have to produce more and better food in a small country. In order to meet all the objectives of sustainable agriculture (increased food and fiber, profitability, efficiency of inputs and environmental concerns), the balance of adequate nutritional levels is an important component. Forty years ago, crop management in India was driven by an increase in foreign imports. Fertilizer has played a major role in improving crop production. Grain production doubled from 98 million tons (MT) between 1969-2007 to a record 212 MT in 2001-2002, while fertilizer use increased almost 12 times from 1.95 MT to 23 MT 2007-08 (Rao, 2009). Despite these positive changes, grain demand is expected to grow by about MT 300 yr-1 by 2025 when the country will need 45 MT of nutrients (ICAR, 2008). With almost no opportunity to expand the agricultural area of more than 142 million hectares, more demand for grain production should be met with increased yields in each area, especially those for major food crops such as rice, wheat and maize.

SSNM provides a method of demand based on the 'supply' of nutritious plants. The SSNM approach aims to increase the profitability of farmers by achieving the goal of maximum crop yield (MEY) of crops. The main features of SSNM are: (Tiwari, 2007) Direct site utilization of nitrogen, phosphorus and potassium and secondary and micronutrients according to soil testing is followed. Proper utilization of existing nutrients, such as soil, residues and compost.

SSNM continues to provide guidelines for selecting the most nutritious economic combination and promotes the efficient and effective use of existing indigenous natural resources such as crop residues and fertilizers (Maitra and Zaman, 2017). The implementation of SSNM strategies should begin with the priority areas that address one or more of the following problems: (Tiwari, 2007) Areas with insufficient or uneven use of fertilizers with low yields show signs of severe malnutrition. Pest problem areas linked to nutrient imbalance or overuse of fertilizer N. phosphorus mines and potash reserves of soil. Areas with evidence of multiple nutrient deficiencies including secondary and nutrient depletion in soil and plants.

The technical features of the SSNM site-specific control system depend on the interaction of three broad and basic elements in order to be effective in its operation. They are divided by knowledge, technology and management. Information on field diversity, spatial or temporal, soil-related structures, plant characteristics, weeds and insect repellent and harvesting data are important details that need to be made in order to master the skill of site management. Apart from this, monitoring crop yields is a very mature part and a sensible start. Several years of yield data may be required to make a good decision. A very diverse product in the field suggests that current management systems may not provide the most favorable conditions for the entire field. The establishment of soil-related features within the field, using soil samples, is one of the most important data. Some factors such as soil texture vary slightly over time, while others, such as moisture and nitrate levels, change rapidly. The decision, therefore, must be taken as to which material to be sampled, how to sample it and how often to sample it so that the interpretation from the database can be made with the utmost certainty. Once the variability has been adequately assessed, farmers should follow agronomic inputs to know the conditions using management recommendations. That's a very specific app control system. We can use technology effectively, in managing site-specific variations. When taking soil plant samples, we should be aware of site linking samples and further we can apply the same to managers. This leads to better use of the installation and avoids any damage.

The possibility of improved accuracy in soil fertility management combined with increased accuracy in application management makes direct soil fertility management more attractive, but some are not allowed in the same field management. For effective use, the concept of accurate soil management requires that, among the diversity of the field there is also a direct and reliable identification and impact on crop yields, crop quality and environment. The higher the dependence on the material world, the greater the power of precision and the greater its potential value. The level of difficulty, however, increases as the temporary part of the local variation grows. Incorporating this hypothesis into soil fertility may support the fact that the regeneration of phosphorus and potassium is very helpful in precise management because temporary variability is low. In N, the temporary fraction of variability can be greater than part of its area, making direct management of N difficult in some cases. There are three key issues with regard to accurate agricultural assessment: economic transfer, environment and technology. The most important fact regarding the analysis of profitability of precision farming is that the value comes from the use of data and not from the use of technology. Potential improvements in environmental quality are often cited as a reason for using sustainable farming. Reduced agrochemical use, nutritional efficiency, increased exploration of how management skills and environmental factors affect plant production (Stombaugh and Shearer, 2000). This assessment provides a straightforward and important answer for the farmer that enables them to make better management decisions (Peletier and Upadhyaya, 1999). Such feedback includes but is not limited to: rapid harvesting and recording of moisture, mapping and moisture mapping, insect-based pest records and annual data planning, farm, field, load and crop. Yield monitoring over time creates a separate GIS database that helps farmers easily identify yield differences within the field, make better pricing decisions, and build local data history. This technology is still being processed and sold to other crops such as potatoes, onions, beetroot, tomatoes.

Variable Rate Technology (VRT)

Variable rate technology (VRT) is used to adjust agricultural inputs according to the specific needs of the site in each part of the field. When machines are used, this requires flexible measuring equipment. On small farms, inputs can be used manually. Fixed price requests require: (a) Correct field position; (b) Appropriate location information; and (c)

Farm equipment is equipped with VRT controls with a DGPS receiver to pinpoint the exact location of the field variance and automatically control the application rate based on pre-obtained input plan maps. There are various uses of VRT technology in the management of location-specific prevention systems. Flexible installation equipment is probably the most widely used technology for agricultural accuracy. About 1,600 flotation fertilizer systems, variable-rate technology (VRT) systems, and on-site tractor systems have been sold.

Nutrient use efficiency (NUE)

"Nutrient use efficiency is a measure of how well plants use the available mineral nutrients. It can be defined as yield (biomass) per unit input (fertilizer, nutrient content). NUE is a complex trait: it depends on the ability to take up the nutrients from the soil, but also on transport, storage, mobilization, usage within the plant, and even on the environment. NUE is of particular interest as a major target for crop improvement. Improvement of NUE is an essential pre-requisite for expansion of crop production into marginal lands with low nutrient availability but also a way to reduce use of inorganic fertilizer" (Hawkesford *et al*., 2016). Therefore, efficient management of precision tools are effective to supply plant nutrients judiciously and enhance nutrient use efficiency.

CONCLUSION

Nutrient management through SSNM practices include the use of optical sensors, chlorophyll radiation, nutrient Expert(NE) and leaf color chart (LCC), process abandonment of models and plant models to assist in the utilization of essential nutrients and thus improve nutrient use efficiency while achieving high yields. Real-time nitrogen management method works best practice for rice and maize, but a real-time combined method that includes preventing nitrogen fertilization such as basal or previous fixed growth stages to prevent nitrogen fertilizer deficiency of nitrogen fertilizer schedule with LCC, SPAD or optical sensor - control of nitrogen fixation appears to be effective. NE software requires some easily available information (even without soil test values) from a farmer or local expert in the real time.

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RESEARCH ARTICLE

Haematobiochemical Alterations Associated with Parvoviral Enteritis in Dog

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ABSTRACT

Canine parvovirus infection is an important pathogen of domestic and wild canids and is prevalent across the globe. It has been affecting the dogs in India after the introduction of exotic breeds in the country. The present study was conducted to establish the haematobiochemical alterations in parvovirus infected puppies. About ten infected puppies and ten healthy puppies were taken understudy in and around Bhubaneswar, Odisha. All the infected puppies were confirmed for parvovirus infection. All the infected puppies were recorded with fever, vomition, loss of appetite, severe diarrhoea with frank haemorrhage and dehydration. The haematological alteration in infected puppies shows leukocytopenia, neutrophilia and lymphopenia. Anaemia was recorded with lower mean values of Haemoglobin, Packed cell volume, total erythrocyte count. The biochemical alterations include a decrease in mean blood glucose, total serum protein, albumin and globulin whereas mean values of the A/G ratio was found to be increased. The haematobiochemical indicators can be an aid to diagnosis followed by early detection through PCR. The vaccine efficacy must be evaluated in endemic areas and infection must be controlled through culling of carriers.

Key words: Puppies, Parvo, Odisha, Anaemia, diarrhoea

INTRODUCTION

Different breeds of dogs have been fashioned out of all pet animals. The increasing trend of People's attachment with dogs both emotionally and socially has laid down the incidence and prevalence of various infectious diseases causing mortality. The reason for the prevalence might be due to the introduction of exotic breeds into different countries. During the last decades, a number of exotic breeds have been introduced in India, where the incidence of Parvovirus infection has become promising for dog's survivability since it causes serious clinical complications. Though commercial vaccination is going on effectively in the country, still several cases are being reported every year. Transmission occurs through the consumption of faecal contaminated objects/food/water. A mostly young puppy under six month is prone to the infection. The common clinical signs include vomition, fever, dehydration and bloody diarrhoea in severe cases. Mostly crossbreds are less susceptible than purebred dogs (Nandi and Kumar, 2010). Several diagnostic techniques have been developed for the detection of infection (Desario *et al*., 2005). But it is not always possible due to the cost, time and sophisticated equipment involved in it. During infection, several haemato-biochemical alterations occur which might be an aid to early diagnosis. Hence the current case study was done through identification of Parvovirus from faeces through PCR and to study the haemato-biochemical alterations in infected dogs in Bhubaneswar.

MATERIALS AND METHODS

A total of 10 dogs of 5-6 month of age with a history of vomiting and diarrhoea were taken in the study for collection of samples from December 2020 to March 2021. A total of 10 healthy dogs of the same age group were taken as control. The faecal samples were collected through swab from the anus and homogenized in PBS and kept at -20^{o}C until further use. The sampling was done in and around Bhubaneswar and sample analysis was done at the Department of Teaching Veterinary Clinical Complex and Department of Clinical Medicine, College of Veterinary Sc and A.H., O.U.A.T., Bhubaneswar. Odisha.

Polymerase chain reaction

The PCR reaction was conducted by using primer published by Buonavoglia *et al*. (2001). The reaction volume was 25 µl which constituted 0.5 µl of 2 mM dNTPS (Fermentas), 2.5 µl of Taq DNA buffer A 10X (tris with 15 mM of MgCl2; Genei, Bangalore, India), 0.25 µl of Taq DNA polymerase (5 U/µl concentration, Genei, Bangalore) and finally 5 µl of lysate was added. Then the rest volume was adjusted to 25 µl by addition of nuclease-free water (Genei, Bangalore). The PCR was conducted from faecal samples as described by Mecrarani *et al.* (1996) with a slight change in the procedures.

Haemato-biochemical analysis

The blood samples were collected in EDTA vials for haematological analysis and clot activator vials for biochemical analysis. The haematological analysis was done following standard methods as described by Jain (1986). The biochemical analysis was done following standard methods as described by Reitman and Frankel (1957).

Statistical analysis

The haematological values of affected cattle were compared with that of healthy control. Data was analysed through one-way analysis of variance (ANOVA) with post hoc analysis by Duncan's multiple range tests using SPSS (20). The results are shown as mean ± SE with significance ($p<0.05$).

RESULT AND DISCUSSION

All the ten infected puppies were confirmed for parvovirus infection through PCR by amplifying at a band size of 583 bp. Previously, also 7.9% canine parvovirus infection was recorded by Ramdas and Latha (2005) through PCR. All the infected dogs were having clinical signs like fever, vomition and lastly diarrhoea and dehydration, which has also been recorded by Banja (2002). During the comparative study of haemato-biochemical alterations (Table 1), all the infected pups were anaemic followed by reduced HB, PCV and TEC. The Hb reduction might be due to severe enteritis (haemorrhagic) leading to blood loss (Mohan *et al*., 1993). The reduction in PCV might be due to haemorrhage and loss of blood through vomiting and diarrhoea (Rai *et al*., 1994; Biswas *et al*., 2005). Previously it has been proved that capillaries and intestinal villi of intestine were damaged by CPV (Haskins, 1998) leading to anaemia. All the infected pups were recorded with leucopenia which is in the agreement with (Biswas *et al*., 2005). Also, neutrophilia and lymphocytopenia were recorded in infected pups. Previously neutrophilia has been recorded by Ramprabhu *et al*. (2002) and lymphopenia has been recorded by McCandlish (1998). This might be due to the concurrent bacterial infections and virus replication in the lymphoid organs respectively (Biswas *et al*., 2005).

During the study, a low blood glucose level was recorded, which might be due to exertion and starvation (Greene, 1984). Also, hypoproteinemia was recorded, which might be due to the leakage of serum protein through damaged capillaries of the villi of the intestine and also due to less absorption through the damaged villi (Ramprabhu *et al*., 2002; Biswas *et al*., 2005). Hyperalbuminemia, hypoglobulinemia and an increase in mean A/G ratio recorded in affected puppies. Previously Greene (Greene, 1984) has reported that hypoalbuminemia is more specific in canine parvovirus enteritis whereas hypoglobinimia is more common in myocardial and occasionally the gastroenteritis form of the disease.

SUMMARY

In dogs, canine parvoviral infection was manifested by fever, vomition, loss of appetite, severe diarrhoea with frank haemorrhage and dehydration. The PCR diagnostic method is a confirmatory diagnosis for canine parvovirus infection. The haematological alteration in infected puppies shows leukocytopenia, neutrophilia and lymphopenia. Anaemia was recorded with lower mean values of Haemoglobin, Packed cell volume, total erythrocyte count. The biochemical alterations include a decrease in mean blood glucose, total serum protein, albumin and globulin whereas the mean values of A/G ratio was found to be increased.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, Director, TVCC, HoDs, Department of Clinical Medicine, College of Veterinary Sc and AH, OUAT, Bhubaneswar, Odisha for providing the necessary facilities to carry out the present investigation. The authors are thankful to VC, Registrar and Dean, MSSSoA for giving permission for designing and conducting the study. The VAS and Para-vets, Govt of Odisha were duly acknowledged for helping in sample collection and pet owners were duly acknowledged for allowing the same.

CONFLICT OF INTEREST

The authors declare no conflict among them.

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Table-1: Haemato-biochemical parameters of Parvovirus affected and healthy puppies

Parameters	Healthy control group	Infected group
Hb (gm/dl)	10.23±0.17	8.12±0.55*
PCV%	46.1±0.88	34.42±1.36**
TEC ($x10^6\mu l$)	6.9±0.22	4.63±0.27**
TLC($x10^3\mu l$)	11.37±0.71	8.68±0.27*
Neutrophil %	68.4±1.13	73.2±2.03
Lymphocyte %	29.2±1.37	23.6±1.94*
Eosinophil %	1.1±0.14	0.5±0.13
Monocyte %	0.6±0.11	1.7±0.17*
Basophil %	1.2±0.15	1.1±0.15**
Glucose (mg/dl)	79.1±2.82	40.1±1.25**
Total serum protein (gm/dl)	6.32±0.017	4.28±0.096
Serum albumin (g/dl)	3.81±0.035	3.09±0.027*
Serum globulin (g/dl)	2.57±0.06	1.25±0.051**
A/G ratio	1.47±0.02	2.48±0.07**

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RESEARCH ARTICLE

Design and Implementation of An Efficient Flash-Type Analog To Digital Converter

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ABSTRACT

The performance parameters like efficiency and speed have major role in deciding the overall performance of ADCs. The generalized flash type ADC is one of the fastest ADC compared to other type. But the flash-type ADC efficiency is degraded due to mismatch of resistors as it uses number of resistors depending upon the size of ADC. This paper presentsan efficient flash-type ADC by replacing conventional resistor with memristor which are tunable to solve the issues of resistor mismatch. Design and Simulation results are carried out in CADNCE to validate the performance of the proposed design compared to conventional flash type ADC.

Keywords: ADC, Flash Type, CADENCE, Memristor

INTRODUCTION

The actual behavior of analog to digital converters cannot be determined from the no. of output bits. As the analog to digital converter uses the operational amplifiers the gain and offset values of the op-amp are also have major role in deciding the overall performance of the ADCs. Similarly other characteristics such as resolution, linearity, signal to noise ratio, bandwidth also have effect on overall performance of ADCs. Hence while designing any ADCs these performance parameters to be verified carefully [1]. Among different type of ADCs provides faster operation as compared to other types of ADCs [1-3]. However as the flash type ADC uses no. of resistors in the design process as shown in Fig.1 the performance of the ADC affected due to mismatch of resistors. Hence in this paper an attempt is taken to replace the conventional resistors by memristor as they are tunable in nature [7-8]. Memristor is a memory resistor which has the can remember its state history. It is a two terminal component and its resistance depends on the magnitude of the applied voltage and polarity hence it's tunable. As flash type ADC suffers from resistor mismatch by using the memristor based resistors the mismatch can be improved by tuning it [14],[15]. As memristor

have several advantages such as easy to interface with CMOS, storage capability, less heat generation and reliability design of flash type ADC with memristor makes overall performance of ADC better. [4-6].

DESIGN OF PROPOSED ADC:

From Fig.1 it can be seen that the conventional flash type ADC uses fixed resistor. Hence when there is mismatch in resistor ADC characteristic changes. Hence in the proposed design the fixed resistors are replaced by memristor which are tunable as its resistance can be tuned with applied voltage to it. Fig.2 shows the design of proposed flash type three bit ADC with memristor. The design if converter consists of series of switches (S_1 to S_{28}), memristor (M1 to M7), comparators and 7:3 encoder. In the proposed work attempt is taken to design individual components i.e., memristor, comparator and 7:3 priority encoder.

The design is made such that the converter can work both in functional and tuning mode by choosing proper switching positions. The individual memristor can be tune by switching on the two switches that connect the memristor with ground and VTUNE. By changing the memristor state variable the initial resistance can be modified. If the state variable is selected as zero then the memristor is on and offers resistance. If the state variable is chosen as one then the memristor is off state and offers off resistance. The reference voltage can be less than threshold value which changes the states (on/off) memristor.

DESIGN OF MEMRISTOR

The design of memristor is basically done by considering the value of memristor that can be switched to different resistance states based on the voltage applied at different time to the memristance material. The relation between voltage and current of memristor offers a linear graph with slight deviation results in hysteresis. [9-12]. A memristor model is designed using cadence tool by using Veriog A Code and an approximate model is obtained. The block of the memristor model is shown in fig. 3

DESIGN OF TWO STAGE OP-AMP

As the two-stage op-amp offers high gain and output swing with good bandwidth, for comparator operation, a 2-stage op-amp is designed as shown in the Fig.4. Transistors M_1 to M_4 constitute the differential (Stage-I) stage of the amplifier. Gate terminals of M_1 and M_2 are the inverting and non-inverting inputs of the operational amplifier. Transistor M_3 and M_4 forms a current mirror circuit to offer better CMR [13]. Second stage of amplifier constituting transistors M_5 and M_6 forms a common source amplifier to provide the additional gain. The gain is determined by the product of transconductance of M_5 and effective resistance of the load. Here transistor M_5act as load and M6 as driver. The biasing of the amplifier is obtained as transistor M_6 and M_7 sink some amount of current depending upon gate to source voltage. Transistors M5 is biased by V_{GS} provided by the current mirror load.

DESIGN OF PRIORITY ENCODER

Following table shows the truth table of a 8:3 priority encoder. It has 8 bit input and 3 bit output. Boolean expressions for individual outputs Q_0, Q_1, Q_2 is obtained as below and using the expressions the circuit for priority encoder is designed using cadence tool.

$$Q_0 = \sum (\sim D6\,(\sim D4 \sim D2\,D1 + \sim D4\,D3 + D5) + D7)$$

$$Q_1 = \sum (\sim D5 \sim D4\,(D2 + D3) + D6 + D7)$$

$$Q_2 = \sum (D4 + D5 + D6 + D7)$$

RESULTS AND DISCUSSION

TWO STAGE OP-AMP FOR COMPARATOR OPERATION

The schematic of two-stage op-amp is designed in Cadence by using virtuoso tool with 180nm technology. The width and length of the transistors is set as below to achieve the better internal gain of the op-amp. Transistor M_1 and M_2 length and width are kept as 1µm and 8µm respectively. Transistor M_3 and M_4 is set to 10µm and 8µm, transistor M_6 is set to 8µm and 12µm. Transistor M7 is set to 8µm and 6µm and M8 is set to 8µm and 1µm. Fig.5 shows the schematic design of two-stage op-amp in virtuoso using 180nm technology.

As the op-amp is designed for performing comparator operation the schematic designed in Fig.5 is converted to symbolical representation and by applying two different voltages (V_{sin} and V_{dc}) at the differential input stage the comparator operation is verified as shown in Fig.6.a. The frequency response of the designed two stage amplifier is also obtained as shown in Fig. 6.b and the gain of the amplifier is found to be 36dB and bandwidth is about 100 KHz.

PRIORITY ENCODER

The output expressions for priority encoder are:

$$Q_0 = \sum (\sim D6\ (\sim D4 \sim D2\ D1 + \sim D4\ D3 + D5) + D7)$$

$$Q_1 = \sum (\sim D5 \sim D4\ (D2 + D3) + D6 + D7)$$

$$Q_2 = \sum (D4 + D5 + D6 + D7)$$

Using above expression schematic of priority encoder is designed in cadence using virtuoso tool and the corresponding top module is obtained as shown in the Fig.7. The Functional simulation results of priority encoder is shown in Fig. 8.

DESIGN OF FLASHTYPE ADC WITH MEMRISTOR

After design and verification of individual blocks, all the blocks has been integrated to obtain the flash type ADC with memristor as per the block diagram of flash type ADC which was described in previous section. Fig.9 Shows the integration of individual blocks to obtain the schematic design of Flash type ADC with memristor using virtuoso tool. Simulation results shown in fig.10 validates the design of proposed ADC which converts the analog input of 3V and its corresponding digital output is obtained as 111. An comparative analysis of the conventional flash type ADC and proposed flash type ADC is done after getting the power and energy report which is shown in fig.11

CONCLUSION

In this paper design and implementation of memristor based flash type ADC is presented. The problem of mismatch of resistors in conventional flash type ADC has been solve by replacing fixed resistor by the memristor as memristor resistance value can be tuned as per the requirement to avoid the mismatch. Simulation results of individual blocks have been presented to validate the proposed design. After comparing with the conventional flash type ADC The overall power of the proposed ADC was found to be 21.8mW which is much less than the conventional flash type ADC.

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Table:1 Truth Table of Priority Encoder

DIGITAL INPUTS								BINARY OUTPUTS		
D_7	D_6	D_5	D_4	D_3	D_2	D_1	D_0	Q_2	Q_1	Q_0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	1	X	0	0	1
0	0	0	0	0	1	X	X	0	1	0
0	0	0	0	1	X	X	X	0	1	1
0	0	0	1	X	X	X	X	1	0	0
0	0	1	X	X	X	X	X	1	0	1
0	1	X	X	X	X	X	X	1	1	0
1	X	X	X	X	X	X	X	1	1	1

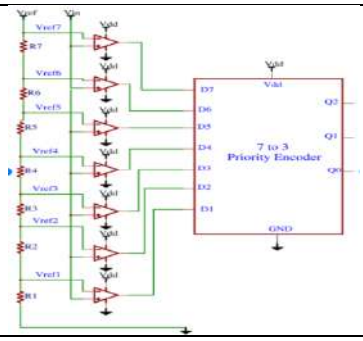

Fig.1 Conventional Flash type ADC

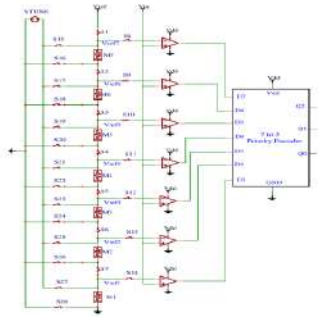

Fig.2 Proposed Flash type ADC

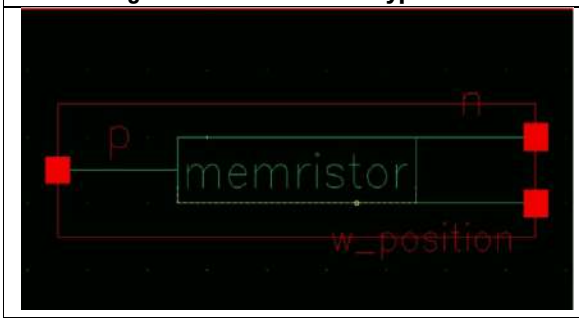

Fig.3 Memristor Model in Cadence using Veriog A Code

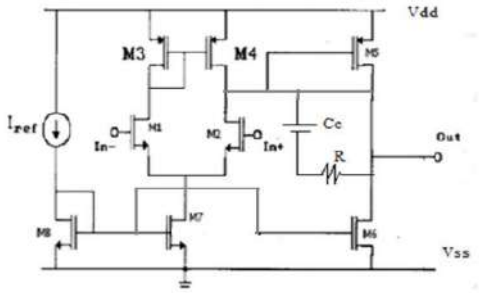

Fig.4 Design of two Stage Operational Amplifier

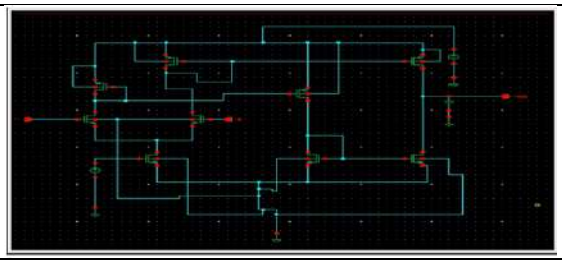

Fig. 5 Schematic Design of Two-Stage OP-AMP

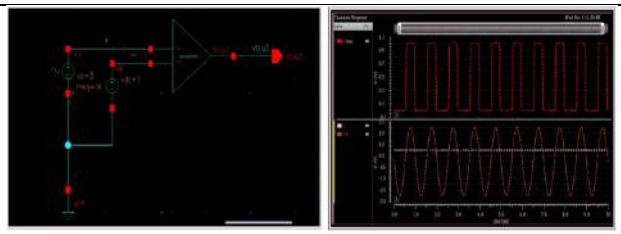

Fig. 6.a Two-Stage OP-AMP as Comparator and its Simulation

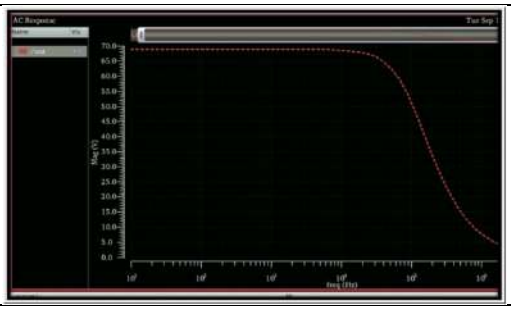

Fig.6.b Frequency Response of Two-Stage OP-AMP

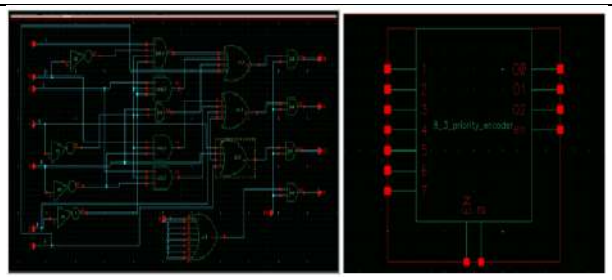

Fig.7 Schematic Design of Priority Encoder and Its Top Module

Fig.8 Priority Encoder Simulation Result

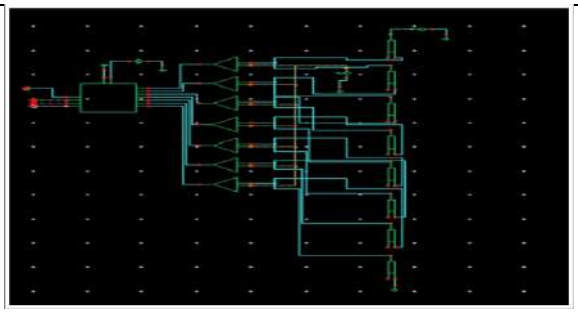

Fig.9 Schematic Design of Flash Type ADC with Memristor in Cadence

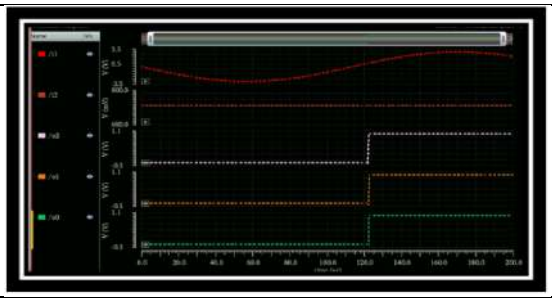

Fig.10 Simulation Result of Proposed ADC

Fig.11 Comparison between conventional Flash Type ADC and Memristor based flash ADC

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REVIEW ARTICLE

Nickel in Soils: Its Distribution and Impacts- A Review

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ABSTRACT

Nickel is trace mineral as essential nutrient group. Nickel uniformly distributed in the soil profile and typical soil nickel contents vary widely based on the parent rock, with elevated levels at surface soils been associated with soil- forming processes and anthropogenic contamination principally ascribed to agricultural and industrial activities. Major concern for the impact and distribution of nickel in soils arises apparently from the role of soil as an ultimate sink for heavy metals and the consequence transfer through the food- chain to crops, fruits and vegetables grown in contaminated soils and their possible consumption by animals or humans. Furthermore, in this review the role of nickel as a nutritional trace element for some animals, micro- organisms and plants, and its implicative deficiency or toxicity symptoms that may arise from the presence of too little or too much nickel is also of interest.

Keywords: Nickel, heavy metal, soil, nutritional element.

INTRODUCTION

Nickel was first isolated from the mineral niccolite by a Swedish Chemist Axel Crostedt in 1751. The name "Nickel" was derived from the term „Kupfenickel" which means „Old Nick's Copper' that the German miners gave to niccolite because of its emission of toxic fumes when heated (Kotov and Nikitina, 1996). Naturally, nickel occurs widely in the environment, being released through both natural and anthropogenic sources, but seldom in its elemental form (DEPA, 2005a; Cempel and Nikel, 2006). Nickel"s natural source to the environment include forest fires and vegetation, volcanic emissions and wind - blown dust, while, the anthropogenic activities resulted in atmospheric accumulation of nickel from combustion of coal, diesel oil and fuel oil, the incineration of waste and sludge as well as, from miscellaneous sources (HC,1991; Clayton and Clayton, 1994; McGrath, 1995; Von,1997). Application of some phosphate fertilizers are also important sources of nickel into environment as pollutants (Kabata- Pendias and Pendias, 1992). Nriagu (1990) reported that industrial emissions of nickel amount to more than 100 times that from natural sources.

Nickel is the 24 th most abundant metal in the earth‟s crust and 5 th most abundant element by weight after iron, oxygen, magnesium and silicon, constituting about 3% of the earth composition. It is a member of 1 st row transition series and belongs to group 10 of the periodic table with other group members that include palladium, platinum and darmstadtium. In its elemental form, nickel is silver- white in colour, hard and lustrous, but in powdery form, it is reactive in air and ignites spontaneously (ATSDR, 2005). It has a density of 8.9 gcm-3, a melting point of about 1455°C and a boiling point of 2732°C. Its relative atomic mass is 58.71 and atomic number of 28. Nickel usually exists in the 0 and +2 oxidation states, but less frequently in the -1, +1, +3 and +4 oxidation states. Nickel possesses high electrical and thermal conductivities; hence, it is resistant to electrical erosion, oxidation and corrosion at temperatures of -20 to +30°C (Coogan et al., 1989; WHO, 1991, Chau and Kulikovsky- Cordeiro, 1995; Higgins, 1995).

APPLICATIONS AND USES

Nickel has many industrial uses such as coinage manufacture, electroplating, nickel alloy production, spark plug and other ignition devices, as well as, electrical resistance heaters and batteries (Kabata- Pendias and Mukherjee, 2007; Kotov and Nikitina, 1996; WHO, 1991). Nickel alloys and nickel plated objects have been widely used in the production of bathroom fittings, kitchen and tableware, consumer white goods and in food processing, as well as in the manufacture of cables and wires, fasteners, motor vehicles, jet turbines, ship building, surgical implants and textiles (Kasprzak, 1987; USPHS, 1993; DEPA, 2005).

The most significant nickel ore are pentlandite; nickel – iron sulphide and garnierite as well as, nickel – magnesium silicate. Nickel is a relatively abundant and naturally occurring metal, widely distributed in the earth‟s crust. Its status in soils is highly dependent on the nickel concentration of the parent rocks, but in surface soils, its content is also a reflection of soil- forming processes and pollution (Kabata- Pendias and Pendias, 1992; McGrath, 1995). The lowest contents are found in sedimentary rocks that comprise of clays, limestones, sandstones and shales, while the highest concentrations exist in basic igneous rocks (Kabata- Pendias and Mukherjee, 2007).

Nickel content in soils varied widely and have been estimated to range from 3 to 1000 ppm; for the world soils, the brand range is between 0.2 and 450 ppm, while the grand mean is calculated to be 22 ppm (Kabata- Pendias and Pendias, 1992; Cempel and Nikel, 2005; Bencko, 1983; Scott-Fordsmand, 1997). Duke (1980a) also reported an average concentration of 86 ppm for the natural nickel content in the earth‟s crust. Values representing the contamination level of nickel in rural soils of the world for various countries have been reported by Chen et al., (1999); Australia (60 ppm), Canada (150 ppm), China (20 ppm), France (50 ppm), Germany (200 ppm), Japan (100 ppm) Netherland (210 ppm), South Africa (15 ppm), United kingdom (60 ppm), and United State of America (420 ppm). Industrial waste materials, lime, fertilizer and sewage sludge constitute the major sources of nickel into soils (McIlveen and Negusanti, 1994). Moreover, nickel is apparently a heavy metal of environmental concern only in urban cities, but could become a problem resulting from decreased soil pH, due to reduced use of soil liming in agricultural soils and mobilization arising from increased acid rain in industrialized areas (Bencko, 1983; Cempel and Nikel, 2005). With decreasing pH, the solubility and mobility of nickel increases, hence, soil pH is the major factor controlling nickel solubility, mobility and sorption, while clay content, iron- manganese mineral and soil organic matter being of secondary importance (Anderson and Christensen, 1988; Ge et al., 2000; Suavé et al., 2000; Tye et al., 2004). Generally, the distribution of nickel in soil profile is uniform, with typical accumulation at the surface soil due to deposition through anthropogenic activities (Cempel and Nikel, 2005). Nickel can also exist in several forms in soils that include; adsorbed or complex on organic cation surfaces or on inorganic cation exchange surfaces, inorganic crystalline minerals or precipitates, water soluble, free-ion or chelated metal complexes in soil solution (EHC, 1991; Bennett, 1982). In the presence of fulvic and humic acids, the complexes are much more mobile, and may be prominent than the hydrated divalent cation in soil solution (ATSDR, 2005).

Transmission through the food chain

Nickel is often mobile in plants, and accumulates readily in plant leaves and seeds (Welch and Cary, 1975), thus, having a high potential to enter the food chain. Therefore, the uptake of nickel by plants is related to its toxicity, which may have possible implications with respect to humans and animals through the food chain.

Nickel in plants

Generally, nickel is not an important element for plant growth and development, but it is an essential micronutrient required for the growth of higher plants (Brown et al., 1987). The phyto availability of nickel has been correlated with free nickel ion activity in soil solution; hence, plant uptake is also dependent on soil pH, organic matter content and iron- manganese oxide (Massoura et al., 2006; Rooney et al., 2007; Ge et al., 2000). Environment Agency (2009e) documented that nickel from anthropogenic sources is more readily taken up by plants than that from natural occurring sources, and that plant species also differ in their tolerance and ability to take up nickel from soils. Nickel toxicity levels vary widely between 25 to 50 ppm (Mishra and Kar, 1974). However, Gregson and Hope (1994) reported that the phytotoxic concentrations of nickel occurred at leaf contents of 10 to 100 ppm depending on the plant species, while, Kabata- Pendias and Pendias (2001) reported phytotoxic range of 40 to 246 ppm DW plant tissue, depending on the plant species and cultivars. The most common plants that have been identified for their tolerance to and hyper accumulation of nickel include cabbage, cauliflower and turnip as well as, leguminosae such as bean and pea (Kabata- Pendias and Mukherjee, 2007). Moreover, Uren (1992) found that besides inhibiting plant growth, other symptoms of nickel toxicity include chlorosis, stunted root growth and brown interveinal necrosis.

Nickel in animals and humans

Nickel is an essential nutritional trace metal based on its deficiency in several animal species, but its functional importance has not yet been clearly documented. In humans, however, nickel˝s role as a trace element has not been recognized nor its deficiency (Scott-Fordsmand, 1997). Dermal absorption of nickel through human skin is quite very limited, and its uptake from soil is rather fewer. Moody et al. (2009) studied an in vitro dermal absorption of radioactive nickel chloride through human breast skin for a period of 24 h with and without a spiked reference soil; the obtained results revealed a mean dermal absorption of 1% with soil and 23% without soil presence. Further studies showed that most nickel applied as a soluble salt is bound within the skin and does not reach systematic circulation (Hostynek et al., 2001; Turkhall et al., 2008), hence, nickel allergy in the form of contact dermatitis is a very common and well- known reaction in animals and humans, and is related to nickel induced hypersensitivity and skin disorders (Samitz and Katz, 1976; USEPA, 1986).

Food intake is the major route of nickel exposure for the general population, while inhalation from air, drinking water, oral and dermal routes could serve as secondary sources of nickel exposure. Nickel naturally occur in foodstuffs at a general range of 0.1 to 0.5ppm, but few number of foods usually obtain nickel during the manufacturing process or through food processing methods that may arise from leaching from stainless steel, the milling of flour or through the catalytic hydrogenation of fats and oils (Clarkson,1988; Solomons et al.,1982). Flyvholm et al., (1984) reported increase of nickel intake of up to 900 µg/ person/ day or more on large consumption of rich food sources of nickel that include dark chocolate and soya products, dried beans and peas, as well as oat meal. The toxic effects of nickel result from its ability to replace other metal ions in enzymes, proteins or bind to cellular compounds (Cempel and Nikel, 2005), and among animals, micro-organisms and plants, nickel is reported by Nielsen (1980a) to interact with at least 13 essential elements namely calcium, chromium, cobalt, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium and zinc. Therefore, prolong exposure to oxides and sulphides of nickel is associated with possible risk to lung and nasal tumours, while systematic effects whose initial symptoms are mild nausea, headache, dyspnoea, and chest pain could be ascribed to nickel carbonyl; these symptoms may disappear or consequently results in severe pulmonary insufficiency. Also, arising from exposure to nickel containing mists and dusts are asthma, pneumoconiosis and irritation of nasal membranes (Kabata- Pendias and Pendias, 1992; El- Hinnawi and Hashmi, 1988; Klein and Snodgrass, 1977).

CONCLUSIONS

Although, nickel is appearing in the environment, but its functional role as a trace element for animals and humans is not yet recognized. Therefore, it becomes trace metal of concern because of its major route of exposure which is either through the dietary intake or ingestion through the food chain which may arise principally from the nickel accumulation in crops, fruits and vegetables grown on contaminated soils and their consequence consumption by animals or man.

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REVIEW ARTICLE

Prospects of Millets in Sustaining Food Grain Needs in India and for Nutritional Security- A Review

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ABSTRACT

Millets are the first food grains known to the mankind. They are cultivated in India from the ancient times. Before green revolution millets are having good production in food grains than all the crops. Millets are having good nutritional values and health benefits than rice, wheat and other crops. They are having adequate amount of proteins, vitamins, minerals, essential amino-acids, and antioxidants which fulfills the daily nutritional requirement of human beings. Nowadays, millet production is decreasing because of improper knowledge and inadequate and untimely inputs supply and minimum support price (MSP) for all millets are not provided by the government. Many of the small millets are in endangered and depleting gradually. Millets are considered as the good alternatives in the famine years. With good nutrient management practices under millet cultivation under degraded land can boost the production of the millets. Because of high nutritional value millets can mitigate the malnutrition of the people. High production of the millets can reduce the hunger deaths in the country because of its short growing season and long duration storage. Millets are the best option for sustaining food grain needs and nutritional security in India.

Keywords: millets, adverse environment, diversified uses, nutritive value, health benefits.

INTRODUCTION

Millets are small seeded grasses that are hardy and grown in dry zones as rainfed crop, under marginal conditions of soil fertility and moisture. Due to its high nutritive value millets are called nutricereals or nutrigrains (Banerjee and Maitra, 2020). Millets are unique for their short growing season they complete their lifecycle within 60-65 days. Millets are one of the crops which reduce the carbon footprint in world (Prasad and Staggenborg, 2009; Brahmachari *et al*., 2018). Most of the agricultural foods were not using by the people because of unawareness among them and millets are one out of them. Millets are also used as feed for bird and cattles and they are having many health benefits and nutritious values (Yang *et al.,* 2012). All millets are having three to five times more nutrient content than

rice and wheat. Rice and wheat provide food security while millets give securities like health, food, nutrition, animal feed, livelihood etc(Kimeera and Sucharitha, 2019).In the year 2019-2020, the total food grain area is 1275.9 lakh hectare, production is 296.6 million tonnes and productivity is2325 kg/ha. Rice, wheat, maize, barley, millets comes under cereals. All millets together with maize and barley come under the category of "coarse cereals". The production of the rice and wheat were 118.4 and 107.6 million tonnes respectively and other coarse cereals production is 47.5 million tonnes in the year 2019-2020(Annual report, 2020-21).

STATUS OF MILLET PRODUCTION IN INDIA

India is the largest producer and largest consumer of the millets in the world followed by the Niger, and china. In India, millets are grown in the area of 8-10 million hectares and producing 10-12 million tonnes of millets every year. In India, Rajasthan, Maharashtra, Gujarat, Uttarpradesh, Karnataka are the leading states of millet production whereas Assam and Haryana are the highest millet consumption states in India (FAO, 2018). Sorghum and pearl millet with production of 8.71 and 8.61milliontonnesrespectively are considered as major millets because of having large area under cultivation by the farmers and all other millets come under the category of minor millets. Minor millets are having enormous nutritive values and health benefits but they are endangered and depleting year by year. India is having 124 million hectares of area under the food grain cultivation and among those millets occupied only 29.2 million acres.

CAUSES OF LESS PRODUCTION OF MILLETS IN INDIA

Following reasons are responsible for less millet production in India

- **Lack of awareness of nutrition and therapeutic potential of millets:** In India, most of the people don't know about the nutritive value and health benefits of the millets. As millets are highly nutritious and rich in fibre, protein, essential aminoacids, minerals and vitamins. Millets are having potential to cure diseases like coronary heart diseases, all types of diabetics, heart diseases, liver diseases etc. millets helps in curing obesity, improving bone health, reduces cholesterol.
- **Lack of input subsides and price incentives from public distribution system:** In India, farmers are not getting subsidies for the inputs like seeds, fertilizers and farm machineries in proper time to produce the millets so many are not interested in millet production. Farmers are not getting the profitable amount as minimum support price (MSP) not fixed like other crops included in the public distribution system (PDS).
- **People have less knowledge about millet consumption:** Before green revolution, millets are made around 40% of total food grains which is more than the rice and wheat. But after green revolution, use of synthetic fertilizers and use of hybrid seeds mainly in rice doubled its production and tripled the wheat production and millet production decreased gradually. Millets are the old grains known to mankind but now the population has very less knowledge about the millets and it uses. Millets are mostly using in rural areas and year by year some millets are getting depleted.
- **Mostly grown as rainfed crop under poor and marginal soils:** In India, millets are mostly grown as the rainfed crop in hilly regions because of its drought tolerant capacity. If Millets will grow in good soils under irrigated areas the productivity will increase compared to other crops.
- **Apart from maize other coarse cereals are not remunerative:** In India, coarse cereals include maize, barley and all the millets. Maize is having largest area, production and productivity among all the coarse cereals. Farmers are getting good amounts for maize compare to barley and all millets.
- **Processing is too long and slow process:** Millets are processed before consuming and for preparing of food. Millets are mostly processed in traditional methods than in machines because millets are highly grown in rural areas and hilly areas. In this are as most of them comes in category of small farmers and they can't afford processing machinery. Millet processing is very long and slow process consists of decorticating, malting, fermentation, roasting, flaking, and grinding.

- **Farmers are growing old traditional varieties:** Compared to other crops millets are having very less number of hybrid varieties. In India, most of the farmers are growing old and traditional varieties of millets. They are not having knowledge about the hybrid varieties of millets and there high yielding properties.
- **Poor nutrient management:** In India, most of millets were grown with no and little fertilizers. Proper nutrient management and judicious application of the fertilizers to the millet crops can boost their production and give good yields.
- **Millet eating habit among the young generation is poor:** Nowadays most of the people are addicted to fast and junk foods by which they are not fulfilling their nutritional requirements. For nutrient requirements most of people and sportsperson were choosing for high protein and low carbohydrates foods like oats, chicken, etc. but nobody is selecting the millets which are having high nutritious value and enormous health benefits.

DIVERSIFIED USES OF MILLETS

- Millets are used as the food for human's consumption, feed for cattle and birds.
- Millets are having enormous health benefits and high nutritive value by which humans can fulfills their daily nutrition requirements.
- Millets are good source of calcium, magnesium, and iron. Millets contain high amount of minerals, vitamins and essential aminoacids, essential fatty acids, phyto-chemicals and antioxidants.
- Millets are specifically anti-diabetic and anti- obesity and plays an important role in the immune system of human body.
- Millets are also used in production of alcoholic beverages, Biofuel and fermentation industries.
- Millets can be stored safelyup to two or more years.
- Millets are often the only cereal crops that grown in arid lands.
- Millets survive better than the other crops in drought condition.
- Millets are the best alternative in famine years.
- Millets needs very less water and less annual rainfall of 350-450 mm compared to the crops.
- Millets has greater crop diversity on farm and it reduces the pest, climate risks and improves farmers over resilience.
- Milletscultivation can keep dry lands productive and ensure future food and nutritional security.

HEALTH BENEFITS OF MILLETS

Millets are having many therapeutic properties which help in preventing health problems such as cardiovascular diseases, lowering blood pressure, decreases tumors, risk of heart diseases, lowering blood pressure, and prevention of cancer, and increase gastric emptying (Gupta *et al*., 2012). Millets are gluten free, these are good choice for people suffering from celiac diseases and patients who are suffering from gluten sensitive by gluten content of wheat and other common cereals grains (Saleh *et al*., 2013). Obesity is major problem in India associated with chronic diseases including diabetics and cardiovascular disease, taking high fibre diet reduces obesity (Alfieri *et al*., 1995). By eating millets blood sugar level and dermal wound healing will controlling by the antioxidants present in it (Rajasekaran, 2004). Millets with high fibre helps in reducing constipation, bloating, stomach cramping with good absorption and digestion (Reddy, 2017). Millets are good in magnesium which reduces heart attacks and rich in phytochemicals which contains phytic acid helps in preventing cardiovascular diseases and lower cholesterol by reducing plasma triglycerides (Lee *et al*., 2010). Phenolics present in millets are effective in progression of in-vitro and prevention of cancer initiation (Chandrasekara and Shahidi, 2011).

Sorghum

Sorghum has many health benefits it can cures the diseases like obesity, Diabetics, coronary heart diseases, cancer, celiac diseases and oxidative stress (Dayakar *et al*., 2017).

Pearl millet

Pearl millet is beneficial due to the presence of high amount of magnesium, it helps in treating stomach ulcers, heart health, good for the bone health and development. It reduces the cancer risk, cholesterol, celiac diseases, and helps in diabetics, preventing gall stones and weight loss. Pearl millet is having anti-allergic properties and it contains all the essential aminoacids (Dayakar *et al*., 2017).

Finger millet

Finger millet has 1.4% of soluble dietary fibre and insoluble dietary fibre of 15.7% (Chethan *et al*., 2007). Finger millets have several health benefits and it is having high content of lysine, valine, threonine and dry weight protein of 8.47g. Brown finger millet has phenolic acid content of 96% which is higher than wheat. Seed coat of finger millet is rich in phenolic compounds and dietary fibre which shows lowering blood glucose and cholesterol. Finger millet helps in losing weight, reduces diabetic risk, anaemia, and improves the bone health (Mathanghi and Sudha, 2012).

Proso millet

Proso millet is having many health benefits and it is rich in vitamins, minerals and essential aminoacids. Pearl millet contains high lecithin which supports the neural health system. It reduces the risk of diabetics and cures the liver diseases (Singh *et al*., 2015).

Foxtail millet

Foxtail millet is rich source of calcium which improves the bone health and good source of fibre and vitamin D (Maitra et al., 2020a). Foxtail millet helps in fight diseases such as osteoporosis and risk of fracture and type-2 diabetics (Singh *et al*., 2015).

Kodo millet

Kodo millet helps in curing the coronary heart diseases, severity of asthma, migraine attacks, atherosclerosis, and heart diseases. It reduces the high blood pressure, diabetics and heart attacks (Singh *et al*., 2015).

Little millet

Little millet contains good amount of magnesium, phosphorus and vitamin B3 (niacin) (Maitra and Shankar, 2019). This helps in fat metabolism, energy production, and body tissue repair. Little millet improves the heart health, lowers the cholesterol and decrease the risk of diabetics (Singh *et al*.,2015).

Barnyard millet

Barnyard millet is good source of highly digestible protein and fibre (Maitra et al., 2020). It is low in calories which help in weight loss. Barnyard millet rich in iron and low glycemic food helps in curing diseases like diabetics, tumor necrosis and hyperlipidemia (Singh *et al*., 2015)

PERFORMANCE OF MILLETS UNDER ADVERSE SOIL AND ENVIRONMENTAL CONDITIONS

Sood *et al.,* (2015) stated that barnyard millet has wide adaptability and short lifecycle so it occupies special place in marginal rainfed areas. This crop can survive in harsh condition make better choice in famine years. Barnyard millet is used as substitute for rice in Himalayan regions. Seghatoleslami *et al.,*(2008) examine the response of prosomillet (*Panicum miliaceum*) to drought stress in different growth stages. Results found that two genotypes had highest harvest indices and WUE. Due to infertile soil and soil salinity in Birjand, grain yield was lower compared to sarbisheh. By this genotype K.C.M-4 proved more suitable for both areas. One experiment was conducted by Niu *et al*. (2017) on semi-arids of north-east of china and observed that application of plant growth promoting rhizobacteria (PGPR) to agro-ecosystem improves the plant growth of foxtail millet in the drought condition and serves as good bioinoculant in arid regions for sustaining production. Han *et al.* (2019) studied the drought tolerance of foxtail millet seedlings by the physiological and molecular changes that are induced by SO_2 fumigation. Results indicated that enhancement of drought tolerance by increasing proline accumulation, promoting antioxidant defence and reducing

stomatal apertures by application of SO_2 in finger millet seedlings. Patil *et al*. (2016) experimented on the new released little millet variety GNV-3 during *kharif* season on Gujarat hilly regions as rainfed crop over the local check variety GNV-2 and national check variety CO-2. Results found that new variety recorded 8.77% (2864 kg ha^{-1}) more grain yield than local check variety GNV-2 (2633 kg ha^{-1}) and 43.92% (1990 kg ha^{-1}) over national check variety CO-2. Rani *et al*. (2017)reported that OLM-203 variety of little millet recorded highest grain and straw yield with the application of RDF 100% + neem cake @ 1t/ha treatment and micronutrient (Zn and Fe).

Nadeem *et al.* (2020) reported that foxtail millet is well responding to the low nitrogen and low phosphate by reduction and enhancement of its root system. Anderson (2020) experimented on delay development of herbicide resistance in semiarid Great Plains. The main objective is controlling of weeds in prosomillet (*Panicum miliaceum*) by using cultural systems, removing selection pressure and eliminating the need for herbicide. Results found that 85% of reductions in pig weed species in both tilled and no-till systems without yield loss of prosomillet. Kalinova and Moudry (2006) were cultivated eight varieties of prosomillet from the year 1998-2000 and 1992-2000. Results showed that protein and amino acid content was influenced by weather during year. Decrease in quality and increase in protein causes during dry conditions. Kumari and Sumathi (2000) observed the effect of finger millet diet on six non-insulin dependent diabetic mellitus subjects. Results found that people who are taking finger millet based diet has low plasma glucose levels it is due to high fibre content, low glycemic response and presence of anti-nutritional factors in finger millet which reduce the starch digestibility and adsorption compared to rice and wheat. Antony *et al*. (1996) mentioned that fermentation of finger millet decreases the starch and long chain fatty acid content and increases the lactic acid 6.5 times and acetic acid content 3.7 times, respectively. These are organic acids produced at the time of fermentation. Barbeau and Hilu (1991) conducted experiment on two wild species and eight domesticated varieties of finger millet on their protein, calcium, iron and aminoacid content. Results indicated that two wild species has significantly higher iron (3.7-6.8 mg/100g), protein (7.5-11.7%), calcium (376-515 mg/100g) and aminoacid content than the eight domesticated varieties.

CONCLUSION

Millets are the high nutritious food by consuming it the people can increase their health benefits and we can decrease the malnutrition problem of the people mainly children's and women's in the country. By cultivation of millets farmers can double their yields and profits by low inputs and cost of cultivation by its drought tolerant capacity and short growing seasons. Processing of millets and making value added products with millets were good idea for the farmers and people living in rural areas to increase their income sources. Millets are mainly grown in the marginal and poor soils and growing millets in good soils with good nutrient management practices can boost their production and ensure the future food needs and nutritional security of India.

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Table 1. Nutritive Value of Millets

Sl. No.	Name of crop	Carbohydrates (g)	Protein (%)	Fat (%)	Crude fibre (%)	Energy (kJ)	Iron (mg)	Calcium (mg)
1	Sorghum	72.6	11	3.2	2.7	67.71	2.6	54
2	Pearl millet	67.5	14.5	5.1	2	62.63	16.9	38
3	Finger millet	72.6	7.3	1.3	3.6	67.55	3.9	344
4	Proso millet	70.4	11	3.5	9	70.04	0.8	14
5	Foxtail millet	60.9	11.7	3.9	7	60.09	2.8	31
6	Kodo millet	65.9	8.3	1.4	9	67.38	0.5	27
7	Little millet	67	7.7	4.7	7.6	66.74	9.3	17
8	Barnyard millet	65.5	6.2	4.8	13.6	65.55	15.2	11

Source: Chandra*, et.al* (2016)

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REVIEW ARTICLE

Management of Weed Flora in Organic Farming- A Review

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ABSTRACT

In agriculture weed management plays a prominent role in ensuring food and nutritional security. Weeds usually compete with crops for various limiting natural resources determining the growth, yield and quality of crop, respectively. Herbicide based weed management is one of the successful methods of weed control. Continuous use of herbicides resulted in soil and groundwater pollution. Moreover, this also reported in development of resistant biotypes and discouraging sustainability in agriculture. Recently, to attain sustainability and ensure environmental safety organic farming is widely gaining popularity. However, in organic farming the use of synthetic input was principally prohibited. Hence, compelled to develop an efficient alternative approach to tackle the weed population. In this view, this review clearly highlighted the potential of integrated weed management approach excluding the chemical herbicides.

Keywords: Weed management, organic farming, crop rotation, establishment methods.

INTRODUCTION

To feed the rapidly boosting population enhanced the reliability of synthetic inputs,*viz.*, fertilizers, pesticides, herbicides etc. (Mondal *et al*., 2021; Praharaj *et al*., 2021; Shankar *et al*., 2021). However, unprecedented use of these inputs was raising serious environmental concerns (Ghosh *et al*., 2020; Zahan *et al*., 2021). Weeds are the most dynamic and difficult to control pests in nature because of their rich diversity(Petit *et al.,* 2018). In general, weeds compete for different factors for growth,*viz.*,light, space, moisture, nutrients and attributes to achieve poor crop yields, respectively(Lalichetti *et al.,* 2021). Multifaceted mechanisms of action of herbicide conferred efficient control of weeds till date. However, perpetual use of herbicides to achieve higher crop yields resulted in building up of

herbicidal residue in the soil(Curran, 2016). Further, these residues were reported to enter the human and animal food chain in several routes triggering many health issues. Raising health concerns among the individuals gave rise to an avenue of organic farming to achieve agricultural sustainability. Organic farming is an agricultural production system without involving the use of chemical inputs and that completely relies upon natural and environmentally friendly processes (Maitra *et al*., 2020). However, it is challenging to control weeds in organic farming because there is no single effective method till date that is efficient and effective as an alternative to conventional herbicides. Hence there is dire need to understand the potential of cultural, mechanical, physical and biological methods of weed control and integrate them according to their availability and utility at the right time for turning out to be an efficient alternative to herbicides. Keeping this in view, the review throws a light on the potential of different management options that confer competitive advantage to crop over the weeds. Therefore, helps in selection of integrated approach of weed management as an alternative strategy to chemical herbicides.

MANAGEMENT OF WEED SEED BANK

Weed seed rain is the foremost reason for the perpetual presence of weeds during the entire year (Schwartz-Lazaro and Copes, 2019). This process of adding new weed seeds to the existing seed bank in the soil is a continuous process since the human beginning of crop cultivation. Exhaustion of weed seed bank should be the primary principle to control weeds in organic farming (Gonzalez and Ghermandi, 2012). However, this is very challenging as production potential ranges from tens to several thousands of viable seeds, respectively. Usually, weed seed banks are of two types *viz.* soil and aerial seed banks. Building of weed seed banks associated with their persistence makes them more troublesome. In general, out of many weed seeds added in a season few of them germinate and are exposed. However, the remaining either decomposes or persists in the soil (Bakhshandeh and Gholamhossieni, 2019). These persisted weeds resurge back when exposed to favorable conditions in the later period of time. Seed dormancy is an attribute to weed persistence for a longer time in the soil (Milligan *et al*., 2016).

Adoption of ideal tillage practices at a right time especially before flowering repeatedly during a cropping season prevents the weed seed rain (Yang *et al.,* 2018). Further, it helps in exposing the weed seeds stored deep in the soil to the external environment and promote their germination for their effective knock down (Benvenuti and Mazzoncini, 2021). Similarly, the exhaustion potential was enhanced when tillage was adopted along with the stale seedbed method, respectively (Marahatta *et al.,* 2017). Crop seed sanitation was another important parameter to be focused to prevent the addition of new weed seeds while sowing the crop (Kaur and Kaur, 2021). In this regard, the potential of covering the soil with organic and inorganic materials cannot be overlooked as they intercept the addition of new seeds into the soil and prevent the germination of already existing seed in the soil seed bank (Balshor et al., 2017).

ROLE OF CROP ROTATIONS IN WEED MANAGEMENT

Raising the same crop or crops of the same family year-round is reported to create an ideal habitat for specific pests resulting in achieving poor crop yield (Dhaliwal *et al.,* 2015). Growing two or more crops of different families on the same piece of land in a repetitive succession unfavor the establishment of crop associated and parasitic weeds (Radzikowski *et al*., 2020). Crop geometry and duration has a significant role in managing weeds. Those crops which are widely spaced and emerge late are more susceptible to weed infestation (Melander *et al*., 2005). The grassy weeds usually compete with shallow rooted crops while broadleaved weeds compete with deep rooted crops, respectively (Amare *et al*., 2014). Rotation of crops with root systems with varied depths provides an opportunity time and act as a biological pump to replenish all the soil factors (Kristensen, 2006). Crops with spreading habit when adopted in a rotation are more competitive compared to those with erect or bunch habit (Maitra *et al*., 2021). Therefore, crop rotations significantly vary the weed flora on the cultivated field in each season and thus limits the development of a resistant biotype.

PRE-CROP EMERGENCE ORGANIC WEED MANAGEMENT

In organic farming, weed control during initial days of crop establishment can be achieved by three methods *viz.* soil solarization, tillage, mulching stale seedbed method (Khan *et al*., 2012) . Soil solarization is the practice of covering

with a thick transparent polythene sheet over the soil during the hottest months of the year (Sareshet al., 2013). This practice helps in the desiccation of perennial seed material and thermo sensitive seed material of annual crops (Wen, 2015). However, maintaining the soil moisture at field capacity determines the effectiveness of this practice. This was due to the ability of water in thermal conduction. In case of black polythene application over the soil disfavours the conduction of absorbed heat to the soil (Dec *et al*., 2009). However, soil solarization for an extended period of time is reported to have a serious negative impact over the soil biology as well (Khalid, 2012). Henceforth, adoption of soil solarization cannot be recommended as a regular practice in organic farming but can be used in specific cases.

Tillage before sowing of the crop is the most common practice aiming to reduce weed infestation (Błazewicz-Wozniak *et al*., 2016). Tillage inverts the soil upside down and exposes weed seeds buried inside the soil to the external exposure prone to desiccation due to hot sun and bird perching, respectively (Duary*et al*., 2016). Grassy weeds are more sensitive to tillage than broadleaved weeds due to thin seed coat (Nikolic *et al*., 2020). Recently added weed seeds to the soil seed bank will be buried deep in the soil and if it is buried beyond the emergence zone those seeds fail to germinate establishing efficient control over the weeds (Hossain and Begum, 2015). Tillage is one of the most prominent and efficient practices in non-chemical weed management. Stale seed bed is an efficient technique in weed management which helps in controlling weeds at pre crop emergence seeds. In this method the favourable conditions necessary for the germination of the weeds were provided before sowing the crop (Sindhu *et al*., 2011). The germinated flush of weeds was knocked down by tillage for 2 to3 times until the surface weed seed bank is exhausted (Holst *et al*., 2007). Further, weed density during initial stages of crop establishment would be under control.

POST EMERGENCE ORGANIC WEED MANAGEMENT

In organic farming the post emergence management of weeds was usually done by mechanical or botanical methods, respectively. Hand Weeding with the help of a khurpi was one the popular methods of weed control (Lundkvist and Verwijst, 2011). This practice is not specific to a certain crop, it is efficient in any crop. However, hand weeding is laborious in nature hence increases the cost of cultivation (Abouziena H.F. and Haggag, 2016). Hand weeding is efficient for both annual and perennial weeds, respectively. Among several weed management practices, weed management using several novel intercultural devices plays an important role in controlling weed population in the cultivated area (Amare *et al.* 2014). The intercultivation buries the weed seedlings inside the soil which attributes to the addition of organic matter into the soil (Onemli, 2004).

On the other hand, the biological entities also help in controlling the weed population. However, they are very much efficient in controlling alien weeds over others (Young *et al*., 2017). Adaptable natural enemies were usually introduced from their respective countries and checking of weed population was observed, respectively (Singh *et al*., 2013). Other than this, weed specific mycoherbicides herbicides were popularized for efficient weed control, respectively. However, this school of thought is not in practice in India (Chutia *et al*., 2007).

ROLE OF CROP ESTABLISHMENT METHODS IN WEED MANAGEMENT

Crop establishment techniques play an important role in managing weed density in organic farming systems. In general, transplanted crops were superior in weed control over directly sown crops (Chongtham *et al*., 2015). This was attributed due to optimum plant size and maturity of the transplanted seedlings while direct seeded crop the weeds would have competitive advantage over the crop. Moreover, main field could be maintained weed free before transplanting and the seedlings would be around 4 to 6 weeks old in their growth hence could easily withstand competitive pressure. Crop Geometry is another important parameter and recorded more weed density in a square geometry over rectangular. Adoption of rectangular geometry encourages quick coverage of the crop over the field limiting the weed population (Chen *et al.,* 2021). Wider the geometry more is the weed growth. However, inter-cultivation is easier at wider crop geometry (Anbarasu*et al.,* 2018). Hence, in organic farms when crops with wider spacing was adopted, frequent inter-cultivation is recorded to get ideal competitive advantage over weeds. Further, intercropping offers ample scope for weed suppression as more number of plants are grown per unit area (Gitari*et*

al., 2020; Maitra and Gitari, 2021). Similarly, when mulching is adopted during sowing of the crops. It disfavors the weed growth and establishment (Amoroso *et al*., 2010). Mulching can be done by organic or poly mulch materials. However, organic mulches control weeds and also improves the soil physical properties while poly mulch was less influential over soil (Teame et al., 2017).

CONCLUSION

A paradigm shift from herbicide reliant weed management to organic weed management is highly essential to ensure sustainability. Non chemical alternatives were mainly based on a principle to exhaust existing seed banks and prevent the entry of new weed seeds. This review clearly highlighted the potential of non-chemical weed management practices and focused on their right time of integration to match as an efficient alternative in managing crop weeds. However, no single practice could act as a suitable alternative to chemical herbicides. Future researchers should align in this direction to choose right management practices and their integration based on spatial and temporal variation.

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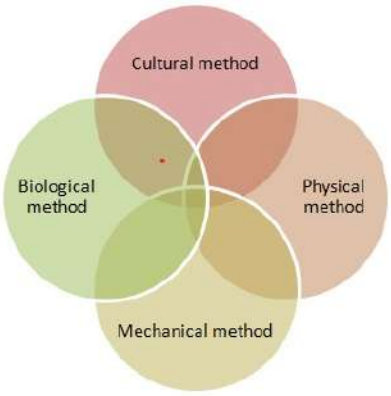

Fig.1: Methods involved in Organic weed management

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RESEARCH ARTICLE

Novel Interventions for Achieving Higher Water Productivity-A Strategic Approach

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ABSTRACT

Water is one of the most important inputs that determine global food production. Scarcity of water resources is challenging to feed a rapidly growing population. Excessive use of water more than its actual recommendation further intensifies this problem. To solve this problem, there is a dire need to enhance water productivity. However, water productivity is majorly influenced by crop type, soil, crop management, economics and associated water losses. As all these factors can be controlled efficiently through proper agronomic management. Henceforth, in this review an attempt was made to discuss the role and potential of different management technologies in improving the crop water productivity.

Keywords: Water productivity, drip irrigation, tillage, mulching.

INTRODUCTION

Globally, rapid increase in population under limited water resources is a growing concern to sustain food security (Liyanage and Yamada, 2017). Yield potential of the crop can be attained with adequate supply of water. Water requirement is essential in all the stages of the crop and water deficit even for a short period of time results in poor plant metabolic functioning and contributes to substantial yield reduction (Biswas and Kalra, 2018). Future insights clearly indicate to bring in additional areas under irrigation to meet the surge in food demand. In this context, the term water productivity is gaining attention.

In general, water productivity is the amount of output received per unit water applied. However, perception of water productivity is scale dependent and changes with the stakeholder (Brauman *et al*., 2013). There are three principles on which water productivity enhancement depends on 1) Increase in economic yield per unit water transpired, 2) Minimize the losses of applied water and 3) Improve the efficiency of water utilization (Prihar *et al*., 2010). Adoption of techniques that satisfy these principles assure water productivity. The amount of water actually

utilized for the attaining crop yield determines the productivity of water (Kumar *et al*., 2021). The amount of water utilized depends on the crop type, cultivar, soil type and management practices (Bryla and Strik, 2007). Proper understanding of all these factors and adopted managerial techniques is a prerequisite for improving water productivity in agriculture.

In the light of above facts this review clearly helps in providing an outlook on various factors associated and techniques involved in agronomic management of crop production to enhance water productivity.

FACTORS AFFECTING WATER PRODUCTIVITY

Crop type

The economical yield of the crop under limited supply of water is determined by the photosynthetic efficiency. The crop type which can assimilate more carbon dioxide during a short period of stomatal opening determines crop water productivity (Lee, 2010). Hence, it was reported that C_4 plants have higher water productivity than C_3 plants, respectively (Wang *et al*., 2012). In a study, maize based cropping system was reported to be have high water productivity over rice based cropping system, comparatively (Devi *et al*., 2020)

Water losses

Minimization of applied water losses is one of the key principles to improve water productivity. Adoption of any method that discourages the leaching, percolation, conveyance or application losses attribute to increase the irrigation water productivity (Moayeri *et al*., 2011).

Soil

Water productivity is also influenced by the soil type and its properties. Soils rich in organic matter or dominated by clay texture retains more soil moisture for a longer time and thereby minimizes the water requirement (Leu *et al*., 2010). Similarly, highly fertile soils confer better growth of the crop and ultimately improves the crop yield even at limited supply of water ensuring higher water productivity (Rashid *et al*., 2016).

Crop management factors

Adoption of any management practice right from selection of cultivar, sowing, nutrient, pesticide, herbicide management etc. till harvesting fitting to changing climatic scenario, that significantly influences the crop growth and development plays a key role in improving water productivity (Jat *et al*., 2011). The adoption of right management practices at the right time provides ideal establishment stands covering the field completely and discourages weed growth ensuring maximum yield with limited water supply (Soni *et al*., 2021).

Economics

Profitability of crops determines the water productivity in economic terms. The adoption of a profitable cropping pattern with minimum managerial costs results in higher economic returns per unit water supplied(Arif and Malik, 2009). For instance, any system that attributes to attain maximum yield with limited water supply and operational costs assures higher water productivity (Islami *et al*., 2012).

TECHNOLOGIES TO IMPROVE WATER PRODUCTIVITY

The technologies involved in improving water productivity focuses mainly on the maximum water uptake through transpiration, efficient use of uptake water in stomatal regulation to allow sufficient carbon dioxide intake and lastly on efficient translocation of photosynthates from source to sink.

Transplanting age of the seedling

In general, the transplanting age of the rice seedling ranges from 25 to 30 days after sowing. However, recent studies indicated that transplanting of younger seedlings of 13 days old reduced the crop duration by 15 days over 27 days old seedlings, comparatively (Li *et al.,* 2020). This might be attributed due to late emergence of tiller from older

seedlings compared to younger seedlings upon transplanting (Pasuquin *et al.,* 2008). This 13 to 15 days reduction in growing period reduces the total water requirement of the crop and thereby improves the water productivity (Singh *et al.,* 2021).

Aerobic Rice

In rice based cropping system adequate availability of water is a key to ensure yield stability. However, in areas with scarce water resources adoption of aerobic rice emerged as a viable alternative. Aerobic rice was observed to save 37 to 45 percent of water over rice cultivated under flooding condition (Kadiyala *et al.,* 2012). Similarly, in another study indicated that due to dry land preparation in aerobic rice recorded low water requirement over conventional transplanting and consequently, improving the water productivity (Sreelatha *et al.,* 2013; Ramulu *et al.,* 2020).

Seed priming

Soaking the seeds in water overnight ensures germination of seeds. Pre-soaking of seeds before sowing results in adequate imbibition of moisture into the seed and initiates germination (Mwami *et al*., 2017). Sowing of pre-germinated seeds in the soil minimizes the water requirement for germination hence saved water and attributed to enhance water productivity (Kumar *et al*., 2018)

Deep tillage

Deep tillage makes the soil rough and improves the soil infiltrability (Abidela Hussein *et al*., 2019). This facilitated enhanced retention of applied water in the soil pores (Sharma *et al*., 2017). In addition, this process promoted the root proliferation deep into soil resulting in efficient uptake of nutrients and water from the soil (Soti *et al*., 2015). This improved water uptake through tillage when associated with adequate carbon dioxide accumulation reported to achieve higher grain yield

Mulching

Covering the soil with organic residues and poly materials improves soil moisture retention capacity and minimizes the water loss from the soil (Minhal *et al*., 2020; Sagar *et al.,* 2020). Mulching protects the soil from direct exposure to sunlight thereby reducing the evapotranspiration losses (Pramanik *et al*., 2015). Further, it controls the weed flush effectively due to inactivation of phytochrome due to poor exposure to light (McKenzie-Gopsill *et al*., 2020). Aerobic rice when cultivated to evaluate influence of mulching indicated enhanced water productivity under mulching over no mulch treatment and reported around 27 per cent of water saving, respectively **(Jabran *et al.,* 2015)**

Nutrient management

Optimum nutrient management is essential to improve the soil fertility status which is essential to attain high crop yield. Organic matter content also plays an important role in improving soil physical characteristics and helps in improving soil moisture retention capacity (Rao *et al.,* 2017). Addition of crop residues at regular intervals along with proper management of fertilizers is essential for attaining more dry matter with limited water supply and hence the water productivity.

Irrigation methods

Recent advancement in micro irrigation was attributed for minimizing losses *viz.* Runoff, leaching, conveyance, application etc. hence popularized as water saving technology (Kumar *et al*., 2008). During the initial period of crop growth in soils with high infiltrability, the sprinkler method was reported to be highly productive (Serem *et al*., 2016). Similarly, adoption of drip irrigation confers better conveyance and application efficiency (Pejic *et al*., 2017). Henceforth, attributes to achieve higher water productivity. For instance, in an experiment, drip irrigation was reported to increase crop water use efficiency by 11 per cent over border irrigation, respectively (Wang *et al.,* 2021).

Water pricing policy

Water pricing is an institutional framework to improve water productivity. These policies are widely gaining popularity. According to this policy the stakeholder is liable to remit charges for water used on volume basis. In Iran, this was observed to trim, the total water usage (Zamani *et al.,* 2021). Consequently, improving the water productivity on farm.

CONCLUSION

Enhancement of water productivity through yield advancement is utmost important to ensure sustainability in food production. This review showed that there are many possible options to improve water productivity. However, the adoption of the most ideal technology for improving water productivity is site specific in nature and depends on the economic and social conditions of a locality or farmer. There is a vast scope for research in innovation and adoption of water saving technologies suited to different agro-ecological regions are essential for further improvement of water productivity.

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RESEARCH ARTICLE

Multi-Sensor Fusion Based on Texture Classification Using Law's of Filter and partial Differential Equation

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ABSTRACT

The process of generating a more informative image from a series of source images is known as image fusion. Navigation and military applications are two of the most common uses of picture fusion. In this case, complimentary photos of the intended scene are captured using infrared and visible sensors. Using fusion techniques, the complimentary information from various source photos must be combined into a single image. The Fused image based on texture and edge information. The raw photos are broken down into texture and edge maps using pyramid structures. The coefficients corresponding to the filtered texture and edge maps are solved using principal component Analysis and the linear relationship between Laws texture extract filters.

Keywords: Image Fusion, KL-Transform, Anisotropic Diffusion, Imaging sensor, principal component Analysis (PCA), Laws texture filter.

INTRODUCTION

Image fusion can be defined as the process of combining many images, or some of their features, to create new modalities or instruments. Many applications, such as object detection, ATR (Automatic Target Recognition), remote sensing, computer vision, and robotics, relay heavily on it [1-5]. Image fusion can be done in a variety of ways. The type of application has a big role in determining which one is best. A simple method for fusion is to synthesis the merged image by averaging the image sources' corresponding pixels. When there are polarity flipped or complimentary characteristics, averaging improves the signal to noise ratio but diminishes the contrast. The visual system of rattlesnakes is one of the most well-known examples of fusion in a biological system. The organs of these vipers are susceptible to thermal radiation. Bimodal neurons merged the IR signals

produced by these organs. Image fusion is utilized in all of these applications to extract more information that isn't available in the separate source photos. In military and navigation applications (for accurate target identification), IR-visible image fusion is very important. The photographs were obtained using visual sensors due to adverse weather conditions after rain, during the winter, and so on. Visible sensors can provide detailed information about the environment, such as vegetation and dirt. Infrared sensors, on the other hand, offer information about the foreground, such as weapons, enemies, and vehicle movements. To improve situational awareness, information from both infrared and visible pictures must be fused into a single image for target detection and localization.

Several fusion approaches have been used in the literature thus far. The most successful methods are multi-scale decomposition methods (pyramid, wavelet) [16], [15], and data-driven methods [13], [12]. However, the fused image may contain artifacts as a result of these processes. Optimization-based fusion techniques [14] have been developed to address these issues. To find the best solution, these strategies require numerous iterations (fused image). Because of the repeated rounds, these optimization methods may over smooth the fused image. Furthermore, edge-preserving picture fusion techniques are becoming increasingly common. For the aim of fusion, these approaches employ edge-preserving smoothing filtering/processes. Guided image filter [10], weighted least square filter [3], [6], bilateral filter [10], cross bilateral filter [8], and 3-D anisotropic diffusion [7] are also popular algorithms in this class. Each source image is decomposed into base and detail layers in most of these approaches [16]. To create the fused image, the manipulated base layer, manipulated detail layer, or both manipulated layers are joined. In the fused picture, the bilateral filter and cross bilateral filter fusion methods cause gradient reversal artifacts, whilst the guided image fusion approach produces halo effects [17].

Three characteristics should be present in an effective picture fusion technique.
1) The majority of the useful information from the source photos must be transferred into the fused image.
2) During the fusion process, it should not lose any meaningful information from the original picture.
3) No artefacts or extra information should be included into the fused image.

To solve the shortcomings of existing approaches and keep the above qualities in mind, a new anisotropic diffusion based image fusion (ADF) using the KL-transform is developed. To extract base and detail layers from each source image, an anisotropic diffusion approach is used. The fused image incorporates useful information from the base and detail layers. This strategy is incredibly effective and simple to put into practice. The majority of the information from the source photos is transferred to the fused image. Fusion loss is minimal. • Fusion artifacts in the fused image are nearly non-existent. • The computation time is reduced.

PROPOSED METHOD METHODOLOGY

In the proposed method, using partial differential equations, the anisotropic diffusion process [17] smooths a given image at homogeneous regions while preserving no homogeneous parts (edges) (PDE). It eliminates the disadvantages of isotropic diffusion. Inter-region smoothing is used in isotropic diffusion. As a result, edge data is lost. Anisotropic diffusion, on the other hand, generates coarser resolution images by using intraregional smoothing. Edges are sharp and significant at each coarser resolution. The flux function is used in the anisotropic diffusion equation to govern the diffusion of an image I as

$$I=c(x,y,t)\,\Delta I + \nabla_C * \nabla_I \qquad (1)$$

where c(x,y,t) denotes the flux function or rate of diffusion, ∇= Laplacian operator,∇ = Gradient operator, and t denotes the time or scale or iteration. (1) is also known as the heat equation. This equation is solved using the forward-time-centralspace (FTCS) scheme. The answer to this PDE is

$I_{i,j}^{t+1} = I_{i,j}^{t} + \lambda[C_N\bar{\nabla}_N I_{i,j}^{t} + C_S\bar{\nabla}_S I_{i,j}^{t} + C_E\bar{\nabla}_E I_{i,j}^{t} + C_W\bar{\nabla}_W I_{i,j}^{t}]$ (2)

λ is a stability constant satisfying $0 \le \lambda \le 1/4$. $\bar{\nabla}_N$,$\bar{\nabla}_S$, $\bar{\nabla}_E$ and $\bar{\nabla}_W$ are the nearest- neighbor differences in north, south, east and west directions respectively. They are defined as

$\bar{\nabla}_N I_{i,j}^{t} = I_{i-1,j}^{t} - I_{i,j}^{t}$

$\bar{\nabla}_S I_{i,j}^{t} = I_{i+1,j}^{t} - I_{i,j}^{t}$ (3)

$\bar{\nabla}_E I_{i,j}^{t} = I_{i,j+1}^{t} - I_{i,j}^{t}$

$\bar{\nabla}_W I_{i,j}^{t} = I_{i,j-1}^{t} - I_{i,j}^{t}$

Similarly, C_N, C_S, C_E and C_W are the conduction coefficients or flux functions in north, south, east and west directions.

$C_{N_{i,j}}^{t} = g(\left\|(\nabla I)_{i+\frac{1}{2},j}^{t}\right\|) = g(|\bar{\nabla}N I_{i,j}^{t}|)$

$C_S^{t} = g(\left\|(\nabla I)_{i-\frac{1}{2},j}^{t}\right\|) = g(|\bar{\nabla}S I_{i,j}^{t}|)$

(4)

$C_{E_{i,j}}^{t} = g(\left\|(\nabla I)_{i,j+\frac{1}{2}}^{t}\right\|) = g(|\bar{\nabla}N I_{i,j}^{t}|)$

$C_W^{t} = g(\left\|(\nabla I)_{i,j-\frac{1}{2}}^{t}\right\|) = g(|\bar{\nabla}W I_{i,j}^{t}|)$

THE TEXTURE & EDGE MULTISCALE TRANSFORMATION

The texture edge multiscale transformation method is obtained by modifying the traditional pyramid-based feature extraction filter. The formula constructs the relationship between the feature extraction filter and the Gauss nominal filter. Therefore, texture edge multiscale transformation is equivalent to the Gauss-based pyramid transformation. In addition, the feature information becomes easier to process, and the feature type may be diverse. The character of the method is perfect for the image fusion process.

The formula (5) only is an objective of the processing method. To satisfy (5), the nominal filter $w^{\cdot}$ and edge filter d_i should be expanded its dimension as the
formula (5) and (6). Through developing, the edge filter is obtained from directional filter d_i.

$w_{new} = (w^{\cdot} * w^{\cdot}) * (w^{\cdot} * w^{\cdot})$ (5)

Multiscale method. The texture filter template and the edge
filter template is designed and carried on each scale

$w_{new} = (w^{\cdot} * w^{\cdot}) * (w^{\cdot} * w^{\cdot})$ (6)

Coefficient of the traditional pyramid. The texture feature and edge feature coefficient can be obtained through the transformation.

The pyramid filter is:
Remembering when the traditional pyramid filter$w = w^{\cdot}new * w^{\cdot}new$

Designed, one reconstruction condition shall be satisfied, keeping the reconstructed image as tiny as errors with the original image. For the texture&edge filter design, the exact condition also should be achieved. Figure 1 is the
The texture filter is obtained by convoluting Laws [7]'s

five feature extraction kernel vector. The following formula is Laws's feature extraction kernel vector. Decomposition and reconstruction transformation of

l_5 = [1 4 6 4 1];
e_5=[-1 -2 0 2 1];
s_5=[-1 0 2 0 -1];
u_5=[-1 2 0 -2 1]; (7)
r_5=[1 -4 6 -4 1];

Texture edge filter. The dashed box must be equivalent to a binominal filter which is adopted in traditional pyramid filter

METHODOLOGY

Figure 1 depicts the ADF approach. These steps are outlined in detail below. The next subsections go through each stage in great detail.
(A) Using anisotropic diffusion, extract the base and detail layers from source pictures.
(B) Using the KL-transform, fuse detail layers.
(C) Use weighted superposition to fuse the basis layers.
(D) Final detail and base layers are superimposed.

RESULT ANALYSIS

The images recorded using visible and MMW imaging technologies are shown in the figure as fused images of various ways and ADF method. The visible image conveys information about people and ships in the vicinity of the water. In order to create the fused image, we require information from both visible and MMW images. There are blocking effects that can be seen. Quality metrics, which are gradient-based objective fusion measures, are examined for a more in-depth evaluation of the suggested ADF approach. If X, Y are the source pictures and F is the fused image, quantitative analysis is performed by taking into account the information contribution from each sensor, as well as the fusion gain or fusion score.

When compared to other methods, the ADF method produces a high-quality fused image (Fig. 5e and Fig6e). In this situation, too, ADF performs admirably across the board.

CONCLUSION

For IR-visible images, a new edge-preserving image fusion algorithm is given. With the use of anisotropic diffusion, each image is first divided into base and detail layers. After that, the fusion process is used on the base and detail layers. The KL-transform is utilized to fuse the detail layers, and the weighted average approach is used to fuse the Laws filter. Using petrovic fusion measures, the ADF method's performance is compared to those of several image fusion algorithms. Our method outperforms the existing methods, according to our findings.

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Table 1. Quality Metric for IR and Visible Image

Iteration	Pyramid	PCA	HIS	ADF
12	1.03	0.7	1.103	0.5
10	0.89	0.9	1.02	0.4

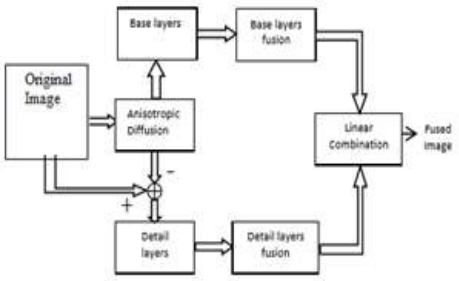


Fig-1: Proposed ADF method

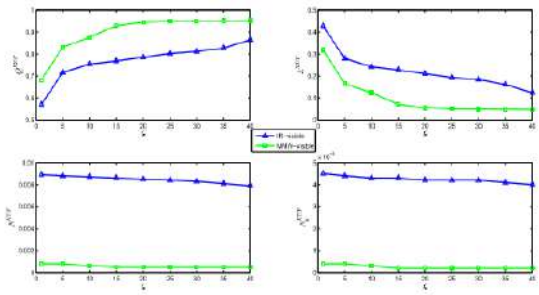


Fig 2:Quality Metric parameter for IR and ADF for K change

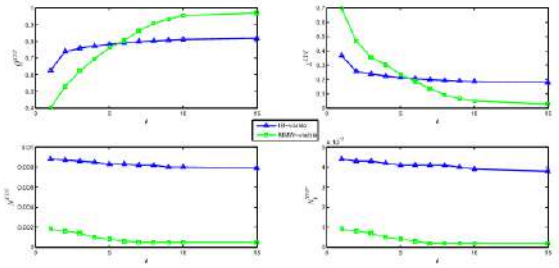


Fig 3:Quality Metric parameter for IR and ADF for t change

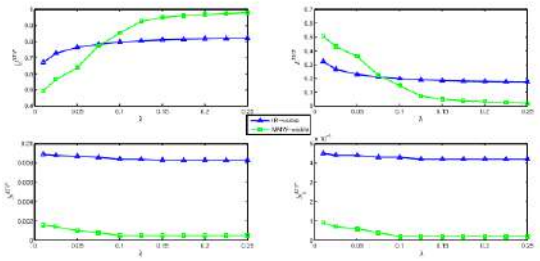
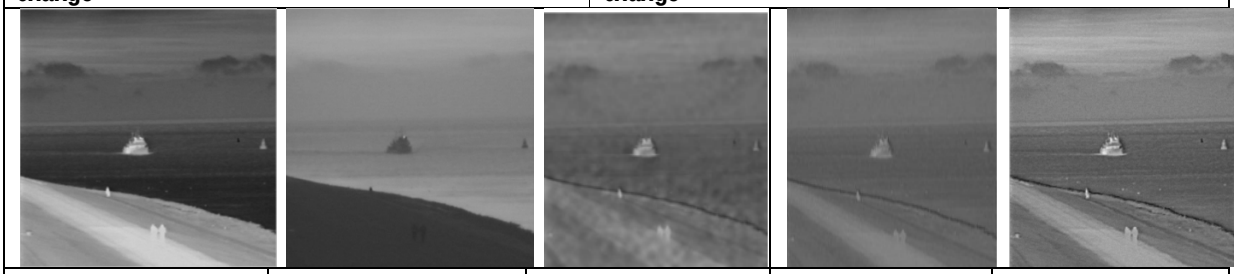


Fig 4:Quality Metric parameter for IR and ADF for lamda change



a	b	c	d	e

Fig 5. (a) IR image (b) Visible Image (c) PCA (d) HIS (e) ADF Method

Iteration 10, Elapsed time is 0.490865 seconds.	Iteration 12, Elapsed time is 0.583595 seconds.

a	b	c	d	E

Fig 6. (a) IR image (b) Visible Image (c) PCA (d) HIS (e) ADF Method

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RESEARCH ARTICLE

Biofertilizers: Role in Crop Production and Soil Fertility Improvement

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ABSTRACT

Current soil management practices rely heavily on inorganic chemical-based fertilizers, posing severe health and environmental risk. Because of their potential significance in food safety and sustainable crop production, the use of beneficial microorganisms as a biofertilizer has become more important in the agriculture sector. Plant growth-promoting rhizobacteria can be used in a variety of ways thanks to eco-friendly methods (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria, and a slew of other beneficial microscopic creatures all contributed to increased productivity. nutrient uptake, plant development, and biotic and abiotic stress tolerance. Biofertilizers mediated crops functional features such as plant growth and productivity, nutritional profile, plant defense, and protection were highlighted in this review, with a focus on its function to trigger numerous growth- and defense-related genes in signaling. To cause cellular response and, as a result, crop improvement, a network of cellular pathways is used. The information gathered through the literature reviewed here will assist us in better understanding the physiological grounds of biofertilizers to achieve long-term sustainability. Agriculture has a role to play in addressing the hazards related to chemical fertilizer use.

Keywords: Bio-fertilizer, types, plant nutrient management, role and functions, benefits

INTRODUCTION

The modern agriculture is based on use of high energy chemical fertilizers and if these are not used judiciously, they cause environmental pollution (Maitra *et al*., 2018; Maitra *et al*., 2021; Shankar *et al*., 2021a). There is enough scope and potential for adoption of integrated nutrient management in which different sources of nutrients such as organic manures, biofertilizers and chemical fertilizers are included (Maitra and Zaman, 2017;Ghosh *et al*., 2020; Sairam *et al*., 2020; Palai *et al*., 2021; Praharaj *et al*., 2021). Among these sources of plant nutrients, biofertilizer plays a significant role in maintaining soil health by increasing diversity and population of beneficial microorganisms (Brahmachari *et al*., 2018; Maidya *et al*., 2021a).

Biofertilizers, a modern agricultural science's gift, delay nitrification for a longer period and boost soil fertility. Biofertilizers improve soil fertility by fixing atmospheric nitrogen in conjunction with plant roots and the absence of plant roots. Without it, insoluble soil phosphates are dissolved and produced. In the soil, some chemicals help plants develop. They are being monitored encouraged to take advantage of the biological system that exists naturally mobilization of nutrients. As the human population grows, so does the demand for more food grains. Fertilizers are utilized to supply food grains by following demand (Maitra, 2020a). A Fertilizer is any material that is used to boost plant growth. Soil productivity is a term used to describe how productive the soil is. Chemical fertilizers are defined as fertilizers made out of raw chemicals in solid or liquid form that is synthesized in factories to meet the nutritional needs of plants. Nitrogen, Phosphorus, and potassium, collectively known as NPK, are commonly found in chemical fertilizers, along with other elements. additional vitamins and minerals (Youssef *et al*. 2014).

Increasing food production from progressively declining per-capita agricultural areas is currently the most difficult issue (Bhattacharyya, 2020; Mazid and Khan, 2014).Fertilizer is plant food, and pesticide is plant medicine (Muraleedharan *et al*., 2010; Maitra, 2020b).During recent years, agricultural production growth has slowed, and the increased use of chemical fertilizers has resulted in unsatisfactory issues on soil health (Mohanta*et al*., 2021) due to a lack of organic matter and a loss of biodiversity of natural fertility (Mahajan and Gupta, 2009; Khare and Arora, 2015) that mainly affect the soil microfauna and flora (Gupta and Singh, 2008). Further, impacts of climate change are also pronounced in crop productivity (Hossain *et al*., 2021).

Plants are unable to absorb all of the nutrients provided by artificial fertilizers (Bhardwaj *et al*., 2014; Maitra *et al*., 2019; Shankar *et al*., 2021b), Some nutrients are fixed in the soil, while others are leached away and eventually combined with water bodies(Mahdi *et al*., 2010). It is vital to establish a balanced and reasonable use of resources to make agriculture sustainable. ingredients that are both cost-effective and nutritious eco-friendly (Venkataraman and Shanmugasundaram, 1992; Mahdi *et al*.,2010); Biofertilizer might be a good solution in that circumstance (Pindi and Satyanarayana, 2012; Borkar, 2015). Plant growth-promoting bacteria (PGPB) are extremely important in agriculture. Indeed, they create metabolites such as plant growth regulators, which help plants develop and absorb nutrients (Bai *et al*., 2002; Salamone *et al*., 2001). There is a large population of PGPB that thrives. in various geographic habitats (Dobereiner *et al*., 1976; Staley,1999; Santos *et al*., 2001; Midya *et al*., 2021b).

BIOFERTILIZERS

The phrase 'biofertilizer,' also known as 'microbial inoculants' or 'bio-inoculants' (Arora *et al*., 2010), comes from the contraction of the term 'biological fertilizer' or a product containing living microorganisms that colonize the rhizosphere alongside the plant is found in the interior of the plant and stimulates growth. increasing access to and adoption of minerals to the host plant (Vessey, 2003; Malusá and Vassilev, 2014). Biofertilizers can fix atmospheric nitrogen and solubilize plant nutrients through a process called biological nitrogen fixation (BNF).such as phosphates and potash; moreover, Plant development is aided by the production of a variety of growth-promoting chemicals has a C:N ratio of 20:1, indicating that it is stable (Borkar, 2015).As a result, their negative consequences on the environment, plants, animals, and human life have shifted the focus away from environmentally beneficial plant preservation (Patel *et al*. 2014). The word "biofertilizer" encompasses a wide range of products. extracting manures from plants (Aggani, 2013).

Biofertilizers are live, ready-to-use mixtures of helpful microbes that can be applied to seed, root, or soil. increases the microbes' availability and utility. As a result, soil health improves (Bhattacharjee and Dey, 2004).The use of organic manure not only helps to control crop yields but also helps to promote environmental awareness. Influence on the nutrition, both direct and indirect soil accessibility by enhancing the physical, chemical, and biological properties, qualities of the soil, and applicable fertilizer efficacy (Kapoor and Pandit 2015). Microbial activity is important in agriculture because it affects the mobility and availability of minerals needed for plant growth and, as a result, lowers the cost of production. (Verma *et al*., 2017) Bio-fertilizers can boost productivity per unit area in a short length of time, consume less energy, reduce soil and water contamination, increase soil fertility, and so on. encourage

biological control and hostility creatures that are phytopathogenic (Yasin *et al*., 2012). Biofertilizers are important not just because they reduce the amount of nitrogen in the soil, but also because they reduce the amount of nitrogen in the soil. not only to reduce the use of chemical fertilizers but also to improve crop output. Agriculture that is both sustainable and profitable. The cost of producing biofertilizers is low and does not pollute the natural environment (Farnia and Hasanpoor, 2015)

Types of bio-fertilizers

Rhizobium

Rhizobium is a member of the *Rhizobiaceae* family. They are Gram-negative, motile, non-sporulating rod-shaped free-living creatures found in soil that can repair. a symbiotic relationship with atmospheric nitrogen They're well-known. as an endosymbiotic nitrogen-fixing microbe legumes' roots. The bacteria attack root hair during seed germination and spreads to the root. Rhizobium is a fungus that grows on the roots of plants and is capable of converting nitrogen into ammonia for plant growth (Yasin, 2012; Palai *et al*., 2021). Because pressures may alter the host plant and symbiotic organisms, the functional state of the legume plant, and the ideal environmental conditions supporting the symbiotic organisms. Rhizobia is a bacterium that lives in soil. Rhizobium has been reported as one of the bacteria which could fix nitrogen taken from the air. The bacterium can fix nitrogen ranging from approximately 100–300 kg per hectare in one crop season, or on average the nitrogen input of soil ranges from 0–60 kg per hectare per year.

Azospirillum

Azospirillum is a heterotrophic and associative plant that belongs to the *Spirilaceae* family. They produce growth-regulating hormones in addition to their nitrogen-fixing ability of roughly 20-40 kg/ha. substances. Even though this genus contains a large number of specific species. It aids the plants in obtaining the nutrient nitrogen from the atmosphere by establishing a symbiotic relationship ship (Essam and Lattief, 2016).Inoculation of wheat with *Azospirillum* sp. increased root dry weight and 1000-kernel weight, increased the number of spikes per plant, grain and straw yields, as well as the number of grains per spike in the wheat. It is a sort of associative microbe that colonizes the root surfaces of plants. It is possible to create a symbiotic organization by putting in place a symbiotic organization that aids the plants in obtaining nitrogen from the air (Abd El-Lattief, 2016).Inoculation with *Azospirillum* resulted in a 29 percent increase in grain yield, as well as increased N (22.8 percent) and P (22.8 percent) in the grains. compared to the control plants, 59.5 percent) and K (34%) (Askary *et al*., 2009).When maize plants were inoculated with *Azospirillum brasilense*, they saw a significant increase in total plant and grain dry weight. In individual experiments, aeromedical was found to be more effective than *seropedicae*. plants that have been grown in nitrogen-deficient soils(Riggs *et al*., 2001). *Azospirillum* inoculation could result in considerable increases in height, leaf number/plant, leaf length and breadth, and fresh and dry weight/plant rice-growing plant (Hossain *et al*., 2015).

Azotobacter

Azotobacter is an important biofertilizer for rice and other cereals, and it can be applied through seed dipping or seedling root dipping. *Azotobacter* is a key player in the nitrogen cycle in nature, as it performs a wide range of metabolic tasks (Sahoo *et al*., 2013). It is capable of producing vitamins like thiamine and riboflavin (Revillas *et al*., 2000) and Indole acetic acid (IAA), gibberellins (GA), and cytokinins are examples of plant hormones (CK) (Abd El-Fattah *et al*., 2013) Plant growth is improved by *A. chroococcum*, which increases seed germination and advances root architecture by (Gholami *et al*., 2009)preventing harmful microorganisms from colonizing crop root systems (Mali *et al*., 2009). Wheat, barley, mustard, sesame, linseeds, sunflower, rice, castor, maize, sorghum, cotton, jute, sugar beets, tobacco, tea, coffee, rubber, and other crops use it as a biofertilizer.(Wani *et al*.,2013).

Cyanobacteria

They can thrive in harsh settings thanks to unique adaptations like the ability to fix nitrogen and resistance to desiccation. Because of the capacity to fix nitrogen from the atmosphere, In the past, cyanobacterial mats were utilized as biofertilizer agriculture in the present (Kumar and Rao, 2012).The increase in total N content can be

attributed to cyanobacteria's nitrogen fixation and nitrate reductase activities, as well as NH4 uptake as well as the uptake of the Cyanobacteria, create amino acids and peptides.(Haroun and Hussein 2003, Adam, 1999).To achieve the same grain yield and quality as the fully fertilized treatment, biofertilization reduced the use of chemical fertilizer by up to 50%. The findings are consistent with those of Pereira et al (2009). Saccharides and nitrogen metabolism may be influenced by growth bioregulators found in the cyanobacterial filtrate, either directly or indirectly (Haroun and Hussein, 2003). In germinated pea, cyanobacterial filtrates can increase the production of GA3 (Drazkiewicz, 1994), as well as auxin and cytokinin.

Vesicular Arbuscular Mycorrhiza (VAM)

Fungi and plant roots form mycorrhizae, which are mutually beneficial connections. Inside the root, VAM fungus pollutes and spread. They have unique structures called Vesicles and arbuscules are two types of vesicles. Plants in severely worn tropical acid soils are likely to show a significant growth response to mycorrhizal fungus. that are deficient in basic cations and P and may cause hazardous effects aluminum levels are high. Plants having a shallow root system would be the most advantageous (Abd El-Lattief, 2016).Due to the presence of distinct morphological structure in mycorrhiza, it forms a symbiotic relationship with more than 80 %of the plant roots.The significance of the use of artificially produced inoculum of two dominant types of mycorrhizal fungi has been increased due to its multifarious role in plant growth, yield, and resistance against the biotic and abiotic stresses. Mutualistic symbiosis is formed by the ectomycorrhizal fungi with many plant species. (Anderson and Cairney, 2007).

Plant Growth-Promoting Rhizobacteria (PGPR)

Differential bacterial taxa are important soil components. Kloepper and Schroth defined Plant Growth Promoting Rhizobacteria for the first time in 1978.Rhizobacteria that promote plant development can fix nitrogen in the atmosphere and the generation of auxin, cytokinin, gibberellins, and other metabolites phytohormones, hydrogen cyanide (HCN), and synthesis of certain flammable materials PGPR also results in the production of some substances. mineral dissolving chemicals, for example, the solubilization of Internal resistances are produced as a result of the phosphorous. Plant development may be boosted through biological nitrogen fixation and the generation of growth-promoting compounds like IAA and Gibberellic acid. The microorganisms that help plants grow are well-known as rhizobacteria that promote plant growth (Satyaprakash *et al*., 2017) The PGPR or PGPR plus AMF co-inoculants can improve fertilizer nutrient utilization efficiency. For P absorption, a synergistic relationship of PGPR and AMF was preferable to 70% fertilizer with AMF and PGPR. A similar pattern was observed in the uptake of nitrogen on a global scale.

The Role of Biofertilizer in Crop Production

To recover and sustain these soils, PSB and other helpful microbial inoculants must be inoculated. the solubilization-effective microbial populations phosphorous that has been chemically fixed and the availability of other macro and micronutrients to harvest a high-yielding, long-lasting crop (Mishra *et al*., 2013).Because of its fast decomposition in soil and efficient nitrogen availability to rice plants, Azolla biofertilizer is employed in rice farming. The *Azolla* application resulted in Rice yields increased by 0.5-2t/ha-1, which is a significant improvement. A 29.2 percent increase in grain yield was realized. *Azotobacter* is a free-living, heterotrophic bacteria. Bacteria repair about 20 to 40 kg nitrogen per hectare per year, and Increases yield by up to 50%. A healthy plant has a healthy environment. Beneficial bacteria should dominate the rhizosphere. On the other hand, on poor soil, dominated plant growth would be hampered if harmful microorganisms were present. Biofertilizers differ from chemical and organic fertilizers in that they do not deliver nutrients to the plant directly. any nutrients to the crops, and are bacterial and fungal cultures. The manufacturing technology for the use of biofertilizers is relatively simple.

Functions of Biofertilizers

Soil microorganisms play an important role in regulating organic matter decomposition and the availability of plant nutrients such as N, P and S. it is primary nutrient and other nutrients are essential nutrients of integrated nutrient management that leads to sensible agriculture. microbial inoculants can be used as an input to increase crop

productivity, the dosage of fertilizers can be adjusted and more nutrients can be gained from the soil. Biofertilizer can be used as a nutrient source for soil which increases soil fertility quality and provides good yield with low input of fertilizer. legumes have been reported to vary from 35-270 kg/ha in a year. it helps in nitrogen fixation in root nodules. The biological N fixation in rice are species of *Alcaligenes, Azospirillum*, *Bacillus, Herba spirillum, Klebsiella, Pseudomonas* and *Rhizobium*. Biofertilizers are living cells of microbes that provide the plantswith nutrients through their root nodule system. The microbes in these fertilizers use different mechanisms to supply nutrients to the plants. They fix nitrogen, phosphate solubilization, phosphate mobilization, and promotion of rhizobacteria. Bio fertilizer uses increases due to it increases soil health and low environmental impact. their application in soil enhances soil biota and minimizes the use of chemical fertilizers (Mishra *et al*., 2013)

Benefits of using Biofertilizers

Beneficial microorganisms can assimilate phosphorus for their own needs, which is then available in sufficient quantities in the soil as its soluble form. *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Fusarium, Sclerotium*, *Aspergillus*, and *Penicillium* are among the bacteria that can cause infections are involved in the solubilization process (Pindi and Satyanarayana, 2012). two kinds of mushrooms *Aspergillus fumigatus* and *Aspergillus niger* were isolated from decaying cassava peels and found to convert semi-solid cassava wastes. Phosphate biofertilizers are made using a fermentation process (Ogbo*et al*., 2010).The plant nutrient demand is met by mycorrhizal mutualistic symbiosis with plant roots. (Kogel*et al.,* 2006). This results in improved plant growth and development, as well as protection against infections and environmental stress. (Lamabam *et al*., 2011.) Isolated from the rhizosphere of a sunflower, Enterobacter and Burkholderia produce siderophores and indolic compounds (ICs) that can solubilize phosphate. (Ambrosini*et al.,* 2012). Biofertilizers make nutrients that are naturally abundant in soil and the atmosphere available to plants. plants. Several field studies have been conducted. These are both effective and inexpensive. inputs that are free of any environmental risks (Sahoo *et al*., 2013; Borkar, 2015). Low production, unexpected climate changes, and low dose are all characteristics of drier agriculture. thus, in this case, chemical fertilizers Rhizobium, in particular, could be used as a biofertilizer. be a link between the removals and the additions of soil minerals in areas where farmers have limited access to able to afford expensive inputs (Das *et al*., 2015).

CONCLUSION

Environmental stress is becoming a serious issue, and productivity is dropping at an alarming rate. Our reliance on chemical fertilizers and insecticides has increased dramatically. supported the growth of manufacturing industries life-threatening toxins that are not only harmful to humans but also have the potential to disrupt the ecological equilibrium. Biofertilizers can aid in the problem's resolution. the challenge of feeding a growing global population at a time when Agriculture is subjected to a variety of environmental pressures. It's true. It is critical to recognize the benefits of biofertilizers to apply them to modern agricultural practices. The new method, which was developed utilizing the powerful tool of molecular biotechnology, has the potential to improve the biological pathways for phytohormone production. These technologies can help give alleviate environmental problems if they are found and transferred to useful PGPRs stresses. However, there is a dearth of information on improved biofertilizer application procedures in the field. one of the few reasons why there are still so many useful PGPRs ecologists and agriculturists don't know about. Nonetheless, recent advancements in technology connected to Plant-pathogen interactions and genomics will aid in the optimization of the essential methods, according to microbial science. The success of biofertilizer research is dependent on the development of novel solutions for the functions of PGPRs and their appropriate application in agriculture. In this field of research, the most significant challenge is since, in addition to the identification of numerous It is necessary to dissect PGPR strains and their attributes. the actual mechanism through which PGPRs function for their exploitation effectiveness in sustainable agriculture.

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RESEARCH ARTICLE

A Review on Long Distance Phloem Transport and Distribution of Photo Assimilates into Developing Organs

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ABSTRACT

Recent plant physiological studies focused on resource allocation and signalling between plant organs and long-distance phloem transport of tiny molecules of produced assimilates in plants. A series of recent researches allow us to learn how phloem mobile macromolecules can play essential roles in regulating the development of plant organs. Phloem tissue mainly consists of complex live cells containing the sieve tube parts and their surrounding cells and translocate photosynthetic assimilates from old leaves to young tissues. Intensive research has revealed that the vascular tissues of the plants transport different genetic product types, and some transportation has the molecular basis of long-range communication. Vascular plant systems experience unique changes to the cell walls which are extremely specialized in water and nutrient transport. The experimental alteration of the source-sink balance increases the understanding of the linkages between sources and sinks. A holistic perspective of sources and sinks, including the molecular mechanisms behind their connections need to be created to bring about an improvement in crop growth and output. The transport of sugars from source-to-sink is one of the major factors of plant development and it depends on the efficient and controlled distribution of sugars throughout the plant organs. However, sugar transfer by phloem may have a significant impact on the environment that changes the interaction between the source and the sink relationship.

Keywords: Phloem, photo assimilates, translocation, partitioning, source-sink relation

INTRODUCTION

For growth, development, reproduction and environmental adaptability of multicellular organisms, cell-to-cell and inter-organ nutrition exchanges information are important (Lanoeu *et al.,* 2014).A wide range of plants evolved through a plant-specific and plasma-specific network links that allow a locally restricted exchange of information (Giehl*et al*., 2009).The vascular network interconnects and is an intrinsic feature of all organs that is used to

translocate or transport for longer distances(Haywood *et al*., 2005). The vasculature network consists of two unique and distinct channels for cell translocation, i.e., xylem and phloem. For cytoplasmic connections between plant cells, in higher plants phloem is a channel system which predominantly assigns organic nutrients, created from "source" (organs which produce photoassimilates) to "sink" (organs which consumes the photoassimilates). The phloem is situated in vascular bundles together with the xylem (dead pipes transporting water from root to shoot) (Knoblauch and Oparka, 2012). The phloem's transport tubes, the so-called sieve elements (SEs), are live cells which are partially degenerated and throughout maturity lose critical cellular components, such as vacuoles, nuclei and ribosomes. Furthermore, the phloem allows quick information transmission across larger distances and phloem thereby create an ongoing symplastic link between the most remote plant parts.(Laugh and Lucas, 2006).The source-to-sink transport of sugar is one of the main factors of plant growth and is based on efficient and controlled distribution of assimilates among plant organisms through phloem. However, sugar transfer by phloem may have a significant impact on the environment that changes the interaction between the source and the sink (Marín-González and Suárez-López, 2012).The distribution of photosynthates in a plant is regulated mostly by sink organ distribution. In some cases, the competition among distinct sink organs develops and the more active sinks are sent to the physical distance (source) and the higher photosynthesis created by certain sheets(Oparka and Cruz, 2000). However, the major sink can be considered as the dominating sink for distribution of assimilates. The article provides an insight into distribution and partitioning of photosynthates in plants.

PHOTOSYNTHATES LOADING IN PHLOEM

The growth of photosynthetic tissues in the development of transport systems has been proven by anatomical investigations (Esau 1969; Gamalei, 2000). The large-vine network develops in the sinking stage acropetally, while the terminal network in the source stage develops basipetally. The duration along the top-bottom-axis of the leaves expands the process (Kollmann and Glockmann,1990). The core formed by cells generated in import and the cell mantle that occurred during exports can be distinguished in big veins (Dengler, 2000). As these veins enlarge throughout the phase of export, they are covered by the same phloemic complexes (Turgeon, 1980). The plant physiologists have investigated the following steps of sink to source transition using isotope and fluorescence tracers, confirming prior evidence that the transition begins at the leaf apex and is finished at the leaf base (Beebe and Evert, 1992). When graded by isotope methods, this chain of structural processes is considered as a practical tool to determine the phases of leaf growth. The movement of the sink–source is linked to the expansion of the leaf, including the formation of the stomata and terminal vein network in consecutive phases of mesophyll cell vacuolisation. These structures are developed within a short time after imports in the particular leaf region have already shut down and exports have not yet been activated. The only way to direct them to the export route, which finally takes place concurrently with the flow itself, is when the growing leaf cells are packed with excess photosynthates. The unwinding in direction from top to base of the leaf provides time for the cavity to be gradually filled with air, the stomata at the respective locus is switched on and the terminal bundles are eventually formed (Behnke, 1994; Kollmann and Glockmann, 1990).

PHLOEM UNLOADING AT SINK

Faster growing tissues such as root and breeding organs use sugar and it is used for the unloading of phloem sieve tubes. There are often no specific accompanying cells in unloading terminals; however, parenchyma cells are sometimes assumed for the latter. Discharge takes place via plasmodesmas, i.e. symplast from the sieve tubes into the surrounding parenchyma. The symplastic loading of all sink areas was thought by numerous researchers to be a generic process (Mezitt and Lucas, 1996; Flora and Madore, 1993) and the identical sugar compositions of all sink sites are indirectly supported by the same idea (Haritatos *et al*., 2000)Another reason for universal unloading is the absence of specific partner cells in these sieve tubes. In contrary to pre-phloem symplast, sugar cannot be accumulated at high concentrations for lengthy periods. The distribution of plasmodesmates conveniently maps the geography of symplast flows in parenchymas. Their density is reduced externally at the root apex from the tube to the meristem of the terminal sieve (Oparka and Turgeon, 1999). With uniformly apoplastic loading due to the specialist companion cells, the idea of phloem transport would seem much simpler; and universally symplastic

release would appear to be passive and without the companion cells. In this regard, the sink sites may also vary, and not all types of tissues have been explored in detail. In ripening seeds and host plant–parasite connections, one participant is uploading and another is unloading. These cases should preferably be taken from one transportation network to another. The evidence for the variable cross-sections of the plasmodesmata should be carefully assessed on the basis of the dextran transport study in the pre-phloem and postphloem symplast (Schulz, 1998; Kuhn *et al*., 1997).The reduced performance of post-phloem symplast plasmodesmata could come from lower pressure in this region of the phenomenon(Zimmermann, 1975). The high mobility and sensitivity of plasmodesmata in plant tissues are well recognized and can result in variation in functional phloem indicators (Fisher and Oparka, 1996; Patrick, 1997).

PARTITIONING OF PHOTO-ASSIMILATES

One of the fundamental factors of plant growth and development is the partitioning of photo-assimilates amongst different organs. The division of assimilates in plants relies on the capacity and the force of the sink. The activity of photosynthesis is an important metric for the source capacity. For assimilation of the division from the source to the sink, sinking strength is needed as the organ is capable of attracting the photo-assimilates (Ho, 1988). Various factors could limit the unloading steps into the sink cells. The import rate to a particular sink organ may be varied by either increasing its strength or changing the power of a competing sink. The import rate to a discharge sink may be dependable on the rate of respiration while the control process may be a storage step in a storage sink. There is no evidence that the import rate is largely restricted by the discharge rate (Kollmann and Schulz,1993). If unloading is not the control step, then mechanisms that are involved in the use or storage of the import of assimilate in sink cells have to be the key components. There is no clear definition of the proportional importance of sucrose syntheses in assimilate partitioning. The main role has been noted by various researchersis either the first or second of these enzymes, depending on the plant organ, tissue or species investigated. (Thiel *et al*., 1998). Sucrose syntheses was proposed as a measure for decreased strength (Sebkova *et al*., 1995).This enzyme could be significant for mobilization of sucrose for product storage synthesis as proposed by Heim *et al.* (1993). Geigenberger and Stitt (1993) have specified, in order to remove the fructose and recycle the sucrose that Su-Sy can influence the movement of sucrose via invertase (Arai *et al*., 1991). Cheng *et al*. (1996) have shown that cell-wall invertase (CWI-2) is the first enzyme to metabolize the intake of sucrose from plant, having a vital function in the optimal distribution of sucrose in a developing maize endosperm. An important stage in determining the carbon flow into the endosperm is the hydrolysis of sucrose in base of the endosperm cells. The lack of invertase activity is connected to the considerable drop of seed weight for mature kernels in invertas is-deficient mutants. The role of the cell-wall invertase in determining the sinking capacity in the bean embryo was examined by Weber *et al*. (1995).In cotyledons and in apoplast endosperm space, they detected a link between the activities of cell-wall invertase in the growing bean seed coat. After discharge from the seed coat, they postulated the hydrolysing of sucrose by means of a cell wall invertase to contribute to the depletion of young seeds.

PHOTOSYNTHATES TRANSLOCATION IN GRAINS AND FRUITS

From the leaves (sources) to the harvestable target organs (sinks), there is a direct influence of dry substances and the auxetic proliferation of sink tissues (Troughton and Currie, 1977; Braun *et al*., 2014). Effective transfer of photosynthate to the sink fruit is very crucial in the formation of fruit crops such as tomatoes, cucumbers and strawberries as they are harvestable bodies. Dry matter is partitioned to the tank by the sink itself, and the target organ's ability to attract assimilates is known as sink force (Gifford and Evans, 1981).The drop strength is determined by the size and the activity of the sink. This latter represents translocation physiological responses (Ho, 1996). Photosynthesis will be loaded into the phloem from the source organ and unloadingfrom the phloem into the sink organ during translocation. This process is influenced mostly by environmental elements such as air temperature, light intensity and CO_2 levels (Lemoine *et al*., 2013). Therefore, it is vital to define the sink-source linkages and reactions relating to the environment in order to assess the yield and quality in developed plants. In tomato, the relationship between the source and the sink and the translocation was explored and determined with respect to environmental circumstances (Ho, 1988; Shishido *et al*., 1999).The presence of photosynthetic was significantly higher

the fruiting inflorescence than the leaves. The influences in photosynthesis translocation in the plants were evaluated using the ^{14}C and ^{13}C tracer methods for growth, inflorescence development, leaf position and environmental circumstances (Nishizawa and Hori, 1986, Hidaka *et al.,* 2014). However, translocation measures generated from the foregoing processes do need tissue removal and damage from plants (Kitano *et al*., 1998). For the production of high-quality and steady fruits in plants, sufficient photosynthesis translocations for each individual fruit are necessary (Kumakura and Shishido, 1994). In reality, even on the same inflorescence, the transfer of photosynthetic to individual fruit may differ. It is commonly established that phloem transport of sucrose is driven by mass flow (Munch in 1930) and by hydrostatic changes in phloem pressure between the source and the tubular system. This is the principal translocational sugar in plants. Active phloem loading via energy consumption of proton/sucrose co-transport, mediated by sucrose transporters (SUTs) and H+/ATPase, maintains relative higher concentrations of the phloem sugar upstream (source side) than downstream (sink side) (Bangerth, 1989).

CONCLUSION

In the present-day agriculture, which is mainly focused on productivity, the physiological study of the crops is very much essential. In physiological point of view, partitioning of photosynthates is a major step involved in improving high yielding varieties. The basis of the subject and the mechanisms of the photoassimilates translocation and partitioning should be studied to incorporate the strategies in to breeding techniques by developing new genotypes. A developed ideotype with good source and sink ratio is desired for maximising the yields. This concept for improving genotypes will enable researchers and producers to provide more in-depth base research for development approach to achieving the objectives of plant breeding by enhancing productivity.

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RESEARCH ARTICLE

Impact of Climate Change on Agriculture and Allied Sector

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ABSTRACT

The alternation in world's climate is due to the heavy population growth and increasing temperature. Now a days climate alternation is considered to be a big issue as climate change affects, directly and indirectly to all the agriculture and allied sector including animal, aquatic animals where it has both the useful and dangerous consequences. A number of studies is being conducted in various ways to know about the climate change and its impact on food sector. The study includes the effects of climate change on agriculture from different aspects like photosynthesis, transpiration and increase or decrease in productivity, which concluded a significant change in crop yield. Besides food sector the climate change is also creates pressure in ecological sector, socio-economic sector, etc. As the greenhouse gases are the primary sources of climate change, the effects are getting worsen with the huge amount of industrial establishment which raise the temperature to an alarm level of global warming.

Keywords: Climate change, impact, agriculture, allied sector, mitigation, adaptation

INTRODUCTION

Climate change is one of the most fearful ecological troubles that facing by the folks of the world, which is associated with food production and natural ecosystem. The adverse effect of climate change is dangerous to the developing country where the land area is low, due to increase in industrialization and people largely dependent on agriculture also (Mendelsohn *et al.,* 2006:Stern, 2006: Nelson *et al.,* 2009; Maitra et al., 2021).Climate change is a critical long-haul change in the arrangement of the climate in a wide range of areas or the total world in a time interval. By the census 2001the developing countries in India about 27.8% of the total 1.02 billion people are found in the urban area and rural area people of a country mainly depend on the water diversity, coastal zones, etc. for their subsistence and these sectors are very much susceptible to the climate change. The causes of climate alternation are mainly divided into two types one is Natural causes and the other one is Anthropogenic or Man-made causes listed below in Table 1.The volcanic eruptions also bring climate change by influencing the atmospheric state along with the atmospheric temperature. Fluctuation in the solar deviation allows climate to increase the temperature from the optimal level. The

earth's orbital tilt consists of extra tilt and less tilt. A more tilt cause hotter summer and too cold in winter and in less tilt vice versa occurs. Global warming results in ozone-depletion and the amount of greenhouses gasses are increasing day by day due to huge industrialization. By deforestation, a huge amount of carbon dioxide lunch into the atmosphere and it unbalance the atmospheric condition and the atmosphere becomes hot. Coal mining is related to natural resources and natural habitat degradation. The burning of fossil fuels will add more amount of carbon dioxide into the atmosphere. Due to the black carbon increase the radioactive forcing also upturn from the previous evaluation amount (Bond *et al.,*2013).

The modern interaction can have negative biological impacts, causing ecological change, loss of ordinary resources, air and water contamination, and obliteration of species and the horticulture add to a few greater natural issues that cause normal debasement including ecological change, deforestation, inherited planning, water framework issues, harms, soil defilement, and waste. From agribusiness an immense measure of methane gas emanation happens, this methane gas is significant ozone-harming substance. The climate change has a various effect on the environment including human and animals. These are as follows: The melting of glaciers, ocean levels rising, flooding occurs in low laying area which causes loss of human life, damage to property, destruction of crops, loss of livestock, and deterioration of health conditions. Environmental factors also lead migratory birds to vary their timing and place. Different sorts of catastrophes such as drylands, heat waves, winters and storms, hurricanes, and typhoons occur each year as a cause of climate change. Long summer can place animals in hazardous housing and continuous heat waves and rise in air temperature cause risk for human health and imbalances the microclimatic conditions.

The immediate effect of environmental change incorporates physiological change, phenotypic change, morphological change, and plant creation. The modifications in plant photosynthesis, respiration, vegetal nourishment, and the activity of plant hormones, etc. are indicated by the physiological changes. The genes are also modified in response to environmental effects in the case of phenotypic alterations. Morphologic alterations include decreased internode growth, leaf size variations, the surface area of the leaves, patterns for branching, shooting reduction and root growth, etc. Physiological changes, phenotypic and morphological changes have a direct effect on plant productivity. The indirect impact of climate change includes soil fertility, irrigation availability, flood/drought, and rise of sea level. With the rising sea levels which are caused by the melting of glaciers, flooding will result, which is a common disaster in India. Climate change affects the water sources so that the water level will decrease and different water sources also polluted so it affects to the availability irrigation. Climate change indirectly affects soil fertility and reduces yields.

GREEN HOUSE EFFECTS AND GLOBAL WARMING

A temperature rise is generated by an increase in greenhouse gas emissions (Fig.1). The heat produced by the earth's surface and atmosphere will absorb these reaction gases, especially by greenhouses gases. Due to the emission of these gasses the ozone layer depletion occurs which affect agriculture either directly or indirectly (*Garg et al., 2001; IPCC, 2001; Krupa; 2003*). The ultraviolet rays are directly exposed to the human body, as well as animals and plants, due to the loss of the ozone layer which induces a various illness in the human body. The temperature of the atmosphere rises as a result of greenhouse effects, warming the climate. The greenhouse effect induced by increased amounts of carbon dioxide, CFCs, and other pollutants cause global warming, which is a continuous rise in the total temperature of the earth's atmosphere.

IMPACT OF CLIMATE CHANGE

In current history, the rate of climate change has accelerated, as has the occurrence of natural disasters like floods, droughts, cyclones (Goswami *et al.,* 2006). Climate change has several unfavorable effects not only on agriculture it also affects to the human, pest and diseases, live stocks, aquatic ecosystem, environment and many more (Maitra *et al*., 2018a; b). Because of climate change, the following sector is mainly affected and the following sectors are the major sector which is included under the agriculture-

- Climate change impact on different Crops.

- Climate change impact on Pest and Diseases of the crop.
- Climate change impact on Live Stocks.
- Climate change impact on Aquatic Ecosystem and Production of Fisheries.
- Climate Change Effect on Forest Ecosystem

Climate change impact on agriculture

Temperature, light, soil, water, oxygen are the important basic elements for a crop. A rise in elevated temperature, alternation in the rainfall, changes in the carbon dioxide level is the key indicator of climate change. Agriculture has a dual response to climate alternation one is susceptible to agriculture and the other response is harmful. Some alternation of climate helpful by the heighten of carbon dioxide level and along with the photosynthesis also increase harmful as increase of carbon dioxide the transpiration rate decrease(Cure *et al*., 1986). Temperature is an important component in reducing output across all environmental variables. Due to over-running soil erosion in the upper layer of a river basin is caused by floods and deforestation. As a result of soil erosion and a loss in soil fertility, plants in that region are unable to thrive.

Climate change will also impact the soil water balance to decrease such that the soil gets drier. Soil water balances will also fall. Drought will arise under these circumstances whenever low precipitation develops to the stage that the plants can't collect enough water to function, since precipitation volumes and quality have declined over the previous several years. The number of raindrops differs according to the varying monsoon patterns. In recent times, the monsoon has produced disruption. In recent days, the monsoon was highly unpredictable. In today's world, it is also difficult to predict the monsoon using satellites, if satellites and other technologies are used to get accurate data. As a result, the plant is unable to obtain sufficient water for its development. Natural disasters will have an impact on plant development and productivity. The stomata-carbon dioxide reaction, together with another plant- and environmental variables, makes it so difficult to anticipate the stomata response to carbon dioxide growth (Rosenzweig, *et al.* 1995; Billah *et al*., 2021).Crops deteriorate from time to time as a result of high temperatures and precipitation. Many weeds are expanding their populations as a result of many climates change factors such as rising temperatures, wetter climates, and increased carbon dioxide levels, among others. This is a new issue that has arisen as a result of climate change. Carbon dioxide levels will eventually rise, which will help C_3 and C_4 plants, which are more vulnerable to carbon dioxide shortages. As carbon dioxide levels rise, the plant's capacity to absorb nitrogen decreases. The soil fertility is impacted by the C: N ratio mismatch.

World agriculture faces a serious yield reduction because of climate variation. By the next few years world will face low agriculture productivity, i.e., 5-10% due to the alternation (Mondal *et al*., 2021; Praharaj*et al*., 2021).Due to the insufficient amount of rainfall, the drought will occur, and due to the drought, the photosynthesis will affect and the plants can't survive well *(*Avila *et al.,*2020; Hossain *et al*., 2021). Some developing countries like India, Afghanistan, Bangladesh, Nepal, etc. asserted due to climate change the agricultural food production will shrink at the level of 20-25%. The Asia-Pacific part may have a tough effect on world rice and wheat yields, and lower yields might put a strain on food security in South Asia, which has a population of 1.6 billion people. Hot will have an immediate impact on yields in regions where temperatures are already close to the physiological maximum for crops(IPCC, 2007).On a global scale, inadequate rainfall has a major effect on crops. Irrigation is mostly used to manage this.

Temperature increases will have an impact on agricultural output, sea level, and the agro-ecosystem, among some other things. This type of detrimental effect will be more dangerous in emerging countries such as India and Sri Lanka (Jayant *et al.,* 2006). Atmospheric CO_2 increase benefited crop like rice, wheat, legume, oilseed crop increase in yield i.e. 10-20% & the harmful results (increase in temperature) are decreased in production in a crop like wheat, mustard, etc. i.e.3-7% According to an Indian agriculture research agency temperature rises 10^0c will destructive loss in yield in wheat 4-5% million tones. In certain areas like Rajasthan, the Pearl Millet yield is steadily decreasing to 10-20 percent due to the increase in the temperature from the optimum plant temperature. The Soybean yield in Madhya Pradesh is 5% lower. Climate change has a huge influence on agriculture supplied by rain. Rainfed wheat

yield 0.45ha in India is increased on rainfed farmland by temperature 0.5 ◦C. The changes in the climate requirements are also affecting chickpea, maize, sorghum, and millet. The impact of climate change in some crops:

A. Rice: In world consumption, the rice crop gets the top position among all the crops. It considers as 90% of the total consumption (Dawe *et al*.,2010). By scientific experiment, it concludes that in India rice yield will reduce from 4.5 to9% by the next few years. Temperature, precipitation, CO2, sun radiation, etc. are the climate variables that are responsible for improved rice growth. The rice spikelet sterility will be increase if the temperature beyond the threshold level, but it has negative effects like reducing the duration. The critical period of rice is flowering, which affects if the temperature increase. It is found that inconsistent in temperature, rainfall has two different types of effect some time it harmful and sometimes beneficial effects. The rice yield will decrease by the decrease of the rice spikelet fertility. It happens in the rice when the temperature rises. The increase of temperature that it will affect in some region yield reduces 3.2% and some regions up to 6% (Zhao *et al.,* 2017).Sometimes rice yield decreased by an increase in temperature. In C_3 plants the yield reduces to 10-18%. Among various regions, the Asia region is very much susceptible to flood.
B. Wheat: World wheat is a vital food crop after rice (Scotti-Campos *et al.,* 2014). It is concluded that a rise in each temperature will impact the wheat production of 5-6% worldwide (Sultana *et al.,* 2009; Asseng *et al.,*2015; Zhao *et al.,* 2017). India contributes 15-20 percent to the international food industry as a significant contributor to wheat. The returns will be cut by 50% in the next years because of hot stress and a rise of 0.5-1% in temperatures (Hatfield *et al.,* 2011). The grain-filling phase over 350c affects and impedes the output. The rise in carbon dioxide levels makes a total of 25% of the yield loss of 29%(Anwar *et al.,* 2007).
C. Maize: Maize is the most susceptible crop to climate change among the 4 major crops (Rice, Wheat, Maize, and Soybean), and due to climate change yield will be reduced to 20-25% *(*Zhao *et al.,* 2017; Rose *et al.,* 2016).Research has shown that the output in China is about 13-15% lower in the spring season because of constant changes in the atmosphere. Among the different climate change factors, the increase of the temperature is the most important factor which affects the reproductive phase as a result the yield will be reduced.
D. Soybean: Some time increase of temperature has two impacts one is average yield reduce and stronger yield reduce 3.1%, 6.8% respectively with a stronger impact of 6.8% (Zhao *et al.,* 2017). Best temperature 23-24^{0}c. Pollen growth, pollen viability, germination, etc are highly affected by an increase of temperature by a decrease in yield(Hatfield *et al.,* 2011).

Adaptation and Mitigation of Climate Change in Agriculture

On the prior studies, it is remarked that the changes in climate in developing countries like India in the agriculture sector are changeable. As consider the impact of climate change it is more destructive in the rabi season agriculture. There is some adoption are taken such as (a) change in the date of planting, (b) use of different plant varieties and plant species, (c) use of alternate crops, (d) use of drought or heat stress-resistant varieties,(e) crop diversification,(f) more use of the water harvest technique, (f) provide loan credits to the farmer, and (g) use of new irrigation technique (Maitra and Pine, 2020). To mitigate the damaging collision of climate change use organic farming, natural farming, and the farmer can do the integrated farming system. In the evolving countries due to great industrialization, a huge amount of greenhouse gases are emitted. In developing nations like India, the mitigation strategy for reducing the damaging impact of rising temperatures would result in late sowing, which is the greatest way to enhance soybean yields(Mall *et al.* 2004).Greenhouse gases not only pass through the industry but rice cultivation is also responsible to emits large amounts of methane and carbon dioxide into the atmosphere. The process of afforestation is the only way to control gas-like carbon dioxide levels. To mitigate another practice such as the use of the conservation tillage, agroforestry, etc. By the above mitigation process, it has a positive impact on agriculture so the farmers are getting benefits and the gases are being under resistor on the atmosphere.

Climate change impact on different diseases of some crops

In agriculture, disease pathogens occupy a vital position (Agrios,2005). Variation in moisture, temperature, the wind is the three major parameters that mostly help pathogen to survive easily and increase their population,

reproduction, etc and also increase the destruction level in the plants. The pathogen frequency will rise if the drought increases its effect (Desprez-loustau *et al.,*2006). In Italy, some exotic species started survival due to the direct result of drier conditions (Garbelotto, 2010). Effects of Rising Temperature: Changes in the growth stage, development rate may be hampered by the alternation in the temperature and precipitation. Alternation in the temperature indicator of the survival of the inactive pathogen (Charkraborty *et al.,*2003). Some bacterial diseases like *Ralstonia solanaceraum, Acidovorax avena,* etc are mostly destructive diseases to agriculture which are caused only by the increase of the temperature. Due to climate change, some destructive diseases are seen in some areas where the disease activity was not seen before particularly in that area (Kudela, 2009). Also increase growth reproduction under the climate change like increase of the temperature (Ladanyi *et al.,* 2010).The increase of temperature some time harmful to some crops like wheat, in the form of rust disease, and beneficial to the forage crop because the forage crop resists the increase of temperature. Some other diseases like a late blight which is caused by the growth up of temperature, and it is controlled by using fungicide.

Effects of Rising of CO_2 Level- It affects both the host and the pathogen. More fungal spore is the result of the increase of carbon dioxide which disturb the pathogen growth. Production of plant biomass occurs due to the increase of carbon dioxide. Some biotrophic diseases like rust are caused by the increase of the carbohydrates in the tissue because accelerate photosynthesis with the increase of carbon dioxide (*Chakraborty et al., 2002*). Conidia reproduction decrease when the CO_2 will increases and sporulation also increase under a high concentration of carbon dioxide.

Adaptation and mitigation strategies

Expectations and evaluations of an increased occurrence of environmental limitations throughout the world, as well as changes in ecozones, may be indicators of global warming-related changes that are already taking place (Milly *et al.,* 2002; Root *et al.,* 2003; Elad *et al.,* 2014). Regardless of changes caused by socioeconomic factors, ranchers should adapt to changing environments in the coming years by using a variety of agronomical procedures that currently work well in current environments, such as changing the planting situation, collecting tasks, substituting cultivars, and any other vital changing or changing. (Rosenzweig *et al.,* 2007). Under hotter environments, yields will develop quicker, coming about in less time accessible for collection of carb and grain construction. As well as changing planting methodologies and cultivar type, land the board frameworks could be adjusted to new environment situations. Converts rainfed to irrigated agriculture is the easiest way, resisting the fact that matters of water accessibility, cost, and contest from altered areas should be thought of (Tubiello *et al.,* 2002; Rosenzweig *et al*., 2004;Elad and Pertot, 2014).

Climate change impact on pest of some crops:

Climate variations impact not just plant disease, but also plant pest activity, as they do not affect pathogens. The rising and falling temperature impacts replicating, developing, and surviving the plague. The increase in the temperature in particular from 1-2^{0}c is considerably expected to result in the addition of more life cycles each season per season *(*Yamamura and Kiritani, 1998). The insect plague is more hazardous in plants than presently, because of climate change (Coakley *et al.,*1999). Severe pests can be observed in India. These pests are comparable to white grub—paddy Uttaranchal, rice leaf blast, and maize borer in Andhra Pradesh; yellow mite, sugar cane wolly aphides, flaming chile, red-gray borer pod, soybean brown, and others. The damage caused by spotted sorghum stump borers has resulted in an unusually severe drought. The frequency of 10c bullfighting increases by 4.17 percent as the temperature rises. For generations, climate changes are increasing the number of plant-insect pests. Adaptation and Mitigation Strategies for Pest Management in a Changing Climate: Environmental change may be regarded as an interrupted cycle of classical approaches performing the current risk and moderating the expected risk due to the effects of environmental change. It is on their physical, social, and economical resources that local publics can alter their pesticide practices. Prospective adoption plans to reduce the risk of spreading the illness and plague and to lessen the adverse effect have been identified.

Sensitization of Stakeholders about Climate Change and its Impacts: India, like all the other emerging countries, advises growers, extension workers, and many others concerned in the management chain about the pest's negative impact on vital crops as a result of climate change. This can be accomplished through promotion, training, and other means. Individual sensitivity is a term used to describe the degree to which people are sensitive to certain things. Breeding climate-resilient varieties: A new type that is resistant to stress has to be created to reduce the impact of climate change. This way, the chances of pests having effects on the plants are lower and the plants will thrive and yield high. Alternation in sowing dates: Research is needed to modify the interaction of host plants in early, late, and normal planting by which farmers may manage and improve their output from the pests.

Rescheduling of crop calendars: Insome cases, the crop rotation will not effective to control the pest, Thus, the agricultural calendar needs to be changed based on climatic change. The infestation of the pest can thus be managed by this technique. The most essential element is soil testing**.** This way we can understand the current soil components and the soil component. Control methods such as IPM for the control of the pest attacks under climate change should be monitored and used. GIS-based risk mapping of crop pests: A geographical information system is the best crop management program that is best for the entomologist to control the pest attack in a different way which is beneficial to farmers.

Climate change impact on Live Stocks

From around the world, the climate is expected to have a significant effect on animal reproduction. Among a variety of environmental variables, heat stress is one of the most important elements impacting animal productivity (Koubkova *et al*.,2002). Impact on livestock health: The impacts of climate change on farm animal health have not been well investigated. Climate change, particularly global warming, is expected to have a significant impact on the health of animals, just as it has on people Animals from the farm. The expanded pathogen continuum raises the animal's illness susceptibility and hence promotes the causative agent's pathogenicity. Climate change affects the animal by different types of disease by the vector, pathogen, and host, etc. Vector**-**Diseases spread by vectors such as indicators like mosquitos, lice, and mites are impacted by variations in temperature and rainfall. Foot and Mouth disease is a severe illness that develops in Andhra Pradesh as a result of temperature fluctuations in livestock. Hosts-When a disease is less severe in younger generations than in older people, the infection is frequent or endemic, and there is lifetime immunity following infection, the illness is said to be endemic. Climate change in Africa in certain important animal illnesses, including several tick-transported diseases, will harm them. (Eisler *et al.*, 2003).

Impact on Feed and Fodder Availability: India occupies a vital position in the livestock population and for their feeding purpose, the major factors are crop residue, weed, tree, leaves, open field for grazing (Dikshit *et al.,* 2010).Climate change has an impact on livestock productivity because it changes the amount and quality of feed available. Climate change is predicted to alter grassland species composition (and hence biodiversity and genetic resources), as well as fodder digestibility and nutritional quality(Thornton *et al*. 2009). If there is a deficiency will occur in the amount and quality of feed to the livestock then it will affect the system. Male reproduction – Several difficulties in male reproduction are identified due to climate change. These issues include reducing sperm motility, increasing the number of dead sperm, increasing aberrant sperm morphology, decreasing testosterone levels, etc.Climate change in women's reproductive causes many difficulties. The difficulties are like reducing fetal growth, reducing oxygen and embryonic water supply, reducing the proportion of growth, decreasing the estrus length. As a result, the animals will be unable to reproduce. Furthermore, the commodity we obtain from cattle will be insufficient. For example, if the cow does not produce enough milk, the farmer who relies only on the sale of cow milk will suffer.

Adaptation and mitigation strategies for climate change on livestock

The vital approval procedures of the impact of climate change on live stocks are –(a) Alteration in production and management system, (b) Breeding strategy

Alteration in production and management system**-**It involves diversification of livestock animals and crops, a mix of livestock with the agroforestry (IFAD, 2010*).*When livestock are subjected to temperature and precipitation conditions, diversification of livestock and crop kinds can enhance drought and heat wave tolerance, as well as increase livestock output. It helps the livestock animal to interpretation to the temperature. It helps to increase the resistance capacity of animals and plants related to the negative impact of climate change(Smith *et al.,*2012). Agroforestry helps to keep the environmental condition, increase production, and mainly maintain the carbon sequence in the environment.

Change in the breeding strategies-Modifying reproductive tactics is generally necessary to enhance productivity. The disease resistance capability of farm animals increases as a result of heat stress (Henry *et al*.,2012).The cattle sector may utilize a variety of technologies to reduce greenhouse gas emissions (Gerber *et al.,* 2013). The level of deforestation increases the carbon level in the atmospherethus, the rate of afforestation must be increased to maintain it (Carvalha *et al.,*2014). This method boosts production while also making plants easier to cultivate. Similarly, erosion control and conservation tillage can help to store soil's organic carbon. It also helps to maintain a level of methane emitted from livestock. Enteric fermentation- The production of hazardous methane gas through practices like animal feeding and genetics. To reduce by feeding antibiotics which is increase the gain and feed intake (Boadi*et al .,*2004) Manure management-The emission of methane gas from cow dung is enormous, thus aerobic digestion reduces the amount of methane gas. increase the system for waste management, keep the storage duration, etc.

Climate change impact on Aquatic Ecosystem and Production of Fisheries

The effects of climate change on aquatic, terrestrial, and air biology, fertility, growth, and biodiversity are well acknowledged. The primary drivers of marine ecological change are over-exploitation of fish resources, pollution, and climate change. Climate change is causing major changes in aquatic ecosystems. Increase in Sea level rise: By 2200, the thermal spillover of saltwater is predicted to increase the sea level by roughly half to 1 mowing to climate change, such as the melting of glaciers. so that it would not be able to take over the area where a significant quantity of delta-area is found such as in Bangladesh, China, Egypt, etc. Because of the increase in the level of the water numerous species of water will be at risk. Some aquatic species such as an anointing pond, belanak, etc. are more affected by climate change. Ocean has become more acidic: The variables of human activity, such as an increase in fossil fuel burning, contribute more carbon dioxide to the water and make the water acidic. The pH level of water and water chemistry will be reduced so that the animals cannot easily survive so that they are severely affected. More severe storms and precipitation can pollute coastally: More water will evaporate due to the changes in climate so that the ocean water warmer. So, when the more moisture-laden meets into a storm system it produces more precipitation. So, if more rainfall will occur in the coastal area, then it increases the runoff and flooding and as a result, it pollutes the water, so the marine animal can't survive well.

Economic crisis of the fish farmers due to climate change: Climate change has had a significant impact on the fish industry, as well as the fish themselves. As a result, individuals who rely on the sea or rivers to make a living are experiencing an economic crisis as a result of climate change. Many various tragedies are witnessed in the sea as a result of climate change, indicating an economic dilemma for fish producers. Fish Population: So according to research studies, the ocean and river fish populations have been declining in recent years. According to a recent survey, global climate variables have a significant impact on the fish population. Because the relationship between climate and fish ecosystem is fragile, it has an impact on fish species' life histories. At this moment, several fish species are on the verge of extinction.

Adaptation and mitigation strategies for climate change in the Aquatic ecosystem

Acidification is mostly caused by excessive levels of carbon dioxide in the atmosphere. So, to manage acidification, afforestation should increase, not decrease, the temperature of the atmosphere, since as the temperature rises, the seawater warms, affecting the life of aquatic plants. Avoid dumping industrial waste, chemicals, and other harmful

elements into rivers and oceans, as these damages the ecology (Shankar *et al*., 2020). Coastal and marine ecosystem management, conservation, and restoration. All governments must also create policies and programs to mitigate the harmful effects of climate change on aquatic ecosystems. A scientific study was conducted.

Climate Change Effect on Forest Ecosystem

These days, the world's climate is changing at an alarming rate. As a result, environmental changes have a major influence on forest ecology, altering tree growth, reproduction, and other factors. The advantages that people get from the natural processes that maintain the environment are referred to as ecosystem services. The changes in forest ecosystem can be classified under various category like: Invasive species: Invasive species suffer from the effects of climate change. Due to forest fires, a large number of invasive plants that are vital for therapeutic purposes are also destroyed. As a result, there is a scarcity of medication being manufactured. As a result, patients are unable to obtain adequate medication. Forest Fire: Fire is a significant effect of climate change as heat rises. It influences the activity of wildlife and speeds up the formation of nutrients. Forest fire will benefit in equilibrating and adversely affecting forest carbon on soil nutrients, and in reducing the macronutrients of the soil. As a result of climate change, the plant and its forests will disappear.

Forest disturbances under climate change: Changes in the atmosphere have an impact on the entire forest ecosystem, as well as the structure and function of the forest. Climate changes have a massive effect on development and repair. This action is mostly triggered by temperature changes. The decreased availability of soil moisture causes a reduction in regeneration activity. Climate change contributes to the deterioration of natural forest resources while also contributing to the growth of greenhouse gases such as carbon dioxide in the atmosphere. Effects of climate change on forest processes: Changes in the atmosphere have an impact on the entire forest ecosystem, as well as the structure and function of the forest. Climate changes have a massive effect on development and repair. This action is mostly triggered by temperature changes. The decreased availability of soil moisture causes a reduction in regeneration activity. Climate change contributes to the deterioration of natural forest resources while also contributing to the growth of greenhouse gases such as carbon dioxide in the atmosphere.

Adaptation and mitigation strategies for climate change in the forest sector

To mitigate the the environmental change and improvement of biodiversity the upgradation of vegetation and land under forest area plays an important role. In this vein, environmental change may cause irreparable harm to unique regional biological systems and biodiversity, resulting in the extinction of a few animal species both locally and worldwide. Because of genuine or expected climatic upgrades and their consequences on typical and financial frameworks, variation is changing in regular or human frameworks. The people around villages must get enough education and knowledge about the tree species and awareness on cutting of trees in the forest. Awareness should bring among the people and address the negative impact of the reduction of the forest. By the “Bonomohatsava” events encourage the people to afforestation and teach the people not to set fire to the forest and not cut the tree in the forest. mate shift will impact ecosystem composition (like the forest) and various mitigation and adaptation measures into various sections for easy understanding and are described as: Factors such as socioeconomics, culture, politics, and geography. Information and knowledge support, use of technology, public participation are the important tools to get a healthy forest and a good biodiversity. The exotic tree species like *Acacia auriculiformis, Acacia mangium, Albizialebbek,* and *Casuarina equisetifolia* used for reforestation purpose. The multipurpose tree species are *Albizia lebbek, Leucaena leucocephala, Azadirachta indica, Artocarpus heterophyllus, Gliridiasepium, Prosopis cineraria and Sesbania grandiflora* etc also improve the soil deterioration and can improve the nutrient content of soil (Panigrahi,2020). To evaluate and compare the climatic protection performance between different country there is an index named as climatic control performance index is being developed.In 2019 index (Fig :2), where no country performed well enough to reach the ranking very good, meaning that no country has yet made it to one of the top three places in the rankings.

CONCLUSION

Agriculture is directly harmed by climate change since farming is largely dependent on the weather. The food output sector is most sensitive to climate change because climate change affects agriculture so agriculture production will reduce. Several adoptions and mitigation strategies are there to control the negative effects, but in some cases, the strategies are not so effective to control the effects. Climate change causes a significant economic loss, primarily affecting farmers. Farmers are strengthening their yields by using adoption and mitigation strategies. Greenhouse gases play a key part in climate change; if greenhouse effects are reduced, climate change will be significantly reduced, as greenhouse gases are the principal cause of climate change. An awareness program is the best way to control because this process can explain the negative impact of climate change on agriculture, health, water, forestry, land, etc. So, awareness programs can control the manmade activity to which is evolved in climate change. Due to fluctuation in the temperature, the humidity there are several diseases are come out in the livestock so the output from the livestock is also reduced. In cattle industry the climate change can be controlled via good management techniques. The pest and diseases are also increase due to increases in temperature, humidity, and other environmental factors which ultimately reduce the growth and development of plants. Climate change is a gradual and man-made phenomenon. The negative consequences of climatic change and its impact can be reduced by reducing the man-made activity and the planet will be safe. Soil erosion and soil fertility are also can be conserved and improved by reforestation and deforestation process.

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Table:1 List of some major natural and man-made causes for climate change.

Natural Causes	Manmade causes
Volcanic eruptions	Greenhouse gases
Ocean current	Deforestation
Solar deviation	Coal mining
Earth orbital tilt	Burning of fossils fuel
Forest fire	Agriculture
	Industrial procedure

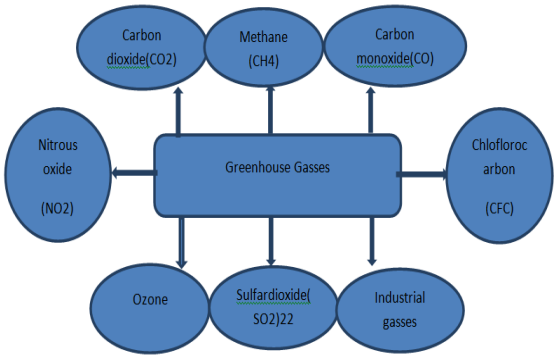
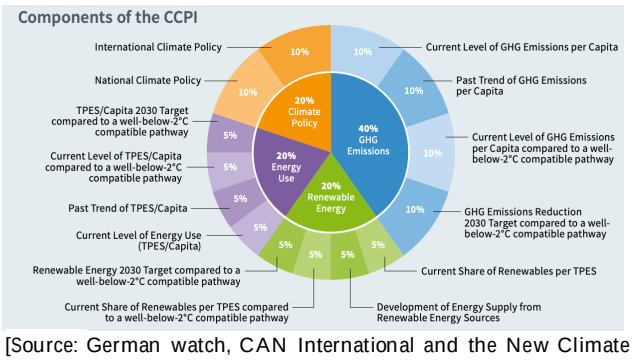


Fig.1: Various greenhouses gasses



[Source: German watch, CAN International and the New Climate Institute. 2019]

Fig: 2 The climatic control protection performance (2019).

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RESEARCH ARTICLE

A Review on Pollinator and Fauna Diversity Associated with Marigold (*Tagetes erecta* L.) – A Universal Trap Crop

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ABSTRACT

A review has been done in the entitle study "A review on pollinator and fauna diversity associated with marigold (*Tagetes erecta* L.) – Universal trap crop". The present review indicated that Marigold attracts a wide variety of pollinators, pest and natural enemies. Marigold also used as a perfect trap crop for the poly-phagous pest *Helicoverpa armigera* in different cropping eco-system. Both insect, non insect, *Apis* and Non- *Apis* pollinators were recorded by different scientist at different location of India. Thus, marigold flower can successfully be grown for aesthetic purpose, praying purpose and as management strategy (Cultural method).

Keywords: Marigold, Insect & Diversity

INTRODUCTION

The most commonly grown flower of India, Marigold is mostly used for different religious and social functions. Different types of marigold are used like African marigold (*Tagetes erecta*), French marigold (*Tagetes patula*) and Signet marigold or golden marigold (*Tagetes tenuifolia*) belonging to family Asteraceae bearing chromosome number of 2n = 24 and 28 in case of African and French marigold respectively propagated by seeds. Marigold basically a native of Mexico but now cultivated in several countries including India sold as loose or as garlands in the market. Marigold is also called as Genda phool in hindi (गेंदे का फूल), Gemda in Bengali (গাঁদা) and Gendu phul or Manda phul in Odia (ଗୋଣ୍ଡୁ). Marigold is specifically used for decoration and landscape use for its variable colour and height. Marigold has also some useful role in integrated pest management. They are used as the major universal trap crop

specifically against Americal bollwarm or gram pod borer, *Helicoverpa armigera.* The present review is collected to understand about the fauna associated with Marigold.

DISCUSSION

Suheel *et al.* in 2017 in their studies has recorded around 18 different species of arthropods associated with marigold belonging to nine different orders and sixteen different families out of which 10 were found to be damaging pests, 4 were found to be pollinators and rest of the 4 were found to be natural enemies or predators of the different pests.

Pests associated with marigold

Among ten different pests, Order Hemiptera was the dominant one comprising of four different genera of insects belonging to 4 different families *viz,* Aphid *Lipaphis erysimi* (Aphididae), Mealy bug, *Drosicha* spp. (Margarodidae), Lygus bug, *Lygus* spp., (Miridae) and Green sting bugs, *Nezara virdula* (Pentatomidae). Order Thysanoptera, Tromidiformea, Lepidotera, Diptera, Coleoptera and Orthoptera each contribute one species each *viz*, Thrips *Thrips tabaci* (Thripidae), Red spider mite, *Tetranychus urticae* (Tetranychidae), Pod borer, *Helicoverpa armigera* (Noctuidae), Leaf miner, *Liriomyza sativae* (Agromyzidae), Hadda Beetle, *Epilachna vigintioctopunctata* (Coccinellidae), Grasshopper, *Bycrotophus longicepes* (Acrididae) (Suheel *et al.* in 2017). Taleb and Sardar in 2008 has recorded tetranychid mites (*T. bioculatus*) as a serious non insect pest.

Pollinators associated with marigold

Sandhya *et al.* in 2020 in their studies conducted near Kathmandu valley, Nepal recorded 21 different species of pollinators belonging to fourteen families of four different orders *i.e.* Hymenoptera, Diptera, Lepidoptera and Coleoptera. They have recorded highest diversity during daytime followed by morning time and evening time. Their observed pollinators were belonging to four different orders where Order Lepidoptera was the dominant one comprising of 10 different genus of insects followed by Order Diptera (7 insects), Hymenoptera (3) and Order Coleoptera representing a single species of insect. Order Lepidoptera represented five different families where family Nymphalidae (*Ariadne merione*, *Pseudolus wedah, Neptis ananta ananta, Algais cashmerensis* and *Vanessa cardui*) was the dominant one followed by Pieridae (*Pieris canidia indica* and *Eurema blanda silhetana*), Eribidae (Nyctemera adversata), Hesperiidae (*Parnara guttata mangala*) and Lycaenidae (*Euchrysops cnejus*)

Order Diptera represented three different families where family Syrphidae (*Episyrphus balteatus, Episyrphus viridaureus, Eristalinus aeneus* and *Eristalis transversa*) was the dominant one followed by Calliforidae (*Calliphora vomitaria* and *Pollenia rudis*) and Muscidae (*Musa domestica*) Order Hymenoptera and Coleoptera represented a single family Vespidae (*Polistes* spp, *Vespa velutina* and *Polistes dorsalis*) and Nitidulidae (*Brassicogethens aeneus*) respectively. They have concluded that suitable foraging area for Order Diptera and Lepidoptera is urban area whereas urbanization may be lead to negative consequences towards wild bee population. They also emphasized for the pollinators' conservation for encouragement of biodiversity of endangered species and growth in agricultural production. Among four different pollinators, two species were belonging to the order Hymenoptera with the same family Apidae *i.e. Apis mellifera* and *Apis dorsata.* Another two pollinators were belonging to order Lepidoptera comprising of two different families *i.e.* Cabbage butterfly, *Pieris brassicae* (Pieridae) and Thistle butterfly, *Vanessa cardui* (Nymphalidae). (Suheel *et al.* in 2017). Shilpa et al. during 2014 in their studies conducted at the Aromatic Garden, Division of Horticulture, GKVK, UAS, Bengaluru has recorded about 13 different species of pollinators foraging on African Marigold (*Tagetes minutum*) belonging to two different Orders i.e. Hymenoptera and Diptera. Three species were observed from family Apidae *i.e. A. cerana indica, A. florea* and *A. dorsata*, from family Scolidae-1 (Scolia sp), Vespidae-1 and Anthophoridae-3) from Order Hymenoptera and Diptera (Sphecidae-1, Muscidae-1, Tachnidae-1 and Syrphidae-1)

Bista and Shivakoti during 2001 reported that marigold is highly suitable for the honeybees as source of pollen and nectar. Similarly, Shilpa *et al.* 2014 reported that three species of honeybee viz. *Apis cerana cerana*, *A. florea* and *A. dorsata* were attracted towards blooming marigold besides four Anthophoridas and individual species from family Vespidae and Scolidae. Comba *et al.* during 1999 observed that marigold were attracted by *Apis mellifera*, *Bombus lapidaries*, *B. terrestris*, two solitary bees, two hoverflies (*Sphaerophoria scripta*), two 7-spot ladybirds (*Cryptolaemus septempunctata* L.), a moth and a small tortoiseshell butterfly, *Aglais urticae*.

Natural enemies associated with marigold

Among four different natural enemies, three were insect species belonging to three different orders i.e. Diptera, Coleoptera and Hemiptera with three different families i.e. Syrphid fly, *Syrphus* spp. Belonging to family Syrphidae, Lady bird beetle *i.e. Coccinella septumpunctata* from family Coccinellidae and one Geocorid bug *i.e.* Big eyed bug, *Geoceris* spp.. One non insect natural enemy was also recorded *i.e.* Spider, *Oxyopes javanus* (Oxyopidae) (Suheel *et al.* in 2017).

CONCLUSION

The present review indicates that Marigold is an effective hub of pollinators, pest and natural enemies. Marigold is also said to be a perfect trap crop for the poly-phagous pest *Helicoverpa armigera*. So it can be understood that growing of marigold flower can be utilized as aesthetic purpose, for praying purpose, as a management strategy and as a hub for pollinators.

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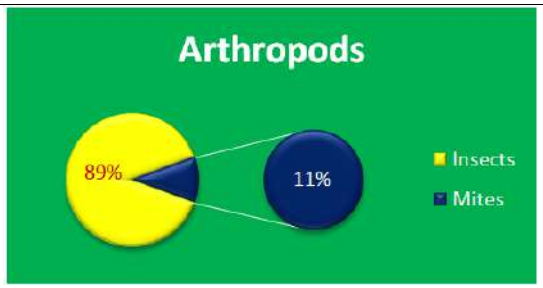
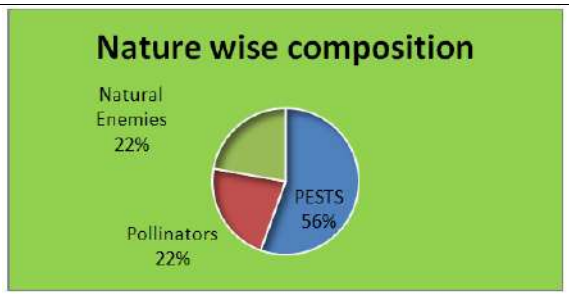


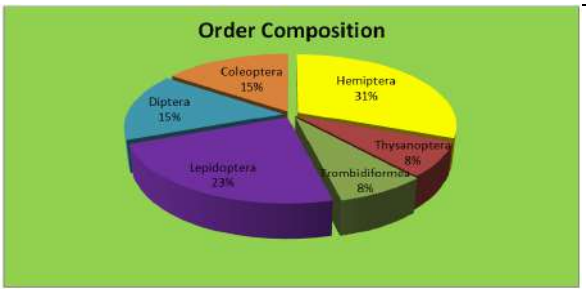
(Suheel *et al.* in 2017)

Fig.1.Arthropods



(Suheel *et al.* in 2017)

Fig.2.Nature wise composition



(Suheel *et al.* in 2017)

Fig.3.Oder Composition

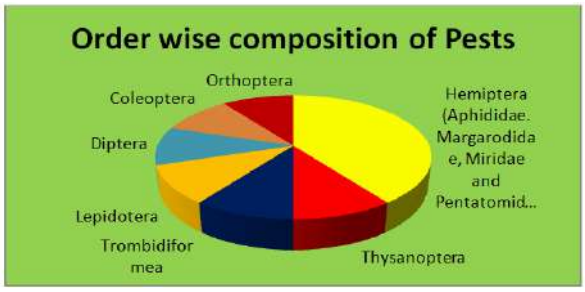
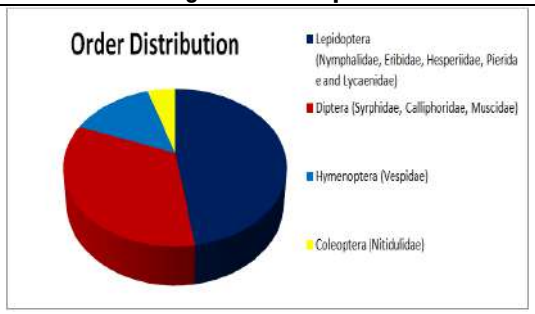
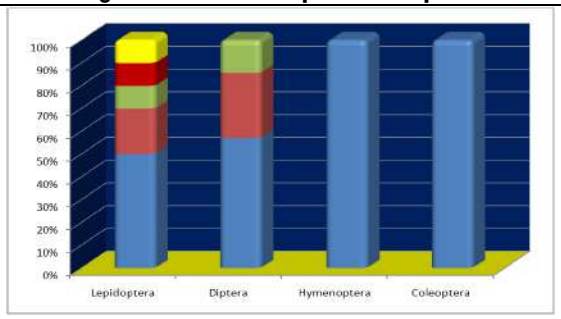


Fig.4.Oder wise Composition of pests



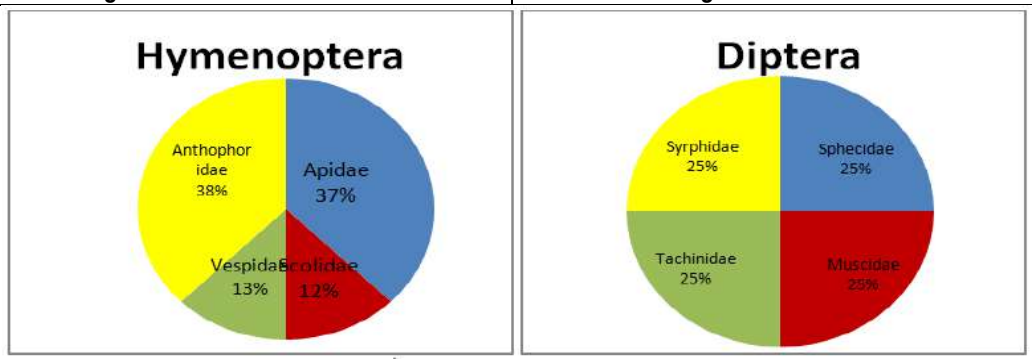
(Sandhya *et al.* in 2020)

Fig.5.Oder distribution



(Sandhya *et al.* in 2020)

Fig.6.Different Pollinators



(Shilpa *et al.*in 2014)

Fig.7.Pollinators associated with marigold

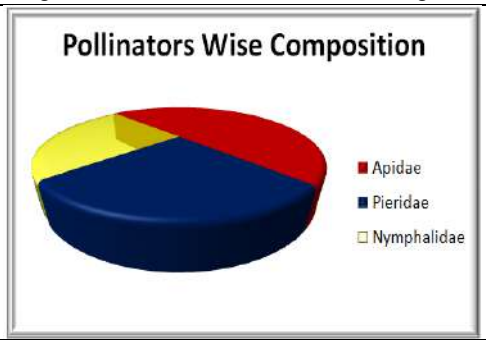


Fig.8.Pollinators wise Composition

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RESEARCH ARTICLE

Face Recognition using Raspberry Pi and Open CV

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ABSTRACT

Human face acknowledgment assumes a significant job in video observation, human-PC interface, customizing various applications. In this paper, the methodological way is dealt, which distinguish and recognize a face from the continuous stream that tracks a face and contrasts it and put away information of known people .Our approach completely ignores the background effect while recognizing a face of an individual. This approach also works on various conditions with different lighting effects which allow to execute results in large aspects of environmental conditions without getting any mismatches. Here a Raspberry Pi and different open source python libraries like open CV, NumPy are used. This framework utilizes Haar Course classifier for face detection in a picture and for facial recognition Local Binary Pattern Histogram (LBPH) is used.

Keywords: Face detection, Face Recognition, Open CV (Open Source Computer Vision Library), Raspberry Pi,Local Binary Pattern Histogram, Num Py.

INTRODUCTION

The process of Human identification is to spot a person based on their unique features. There are various types of human verification procedure are present on this commercial world, in which Password Verification Number(PIN) is mostly preferred .But this method is most likely to be theft or forgery because most of the people keep the pin with their date of birth or any special dates. So, the focus on Biometric has been increased due to its unique features [1]. The feature such as face detection, fingerprint, iris, palm etc. Human movement is a significant worry in a wide assortment of exercises, for example, human observation, human-machine interface, [2], [3], and face recognition based database management [4]. But in recent years, Artificial Intelligence and other sophisticated development had lot of applications which made recognition easier and economical.. So face recognition is recognizable proof for its business and law implementation applications. The meaning of facial recognition alludes to a subset of PC innovation that recognizes human faces in computerized pictures. The algorithm of face detection values on human

face in the image , which may also contains various other objects like landscapes etc .Open CV is most preferred algorithms for identifying .And some other algorithms using Open CV are LBPH, Eigenface, Fisherface.Now, the application of face recognition is increased in our lives. One of the practical applications where it can be implemented is in ATMs where the transactions can be made safe by using face recognition. The face capturing assessment with Exceptional Occlusion Handling (EOH). Is achievable on practical conditions [5]. And when it is used in security system, such as CCTV surveillance there is no need to see the whole video of the theft, but with the help of the face recognition, will show the parts of video where the crime took place. And face recognition is also very useful to include the quantity of individuals in a spot with the help of edge contrast and chromatic components to find people's face[6].If face recognition is used in educational institutes for attendance, it becomes more efficient and easy to record the student's attendance which is called as Personal Component Analysis (PCA).It also helpful for the faculty to maintain a record of students in-out information[7].Face recognition has lot of potential in hospitals and medical emergency, where we can track the movement of unauthorized access of patient[11].

System Architecture

In this system we used Raspberry Pi and Python is used for coding. And OpenCV and Numpy are the open source libraries of python are applied to process face recognition algorithm. The Haar Cascade classifiers are used to detect face from an image and the Local Binary Pattern Histogram (LBPH) are used to recognize a face from the given database. The whole process is categorized as:

- Detection and Data Gathering
- Training
- Recognizing

Detection and Gathering

As of late face detection is widely used due to its interaction and application in the world of computers. Face detection is also used to detect multi face irrespective of the background noise. The first step is to detect a face by using Haar Cascade classifier and then the captured information is stored in a dataset. Using Haar based cascade classifier for object detection is mostly used and implemented by OpenCV

Training

In this part, the trainer takes the information from the data set and able to specify persons. In the trainer system the input images are converted to grayscale format. Although the system does not need colour images but, it becomes difficult to identify the edges in colour images and considered as noise to the system. So, gray scaling is important, because it makes the image processing algorithm faster and efficient [9]. At the point when a RGB picture is changed over into a grayscale design the 3D pixel qualities are changed over into 1D value. Therefore, we should manage less information if gray scaling is utilized.

Recognisation

The camera takes the input through a video stream and imported to python code of the raspberry Pi as a 2D matrix. Numpy is used to processes this 2D matrix [10]. At first, the face of the person/image is taken avoiding the background and compared with sample photos stored in the trainer. If any match found, the name is displayed along with the square mark tracking the image.

System Performance

Face Detection

The main objective of the work is to identify/detect the face using Haar Cascade Classifier algorithm. The detected image is shown in blue square mark as in the following Figure.

Data Gathering

A database of different images is maintained and an authorized system is run to get the nearest identify of personalities in quick time. A few samples were gathered as shown in the following Figure.

Trainer

In this phase, all the user data is taken from dataset by using a specific OpenCV function. The result will be a .yml file that will be saves on a trainer "trainer/" directory. In this segment the images are converted into grayscale image to make the system fast and efficient. The 3D values are converted into 1D value.

Face Recognisation

In this segment, the image is taken as a input and checked with the samples present in the dataset which was stored, if the match found ,it will be tracked with a square along with the name and confidence level of matching, according to the python code written as the following Figure.

CONCLUSION

Security is the most concerned problem and this system can give wide impact on the lives of the general public. It has found a lot of applications in the present era of communication, where the numbers of users are increasing day by day. So an automated face detection classifier will be a proper solution .And it has a lot of applications in security, educational, health sectors.

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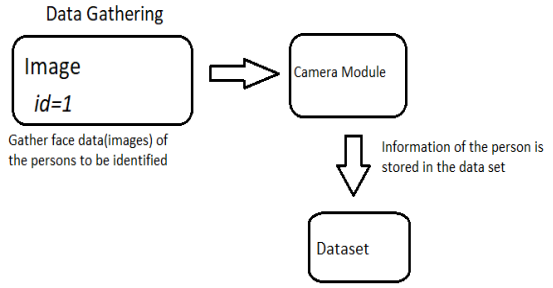

Figure 1. Detection and Data Gathering

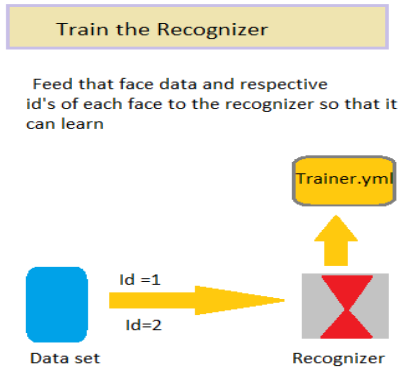

Figure 2: Training the Recognizer

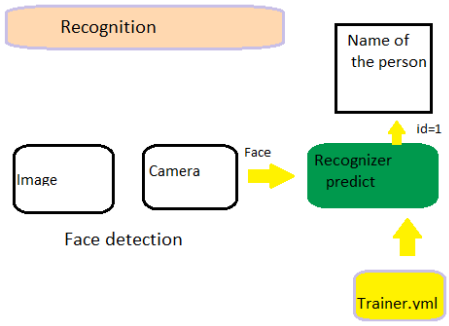

Figure 3: Recognition

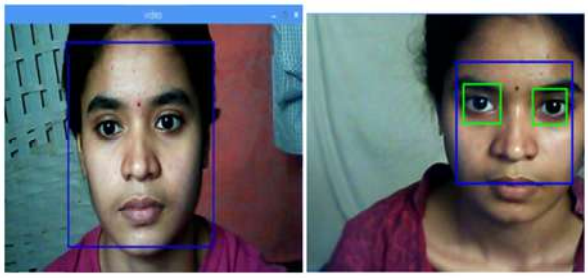

Figure 4: Detecting Human Face.

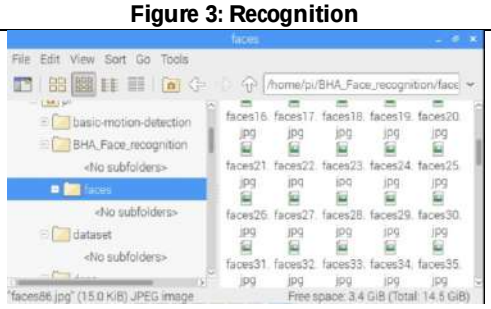

Figure 5: Gathering the Samples

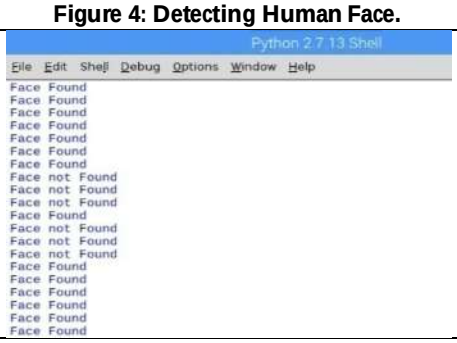

Figure 6: Detecting a Face

Figure 7: Converting from Colour Images to Grayscale

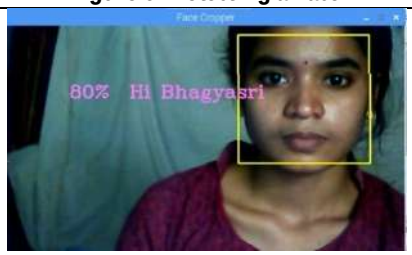

Figure 8: Recognized Face

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RESEARCH ARTICLE

Legumes in Cropping System for Agricultural Sustainability

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ABSTRACT

Achieving food security for a country like India is very important where day by day population is expanding rapidly. In agricultural and food systems, producing in a sustainable way is, thus, becoming increasingly of prime importance. Legumes play several important roles in agriculture as well as society by lowering the risk of climate change, reducing the net release of greenhouse gases into the atmosphere due to human activity, meeting the increasing demand of food and food and nutritional security of the smallholders. Legume crops are considered as vital food crops with several beneficial effects on human health and also play a prime role by providing multiple advantages towards agricultural sustainability. Hence, an attempt has been taken to look into the prominent presence of leguminous crops under the present consequences where agricultural sustainability is in top priority in the developing world.

Keywords: Legumes, food security, sustainability, biodiversity, soil health

INTRODUCTION

The population is growing by the day throughout the world and will reach tenbillion people in the next decades (Stagnari *et al*., 2017), posing plenty of global issues. Various types of cropping systems such as sequential cropping, mixed cropping, intercropping, relay cropping, ratooning etc. are to be taken into consideration for maximization of agricultural productivity (Ghosh *et al*., 2020; Maitra, 2020; Shankar *et al*., 2021a,b; Mondal *et al*., 2020). Cropping systems, an important component of a farming system, represent a cropping pattern used on a farm and how it interacts with farm resources, other farm operations, and available technology which define its building (Mohanta *et al*., 2021; Praharaj *et al*., 2021). In certain cropping systems, many crops are cultivated in the same field at the same time or at short intervals. This method that relies on climate, soil, and water availability must be developed in order to achieve maximum output levels by minimizing the use of available resources (Sairam et al., 2020;Hossain *et al*., 2021). The agricultural system should produce adequate food for the household, livestock fodder, and monetary revenue to cover domestic and cultivation costs. The adoption of intensive cropping might help achieve this goal. Intercropping and multiple cropping are two examples of intensive cropping techniques (Rana and Rana, 2011; Gitari et al., 2020; Maitra and Gitari, 2020).

Legumes belongs to Leguminosae family and consists of 800 genera and 20,000 species and among the all represents the third largest family among flowering plants (Meena *et al*., 2016). Legume crops have the potential to play a major role in this setting by providing numerous functions in accordance with sustainability principles. In addition to being a vital global source of high-quality food and feed, legumes help to minimise greenhouse gas emissions by emitting 5-7 times less GHGs(Greenhouse gases) per unit area than other crops (Stagnari *et al*., 2017; Palai *et al*., 2019). Sustainable agriculture refers to farming methods that can sustain their production and value to society indefinitely.

LEGUMES AND ITS ROLE IN FOOD SECURITY

Food security, climate change mitigation, and controlling growing energy consumption will all be major problems in the future years. In agricultural and food systems, producing in a sustainable way is thus becoming increasingly essential. As a result, legumes were created in order to improve food security and feed the world's undernourished people. Many varieties of legume crops are grown across the world, including groundnut (*Arachis hupogaea*), red gram (*Cajanus cajan*), chick pea (*Cicer arietinum),* horse gram (*Macrotyloma uniflorum*), soybean (*Glycine max*), green gram (*Vigna radiate)*, and black gram (*Vigna mungo*). Globally and regionally, concerns have been expressed regarding the sustainability of agricultural output at current levels due to a lack of additional crop production area and diminishing soil fertility. As a result, efforts for improving and maintaining agricultural production must focus on exploiting limited land and nutrient supplies more effectively than in the past. This goal can be fulfilled by introducing an extra population of a second crop by an appropriate change in the main crop's typical planting geometry, a practise known as intercropping (Renu *et al*., 2018).

Legumes are high in plant protein, fibre, carbs, vitamin B, iron, copper, magnesium, and zinc, and they play an important part in societal food security. Legumes are low in fat and saturated fat-free, and because they are produced from plants, they do not contain cholesterol. Legume has a low glycemic index and offers around 115 calories, 20g of carbohydrates, 7-10g fibre, 9-10g of protein, and 1 g of fat per serving (Polak*et al*.,2015).In developing countries legumes provide essential proteins, key vitamins, and minerals essential in the diets of millions of people (Otieno *et al*., 2020). Seed of different legume crop shows different chemical composition and the average energy content of legumes is330–374 kcal/100 g dry matter and depending on the species proteins content in legumes varies between 20 and 35% (Martin-Cabrejas, 2019).

When compared to cereals, legume has a high protein content and is abundant in the amino acid lysine as well as sulphur amino acids like methionine, both of which are scarce in most plant diets(Martin-Cabrejas, 2019).Due to the enormous benefits of legumes, it became the essential component of modern agriculture and it can bring a great change in maintaining the food security in the coming centuries.

LEGUMES IN CROPPING SYSTEM

For the concern towards multiple sustainable service in agriculture legumes plays a vital role. Legume crops are highly enriched in nutrition's consumed globally; as compare to other crop they release less greenhouse gases per unit area. Due to symbiotic relation with nitrogen-fixing bacteria in root nodules, Legumes are one of the appreciated crops in crop rotation. Many research has recently focused on pairwise study of legume and non-legume crops to identify rotational advantages and disadvantages to get maximum yields and benefits.

Legumes in sequential cropping

Lentils have been used in farming systems since prehistoric times. Legume is a natural mini-nitrogen producer in the field, and farmers can help increase indigenous nitrogen production by cultivating these crops (Ghosh *et al*., 2007). Legume assists in the solubilization of insoluble P in soil, the enhancement of the soil physical environment, activation of soil microbial activity, and the replenishment of the organic matter. In India, in which the average intake of plant nutrients from synthetic fertilizers is comparatively lower on a national scale, the potential for utilising direct and residual fertility related to legumes is noticeable. Legumes in sequential cropping plays a great role in increasing the monetary benefits to the farmers also supply nutrients to the other crops. When we cultivate

rice in sequential cropping after the harvest of rice the land remain unused for several months and there was less availability of the soil nutrients. So, after harvesting rice, we cultivate legume crops, which create a relationship with nitrogen-fixing soil bacteria, resulting in the formation of nodules in the plant roots, where those bacteria converted the atmospheric nitrogen to ammonia (NH_3) which the crop may use for its growth and food production. After the harvest of legume crops the residues of harvested crops were left behind than after, different primary grains and cereals were cultivated after those crops in that same field where paddy and legumes were cultivated and harvested and the grains and cereals absorb nitrogen from the soil and also there will be minimal number of synthetic fertilizers will be required to the crop.

The sequential cropping system with legumes helps farmers a lot in the time where the cost of nitrogen fertilizer is expensive and unavailable, then the farmers depend on the legume crops for the maintenance of N cycle. So, growing of legume crops is very much beneficial for the farmers in the recent days to get better yield.Green or brown manuring is the practice of returning plant debris to a soil which increases soil organic matter, improve soil fertility and reduce weed population. Green manuring is the process of ploughing any green manure crops, fragile twigs, or leaves when they are still green or shortly after flowering (Maitra *et al*., 2018). Green manuring, or the practice of cultivating herbaceous green-manure species in rotation with economically important crops, has been practiced for generations(Nair, 2005). The relevance of green manuring stems from the organic matter it adds to the soil, which is widely recognized as one of the most critical components for real soil fertility (Meena*et al*., 2019). Green manuring (GM) with legumes boosts soil nitrogen levels through biological nitrogen fixing. The most frequent GM crops are sunnhemp (*Crotolaria juncea*) and *dhaincha* (*Sesbania aculeata*), while *Sesbania rostrata* has the highest atmospheric N_2-fixing capacity and can totally replace urea-N in rice farming(Dwivedi *et al*., 2017). Some common examples of legumes used in green manuring arealfalfa, sunhemp, *Sesbania*, beans, sweet clovers, guar, lupins, vetches, peas, soybeans.

Brown manuring is a 'no-till' version of green manuring, in which a non-selective herbicide is used to desiccate the crop (and weeds) at flowering stage rather than cultivating or tilling (Maitra and Zaman, 2017).Brown manuring in rice is the process of cultivating *Sesbania* spp. and rice together, and then using a weedicide 2, 4-D to kill the Sesbania plants when they overrun the rice plants in height after around 25 days of co-culture. Brown manuring is the application of a post-emergence herbicide to green manure leaves, resulting in the loss of chlorophyll and the appearance of brown leaves (Tanwar *et al*., 2010).Legumes are preferred because, with the help of their nodule bacteria, they can fix atmospheric nitrogen and examples are Sunhemp, Dhaincha, Mung, Cowpea, Lentil. (Phukanand Bora,2012). Plant leftovers are kept on the surface to assist maintain surface cover and soil structure and the amount of organic matter in the soil has grown.

Legumes in intercropping

Legume a key functional group, provide high agroecological services(Duchene *et al*., 2017).To protect their revenue and profit, most farmers used an intercropping strategy, combining legumes with other crops. The goal of the legume-based intercropping method is to produce maximum output from a unit area while utilising the fewest accessible resources that a single crop or sole crop would not be able to use (Zhang *et al*., 2011, Ram and Meena, 2014; Maitra *et al*., 2019; Maitra and Ray, 2019; Manasa *et al*., 2018; Manasa *et al*., 2020).Because, legumes have the inherited capacity to fix nitrogen, they make the crop more valuable as a green manure crop, which is much more beneficial to the crop for their growth and development during the time of inter cropping, and nitrogen is mineralised from the residues left after the harvested legume crops (Maitra et al., 2020; Sarath Kumar and Maitra, 2020). As legume crops increase soil fertility through BNF (Biological Nitrogen Fixation), they also have the ability to minimise competition for available N in the soil owing to the more competitive nature of cereals and aid in the effective utilisation of available nitrogen (Layek *et al.,* 2018). Intercropping legumes with other crops such as grains gives a wide range of adaptability and aids in agricultural sustainability by providing higher output than a single crop. Without use of chemical herbicides, the practice of intercropping with legume can reduces the weeds with a reduction range of 20–

75% (Maitra et al., 1999, 2001; Lorin *et al*., 2015). Legume crops can also act like a living mulch, which are potentially outstanding and increase the competitiveness of the intercrop against the weed plants (Naudin *et al*., 2011).

LEGUMES IN SOIL HEALTH IMPROVEMENT

Crops require a sufficient amount of nutrients for optimal growth and development as well as optimum output. Nitrogen is the most critical nutrient for the crop, but when the soil is depleted due to the continuous application of inorganic fertilisers to the soil as well as the crops, the availability of nitrogen and all necessary nutrients decreases. By fixing nitrogen organically, legume crops provide a low-cost means of replenishing nitrogen in the soil, improving soil fertility and overall crop yield. Practice of continuous cereal-cereal system declined the productivity and pulses can check this trend by improving chemical, biological, and physical properties in the soil (Savci, 2012).
Legume-based systems boost soil fertility in various ways by increasing SOC and humus content, as well as N and P availability in soil and grain legumes can raise SOC in a variety of ways, including by contributing biomass, organic C, and N, as well as by producing hydrogen gas as a by-product of BNF, which supports the formation of bacterial legume nodules in the rhizosphere (Stagnari *et al*., 2017).

Soil physical properties

There are three main soil physical properties those are, bulk density, porosity, combination stability and soil texture and those properties are associated with water related methods such as aeration, runoff, erosion and infiltration rate Juma *et al*.(2005).By cultivating the legume crops it enhance the soil physical properties by becoming a soil conditioner (Lal, 2012).Those crops were grown as because it protects the soil from nutrient loss which were present in the soil and also helps in control of erosion. Legume crops helps in balancing the soil C/N ratio and also helps in preserving the soil organic carbon. Adding legume residues to the soil also plays a vital role in enhancing soil natural carbon and also helps in carbon sequestration from the atmospheric carbon dioxide (Ayraza *et al.* 2007).

Soil chemical properties

The soil pH, nutrient levels, and soil organic carbon content are the three primary causes for soil fertility. Leguminous crops improve soil chemical properties through nitrogen fixation and root biomass. A legume-based intercropping method aids in the adjustment of the pH of the soil's rhizosphere sector. Leguminous green manure is also an excellent source of organic matter for the soil. Green manure contributes to the increase of soil nitrogen and the renewal of soil natural carbon, as well as the enhancement of soil chemical characteristics. Bacterial siderophores present in root of legume crops can chelate heavy metals such as Al, Cd, Cu, Pb and Zn to form nontoxic complexes to plants (Yan *et al*., 2020). Crop rotation with legume crop enhance P nutrition of subsequent crop by improving soil chemical P availability and increased P uptake microbiologically (Alvey *et al*., 2001). Several legume crops tend to produce a vigorous taproot which reaches up to a deep under the soil surface, which open channels deep into the earth. Earthworms and the tunnels they make are influenced by nitrogen-rich legume leftovers. Root channels and earthworm burrows which increase the soil pores, and also promote air circulation and deep-water percolation.

Soil biological properties

Legumes are one of the most important components in any cropping system for increasing soil microbial biomass. Those crops contribute significantly to nitrogen cycling and soil organic matter decomposition, which aids in agricultural sustainability and production (Graham and Vance, 2000). Some of the microorganisms that participate in the leguminous cropping system in the zone known as the rhizospheric zone also contribute to crop growth enhancement and crop yield maximisation (Israel, 1987).

LEGUMES AND AGRICULTURAL SUSTAINABILITY

Legumes have significantly important role in nitrogen fixation, the release of high-quality organic matter into the soil, and the improvement of soil nutrient cycling and water retention. Because legumes may be utilised as a growing crop or as agricultural waste, they offer a high potential for conservation agriculture.

Biological nitrogen fixation

Excessive use of chemical fertilizers has thrown the nitrogen cycle into disarray, resulting in nitrate buildup in soil and water, as well as nitrogen oxide poisoning of the atmosphere. Chemical fertilizer replacements should be done on an emergency basis to preserve a sustainable agricultural system. Legumes use a microbiological process known as biological nitrogen fixation (BNF) to convert atmospheric nitrogen into a plant in useable form (Raza *et al*, 2020).Biological nitrogen fixation (BNF) first time discovered by Beijerinck in 1901 (Swain and Abhijita, 2013). Plants can assimilate NH_3 to produce the nitrogenous biomolecules (Wagner, 2011).After germination rhizobia present in the soil or added as seed inoculum invade the root hairs of legume seeds and move through an infection thread toward the root. The bacteria multiply rapidly in the root, causing the swelling of root cells to form nodules. BNF eliminates or minimises the requirement for synthetic nitrogen (N) fertilisers, saving fossil energy resources for fertiliser production and transportation, and indirectly lowering greenhouse gas emissions (Zander *et al*., 2016). The symbiotic fixation of atmospheric N_2 by Rhizobium species and legumes is a renewable source of N for agriculture and the estimated values for different legume crops are generally ranging from 200 to 300 kg of nitrogen per hectare per year (Zahran, 1999). The nitrogen fixation in root nodules of leguminous plants is synchronized with the rate of plant growth, as it is directly dependent on the translocation of carbohydrates from the leaves. Careful adjustment between supply and demand of nitrogen is the main reason for greater efficiency of symbiotic nitrogen fixation. The comparison of chemical and biological nitrogen fertilizers in terms of economic and environmental costs shows that BNF is a cost-effective, sustainable, and environment friendly option to meet nitrogen requirement of an agroecosystem.

N fertilizer demand and GHG emission

In agricultural systems based on mineral N fertilisation, legumes can reduce greenhouse gas (GHG) emissions such as carbon dioxide (CO_2) and nitrous oxide (N_2O) and also play a key role in carbon absorption in soils and reduce overall fossil energy inputs in the system (Stagnari *et al*., 2017). According to Jeuffroy *et al*. (2013), legume crops produce 5-7 times less GHG per unit area than other crops. They found that peas emitted 69 kilogramme N_2O per hectare which is significantly less than winter wheat (368 kg N_2O per hectare) and rape (534 kg N_2O per hectare).Legume-based green manuring reduces GHG emissions in two ways: first, by converting plant carbon to SOC, and second, by reducing the need for nitrogen fertilizers, resulting in fewer N_2O emissions (Nair *et al*. 2015).The crop residues derived of leguminous plants are supposed to be more efficient to stabilize soil carbon (Rani *et al*., 2019). When cultivated in rotation with rice, which is considered a "high input-high loss-high pollution" agricultural system, leguminous plants have also been proven to lower carbon footprints by regulating greenhouse gas emissions (Cai *et al*. 2018).

Potential N supply through legumes

Legume crop act as an economic as well as eco-friendly source of N supply to the soil and thus symbolize their potential to replenish nutritionally exhausted soils (Rani*et al*., 2019). According to some researchers, getting nitrogen from legumes could be more sustainable than getting it from industrial sources (Crews and Peoplesb, 2004).

Biodiversity enhancementandagricultural sustainability

Legume crop increased diversity in cropping system which also increased above and below ground biodiversity including decomposer invertebrates like earthworms and Collembola, as well as pollen- and nectar-gathering wild and domesticated bees and bumblebees (Zander *et al*., 2016). Legumes will continue to play an important role for supplying plant nutrients and establishing a self-regulating ecosystem which is based on a diverse range of organisms (Karnja *et al*., 2011). In order to remain productive and sustainable in the long run farming systems will be necessary to replenish the reserves of nutrients that are removed or lost from the soil (Kakraliya*et al*., 2018). legumes reduce both nutrient runoff and climate emissions by reducing fertilization through two mechanisms: biological nitrogen fixation during the year of legume cultivation and the residual nitrogen for the following crop (Lotjonen and Ollikainen, 2017). The cultivation of legumes significantly increases soil C sequestration as forage, green-manures and cover-crops which return to the soil large amounts of organic C and N their propensity to produce

more belowground biomass and drop leaves (Stagnari *et al*., 2017). Rhizobial metabolites such as riboflavin andlumichrome present in the root of leguminous plant are generally involved in chemical cross-talks leading to plant growth promotion and also improved immune response to biotic and abiotic stresses (Jaiswal *et al*.,2021). Crop rotation with legumes, rather than monoculture production, produces better economic and environmental results. Crop rotation with legumes also minimizes profit fluctuation caused by unpredictably changing weather (Lotjonen and Ollikainen, 2017). To reclaim the contaminated soils rhizobial siderophores are important for agricultural use and soil microbial communities which affect the sustainability of agricultural ecosystems, are supported bylegume based cropping systems (Karanja *et al*., 2011). Legume crops are also provided other benefits like break pest and disease cycles and weed management, improved soil quality, more effective fertilizer utilization (Rajala *et al*. 2006) and increased biological diversity in soil (Jensen and Hauggaard-Nielsen 2003). Improved soil quality and drought resistance through deep root systems, and give support for pollinating insects. When grain legumes were grown in rotation with cereal crops and oilseeds crops mineral fertilization, pesticide use, and cultivation costs decreased while productivity of subsequent crop improved (Nemecek*et al*., 2008). Many countries can improve their protein and nitrogen demand by increasing use of legumes (de Visser *et al*., 2014).

Different role of legumes on cropping system illustrated by Jensen *et al*. (2012) such as(a) the emission of the greenhouse gases such as carbon dioxide and nitrous oxide (CO_2 and N_2O) getreduced in leguminous crop comparison to emissions from nitrogen-fertilized crops,(b) reduce use of fossil energy for the production of food and feed, and(c) increases carbon sequestration in the soil.They also required fewer external inputs which is a key part of food-system sustainability: "Thus, legumes could play a critical role in increasing the sustainability of cropping systems at the farm level."

CONCLUSION

As we saw that cropping system with legume crops had a great role with each other and also had the beneficial effect on the crops which were grown with those different cropping systems which helped in the agriculture sustainability by fulfilling the needs of the human being and also by balancing the ecosystem and having minimal effects on the environment. Due to rising demand for plant products such as protein and oils, as well as increased economic and environmental constraints on agro-ecosystems, grain legumes are expected to play a significant part in future cropping systems.

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Table1: Nitrogen fixation by different legume crops

Grain legume	N-fixied (kg ha^{-1})
Black gram (*Vigna mungo*)	16-79
Mung bean (*Vigna radiata*)	19-54
Rice bean (*Vigna umbellata*)	13-30
Groundnut (*Arachis hypogaea*)	150-200
Pigeon pea (*Cajanus cajan*)	120-170
Cowpea (*Vigna unguiculata*)	14-35
Common bean (*Phaseolus vulgaris*)	20-60

[Sourse: Gogoi *et al*., 2018]

Table 2: Percentage of nitrogen fixedby different Legumes of their total requirement

Legume	Plant-N Derived from Atmosphere (%)	N Fixed Symbiotically (lb/A)
Alfalfa	**80**	**267**
Sweetclover	**90**	**223**
Faba bean	**90**	**267**
Field pea	**80**	**178**
Lentil	**80**	**134**
Soybean	**50**	**134**
Chickpea	**70**	**108**
Dry bean	**50**	**62**

[Source: Saskatchewan, https://www.saskatchewan]

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RESEARCH ARTICLE

Dehydration and Rehydration of Potatoes

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ABSTRACT

In this study, potato slices of 1mm,3m and 5mm) were carried out at 105°C in a tray dryer using hot air at a flow rate of 1.7 m/s and 10% relative humidity.. Also, the drying rate was found to increase with temperature and the blanched slices dried faster than unblanched slices. Dryinghas effect on the product quality for index. Longer blanching time and lower drying temperature resulted in better colour retention and led to chips of lower browning index. It was also established that blanching reduced the hardness and shrinkage of the product; however, the use of different blanching periods did not show much affect on the product hardness.

Keywords: Dehydration of potatoes, rehydration potatoes, blanching of potatoes.

INTRODUCTION

Potato is rich in enzyme namely peroxidases and being a high moisture food cannot be sun-dried, as the traditional sun drying is a slow process and susceptible to fungal growth. It may also result in the loss of product quality from colour degradation, microbial growth and poor rehydration etc. Tray dryer for drying of potato cubes was used. The potato slices spread on trays at an acceptable thickness so that the product can be dried uniformly. Heating may be produced by hot air stream across the trays. Dehydration involves simultaneous transfer of heat and mass in which heat penetrates into the product and moisture is removed by evaporation into an unsaturated gas phase. Owing to the complexity of the process, no generalized theory currently exists to explain the mechanism of internal moisture movement. Dehydration is the most convenient method of preserving perishable. The main objective pursued in this paper is to investigate the single layer drying behaviour of potato slices experimentally in a convective Tray dryer and also to perform mathematical modelling by using single layer drying models in the literature. Drying experiments of potato slices with the thicknesses of 0.5,1 and 2 mm were conducted at inlet temperatures of drying air of 50, 60 and 70 °C and with drying air velocities of 1 and 1.5 m/s. It was concluded that potato slices with thickness of 1 mm would dry perfectly in the range of 460–740 min, while those with thickness of 0.5 mm would dry in the range of 280–520 min in these drying conditions in the convective type Tray dryer.

The experiments were conducted at 50, 60, 70°C for controlled and blanched sample of potatoes in a tray dryer. Fresh potatoes were peeled, washed in clean water and sliced uniformly into three thickness (i.e., 1mm, 2mm, 5mm of thickness) and labelled which was used for moisture loss determination at different temperatures. The present study examines blanching of potatoes caused starch gelatinization which sealed the dehydrated tissues. Incomplete gelatinization of starch contents caused lumpiness when reconstituted. Forced drying also produced other undesirable effects.

REVIEW OF LITERATURE

The literature related to drying and dehydration of Potato slices and related research findings

Ghosh and Gangopadhyay (2004) studied the effect of drying methods and rehydration kinetics of potato slices at 10°C, 35°C and 60°C. Low temperature dehydration was found to improve the rehydration characteristics of potato slices.

Olawale and Omole, (2012)conducted experiments on drying of sweet potato slices between 50C and 80C in a tray dryer using hot air at a flow rate of 2.5 m/s and 10% relative humidity.. The eight models investigated fitted theexperimental data of the six sweet potato samples between 50°C and 80°C adequately.

Ibrahim et al., (2012). Reported that the experiments were conducted on potato slices with thickness of 8 mm at 65°C with an air velocity of 2.0 m/s.Potato slices were pre-treated with citric acid solution (1:25 w/w, 3 min, 20C) or blanched hot water (3 min, 80C)prior to drying. Besides, the untreated samples were dried as control. The shortest drying time was obtained with potatoes pre-treated with citric acid solution.

Bailon, (2017) Unblanched potato cubes (0.9 × 0.9 cm) were dehydrated in a multi-tray dryer with and without draft tube.Potato cubes dried in tray dryers with temperatures used for drying in the annular region were (60, 70 and 80 °CNo constant period was observed in the drying curve during the drying process, for potato cubes dried in tray dryers.

MATERIALS AND METHODS

Raw materials

Fresh Potatoes (Solanum tuberosum) purchased from the market of Paralakhemundi. It was taken care that all the potatoes taken have nearly the same size and shape i.e., big and round which makes it easier for slicing.

Chemicals Required

Distilled water used for all blanching purposes.

Peeling and Slicing

The potatoes were thoroughly washed and then peeled carefully by using peelers the peeled potatoes were then sliced using different slicers to obtain different thickness of slices. Three different slices of three thicknesses (1mm, 3mm, and 5mm) as shown in plate no. 3.4.

Sample Preparation of Potato slices

The washed and sliced potatoes were kept in water to prevent blackening. After all slicing was done then slices were taken out and spread on a towel and excess water was wiped, then it was taken for hot water blanching using distilled water (Plate No.1).

Blanching

The potato slices are immersed in distilled water at 98°C for 4-5 minutes. This temperature and time are very essential to maintained and are monitored using thermocouple and stopwatch. After the time the water is strained out by the help of a strainer. The blanched slices are put into a tray with ice and water and the temperature is lowered to room temperature rapidly. This stops the cooking process if it's initiated in the slices. Then the potato slices are taken out and placed on a cloth or towel and extra water is wiped out. Lastly the slices are measured using weighing balance and each sample is exactly kept at 600gms. For the control samples blanching is not required it is directly kept in tray dryer for drying.

Tray drying

The trays of the dryer were first weighed before loading the sample and then again weighed after loading the sample. The dehydrated Potato slices were taken out from the tray dryer as show below.

Observation of dehydration of potato slices

The time and temparature readings of potato slices were taken for every 30 minutes by taking the trays out weighing them and turning the potato slices and placing them inside the tray dryer. The weight of the sample was calculated by subtracting the weight of the tray from the recorded value. This procedure was repeated for 6 hours.

Packaging of De-hydrated Sample

The dehydrated potato samples were immediately put into foodgrade polythene packs. One pack for each sample and were labelled accordingly. While packing care was taken that after filling in the sample excess air in the pack was fourced as much as possible then they were sealed. The polybags were stored in dessicator to prevent any further moisture absorption.

Rehydration of the Sample

The Dehydrated sample was then re-hrdrated at different temperatures. Each sample at three different temperatures namely 10°C, 30 °C and 60 °C for 6 hours. The re-hydration process was carried out in a water bath with beakers and in the refridgarator for the 10°C temperature effect. The increase in weight was recorded and graphs were plotted.

Measurement of moisture content :

After that weigh the sample and take the reading. The moisture content is determined by weighing the sample before and after the drying and determining the difference.

Initial Moisture content = $\frac{initial\ weight - oven\ dried\ weight}{oven\ dried\ weifht} * 100$

With the readings obtained by the experimentation were plotted on graphs to show the drying time curve and measure the moisture loss during the period.The graphs were plotted by taking the horizontal axis or x- axis as time which is in minutes and the vertical or y-axis was taken to be moisture content.

Observation

The graphs show the dehydration of different samples of different sizes, namely A (thickness 1mm), B (thickness 3mm) and C (5mm) each at 50°C, 60°C and 70°C in both blanched and unblanched conditions. These graphs show three curves constantly going down with the increase in time. The moisture content decreases with increasing drying time. The time taken to dry each sample to 10% moisture content can be found out. Out of the 18 different conditions plotted in 6 graphs show that size A has the best dehydrated product at 50°C, average product at 60°C and a below average product at 70°C.Size B has the best dehydrated product at 60°C, average products at 50°C and 70°C. Studying size, C has the best dehydrated product at 70°C, average product at 60°C and a below average product at 50°C. In all conditions the unblanched sample differ by a little margin than the blanched ones. But it can be clearly pointed out that blanched samples showed better drying quality.The unblanched samples turned black and looked very ugly

while the blanched samples retained their colour after drying. Despite of the looks all the samples were edible (if normal water was used in place of distilled water).

RESULTS AND DISCUSSION

From the graph it was concluded that the initial moisture content of the potato sample was 82% and it was gradually reduced to 10% moisture content by drying at 60°C. From the graph it was concluded that the initial moisture content of the potato sample was 82% and it was gradually reduced to 10% moisture content by drying at 60°C. The graphs show rehydration of samples of three different sizes taken earlier as A 60mm x 40mm x 1mm), B (60mm x 40mm x 3mm) and C (60mm x 40mm x 5mm) (each dehydrated at 50°C, 60°C and 70°C in both blanched and unblanched conditions. The rehydration is done at three different temperatures 10°C, 35°C and 60°C. These graphs show the rehydration of all possible 54 conditions in 6 graphs. Each graph plots 6 curves that show rise in moisture content with increase in rehydration time.

This analysis of the graphs show that the samples dehydrated at lower temperature (50°C) show most rehydration as compared to 60°C and 70°C. The samples rehydrated at 10°C show slightly more rehydration ratio as compared to the 35°C and 60°C. So, the sample showing the highest rehydration rate was A(50°C) rehydrated at 10°C and A(70°C) rehydrated at 35°C, the same counts for both blanched and unblanched samples. While comparing blanched and unblanched samples the branched samples show more rehydration ratio than the unblanched samples.

From the graph it can be concluded that the initial moisture content of the sample was 10% which increased to different moisture contents depending on the various conditions when rehydrated at 10°C for 360 mins. From the graph it can be concluded that the initial moisture content of the sample was 10% which increased to different moisture contents depending on the various conditions when rehydrated at 10°C for 360 mins.

CONCLUSIONS

The Thin- layer potato slices have been dehydrated at temperatures of (50^{O}, 60^{O}, and 70^{O}) and rehydration temperature (10^{O},35^{O} and 60^{O}) with sizes of 1mm, 3mm and 5mm. The conclusion drawn from the results are as follows

- The time of achieving desired moisture content (10% w.b) was observed to increases with thickness and the mass of the slices decreases with drying temperature. The blanching of the potato slices enhanced the drying rate.
- Samples dehydrated at high temperature could not absorb more water as compared with low temperature dehydrated of tray dryer sample.
- During Dehydration, it was observed that the degree of shrinkage of potato slice by the low temperature drying was greater by-high temperature drying.

The structure destruction increased with temperature as reflected by rehydration ratio. However, the qualities of the dried slices were found to be good.

SUGGESTION FOR FUTURE WORK

- Blanching of Potato can also be done using a variety of solutions to determine which solution is more or less effective.
- Research for dehydration and rehydration of other fruits and vegetables can be conducted by using this experiment as a reference.
- Though good feeds have been achieved in drying technology there is a need for further development of cost effective and energy efficient methods of drying.
- An accurate method for determination of the parameters used for calculating those indices should be developed.

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Plate No.1 Blanching of potatoes using distilled water

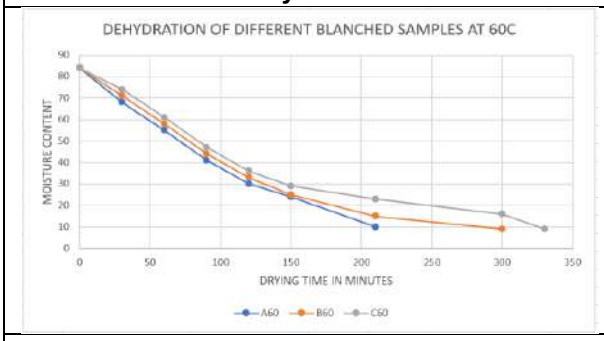
Plate No. 2 Potato slices after dehydration in tray dryer

Plate No. 3 Observation of potato slices before and after dehydration

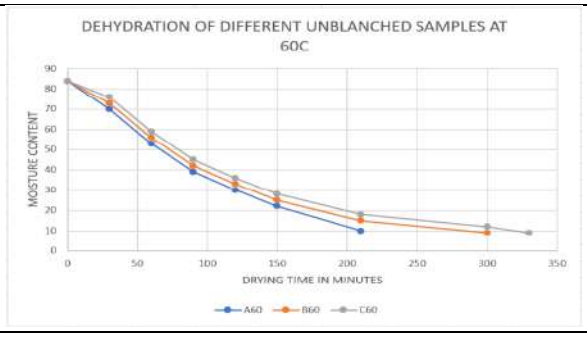
Plate No. 4 Packaging of Blanched Potato slices

Plate No. 5 Potato slices inside the water bath for Rehydration

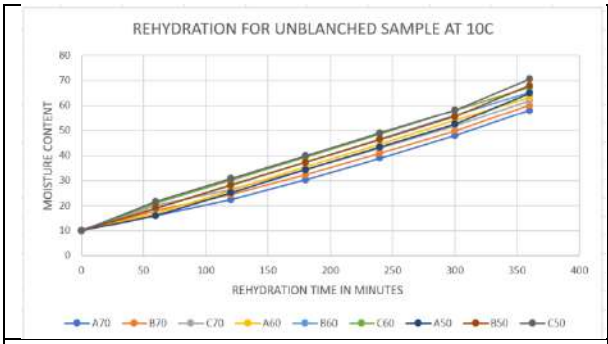
Plate No. 6 Potato sample after the Rehydration



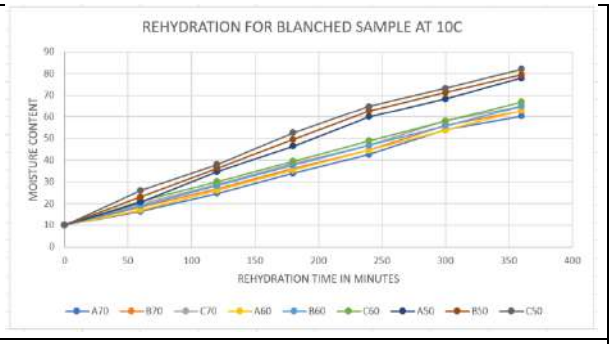
Graph.1. Dehydration of Different blanched sample at 60°C



Graph.2.Dehydration of different unblanched sample at 60°C



Graph.3. Rehydration for Unblanched sample at 10°C



Graph.4.Rehydration of blanched sample at 10°C

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RESEARCH ARTICLE

Estimation of Irrigation Water Requirement of Zucchini Squash (*Cucurbita pepo* L.) under Protected Cultivation Structures and in Open Field Conditions

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ABSTRACT

Zucchini (*Cucurbita pepo* L.) is one of the members of Cucurbitaceae family. It also regarded as a highly polymorphic vegetable. Indian climatic condition has unique advantage of commercial cultivation ofexotic vegetables. Zucchini can be grown round the year adopting different protected cultivation techniques such as drip irrigation, plastic mulch, poly house and shadenet house. Protected cultivation methods provide protective measures for successful production of off-season summer squash for early market around the year. Information of accurate amount of water required to raise the crop round the year using different protected cultivation methods are inadequate. Therefore study conducted to estimate the irrigation water requirement of Zucchini crop under different protected cultivation methods. Crop water requirement was estimated using the FAO-56 Penman-Monteith equation considering the 3 years locally recorded weather parameters. The Zucchini crop grown in open field requires 32 per cent more irrigation water in comparison to the crop grown in field covered with plastic mulch. Study also reports Zucchini crop requires up to 30 per cent less irrigation water to grow in polyho use in comparison to shadenet house conditions.

Keywords: Polyhouse, Shadenet house, Plastic mulch, drip irrigation, evapotranspiration

INTRODUCTION

Zucchini (*Cucurbita pepo* L.) or locally called summer squash is belonging to family *cucurbitaceae* and is originated from tropical America. It is fast growing exotic vegetable crop and slowly becoming popular in India. It may be consumed as cooked vegetable or as salad in the immature stage. It is a rich source of vitamin C and minerals. It can be cultivate throughout the year with small scarification of yield during the extreme weather conditions (Singh and Saxena, 2020). The Zucchini crop can be cultivated during extreme weather conditions using

protected cultivation methods such as drip irrigation, plastic mulch and protected cultivation structures (poly house and shadenet house) with higher productivity (Maitra et al., 2020). Drip irrigation systems can provide water and nutrients directly to the crop at the root zone, results in higher yield with saving water by increasing the irrigation efficiency (Santosh et al., 2017). Various biotic and abiotic factors influence the productivity of exotic vegetables. Use of plastic mulches in Zucchini cultivation offers a reliable and practical solution to the problems under the various climatic conditions (Sing et al., 2014).Cultivation of vegetable under polyhouse and shadenet house permitted to control the micro climatic parameters of structures partiallyto get early and off-season yield.

Information on precise amount of irrigation water require to raise zucchini crop is one of the important factors to harvest greater yield under different protected cultivation methods. The FAO-56 Penman-Monteith equation can be used to calculate the crop water requirements of Zucchini crop under different protected cultivation methods. Required weather parameters to calculate the crop water requirements were measured and recorded for consecutive three years (2018-2020). Current study aims to determine the irrigation water requirement of the Zucchini crop under drip, plastic mulch, poly house and shadenet house.

MATERIALS AND METHODS

Current study conducted to estimate irrigation water requirement of Zucchini crop under different protected cultivation methods using the climatic data measured and recorded data from Meteorological Department, Centurion University of Technology and Management (CUTM) Paralakhemundi. The meteorological site is located on the flat land at18°47' N latitude, 84°06' E longitude and altitude of 116 m above mean sea level. The irrigation water requirement of the crop mainly depends on the water transpired by the plant and the amount of water evaporating from the soil surface. The daily irrigation water requirement of a Zucchini crop grown under different protected cultivation measures can be estimated by using the following equation

$WR = ET_0 x\ K_c\ x\ Wp\ x\ A$

where,
WR = Crop water requirement (L day^{-1})
ET_0= Reference evapotranspiration (mm day^{-1})
K_c = Crop co-efficient (Table 1)
Wp = Wetting fraction (taken as 1 for close growing crops)
A = Plant area, m^2 (i.e. spacing between rows, m x spacing between plants, m).

The daily meteorological data recorded for the years 2017-2020 were used to compute reference evapotranspiration (ET_0). The modified Penman-Monteith method suggested by Allen et al.(1998) was used to compute reference evapotranspiration (ET_0). The crop evapotranspiration (ET_c) value will vary for Zucchini cultivation under plastic mulch and will be reduced by 10% to 30% depending on the number of irrigation. The K_c value of plastic mulch applied Zucchini crop at the initial stage (K_{cini}) is often low as 0.10 (Allen et al., 1998). The K_c value at different stages of growth and under different protective measures were tabulated in Table 1.The product of daily reference evapotranspiration value (ET_0) and crop growth stage-wise Kc values gives the daily ET_c values.

RESULTS AND DISCUSSION

Reference evapotranspiration (ET_0) values for local climatic conditions were estimated using FAO-56 Penman Montieth equation for three different years (i.e. 2018, 2019 and 2020) and values were averaged to calculate the crop evapotranspiration (ET_c). Estimated ET_0 values were tabulated in Table 2. Maximum daily average ET_0 values were found in Mayand the lowest values were found in December, which was the trend that got followed during all the

three years. Maycoincides with the dry season characterized by dry wind and high temperature. On the other hand, during the winter season, reference evapotranspiration (ET_0) tends to fall to a minimum value of 2.2 mm day^{-1} in December. This decrease in $ET_0$is due to lower temperature and reduced solar radiation during this period. Average ET_0values are gradually increasing from January to May (2.5 mm to 5.2 mm) further, which was decreasing in trend from June to December (4.3 mm to 2.2 mm).Zucchini crop usually grown round the year, therefore irrigation water requirement of the crop can be found out for different crop seasons. This is around 90 day's crop and it can be grown in any month of the year. Figure 1presents the ET_0 values of different crop season for the different years. From the Figure 1, it can be found that maximum ET_0 values computed for the February-April and May-July crop season.

The product of ET_0 and K_c values gives crop evapotranspiration (ET_c) and product of ET_c and plant area gives the irrigation water requirement (WR). The Table 3 presents the values of monthly average of daily ET_c and WR for Zucchini crop grown in open field condition and surface covered with plastic mulch for different growing season. Total irrigation water requirement (WR) of Zucchini crop grown in open field was estimated as 40.1 L day^{-1}, 65.9 L day^{-1}, 74.6 L day^{-1} and 51.4 L day^{-1} for November-January, February-April, May-July and August-October crop duration respectively. Whereas the total WR for Zucchini crop grown with plastic mulch was estimated as 27.5 L day^{-1}, 49.2 L day^{-1}, 50.7 L day^{-1} and 35.2 L day^{-1} for November-January, February-April, May-July and August-October crop duration respectively. Irrigation water requirement of Zucchini crop under plastic mulch for various crops season was 32 percent less in comparison to the WR requirement for growing zucchini in open field condition without applying plastic mulch. The saving of water from the application of plastic mulch was found for other crops (Tiwari et al., 1998 and Tiwari et al., 2014).

The Table 4 presents the values of monthly average of daily ET_c and WR for Zucchini crop grown under polyhouse and shadenet house conditions for different growing season. Total irrigation water requirement (WR) of Zucchini crop grown under shadenet house conditions was estimated as 46.8 L day^{-1}, 88.9 L day^{-1}, 91.4 L day^{-1} and 79.5 L day^{-1} for November-January, February-April, May-July and August-October crop duration respectively. Whereas the total WR for Zucchini crop grown under polyhouse was estimated as 34.4 L day^{-1}, 60.4 L day^{-1}, 74.6 L day^{-1} and 62.1 L $day^{-1}$for November-January, February-April, May-July and August-October crop duration respectively. Water required to grow Zucchini in shadenet house requires up to 30 percent more water in comparison to zucchini grown in polyhouse conditions. Same results were reported by Santosh et al., 2017 for lettuce crop.

CONCLUSION

Cultivation of Zucchini crop under protected cultivation methods requires less irrigation water in comparison to the open field condition. More than 32 percent seasonal water requirement needed to grown Zucchini in open field without applying plastic mulch in comparison to applying plastic mulch. Growing Zucchini in polyhouse reduces irrigation water up to 30 per cent in comparison to grow under shadenet house conditions.

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Table 1. Crop coefficients and plant spacing of a Zucchini crop under drip, plastic mulch poly house and shadenet house

Sl. No.	Protected cultivation measures	$K_{c\ ini}$	$K_{c\ mid}$	$K_{c\ end}$
1	Open field condition	0.50	0.95	0.70
2	Plastic mulch	0.10	0.81	0.60
3	Polyhouse	0.50	0.95	0.70
4	Shadenet house	0.50	0.95	0.70

Table 2. Monthly average of daily reference evapotranspiration (ET_0) during the study years (2018-2020) for open field condition.

Month	Reference evapotranspiration ET_0, mm			
	2018	2019	2020	Average
January	2.5	2.5	2.4	2.5
February	3.2	3.0	2.9	3.0
March	4.2	4.0	3.3	3.8
April	5.1	5.2	4.8	5.0
May	5.3	5.4	5.0	5.2
June	4.4	4.4	4.3	4.3
July	3.6	3.6	4.0	3.7
August	3.4	3.4	3.6	3.5
September	3.3	2.8	3.2	3.1
October	3.0	2.8	2.8	2.9
November	2.9	2.6	2.7	2.7
December	2.2	2.2	2.3	2.2

Table 3. Crop reference evapotranspiration (ET_c), mm day^{-1} and irrigation water requirement, L day^{-1} of Zucchini crop for different crop seasons in open field conditions

Months	Open field		Plastic mulch	
	ET_c, mm day^{-1}	Irrigation water requirement, L day^{-1}	ET_c, mm day^{-1}	Irrigation water requirement, L day^{-1}
November -January season				
November	1.3	0.34	0.3	0.07
December	2.1	0.53	1.8	0.45
January	1.7	0.43	1.5	0.37
Total	160.3	40.1	110.0	27.5
February -April season				
February	1.5	0.38	0.3	0.08
March	3.6	0.91	3.1	0.77
April	3.5	0.88	3.0	0.74
Total	263.7	65.9	196.7	49.2
May-July season				
May	2.6	0.65	0.5	0.13
June	4.5	1.12	3.8	0.95
July	2.6	0.65	2.2	0.55
Total	298.5	74.6	202.7	50.7
August -October season				
August	1.7	0.43	0.3	0.09
September	2.9	0.74	2.5	0.63
October	2.0	0.50	1.7	0.43
Total	205.4	51.4	140.9	35.2

Table 4. Crop reference evapotranspiration (ET_c), mm day^{-1} and irrigation water requirement, L day^{-1} of Zucchini crop for different crop seasons under polyhouse and shadenet house conditions

Months	Shadenet house		Polyhouse	
	ET_c, mm day^{-1}	Irrigation water requirement, L day^{-1}	ET_c, mm day^{-1}	Irrigation water requirement, L day^{-1}
November -January season				
November	1.6	0.41	1.2	0.30
December	2.3	0.57	1.8	0.44
January	2.2	0.54	1.5	0.37
Total	187.4	46.8	137.7	34.4
February -April season				
February	2.1	0.52	1.4	0.34
March	5.2	1.29	3.5	0.87

April	4.4	1.11	3.1	0.77
Total	355.5	88.9	241.7	60.4
May-July season				
May	3.0	0.75	2.3	0.57
June	5.0	1.25	4.4	1.10
July	3.9	0.97	3.0	0.76
Total	365.6	91.4	298.5	74.6
August -October season				
August	2.5	0.63	1.9	0.49
September	4.9	1.23	3.7	0.93
October	2.9	0.73	2.4	0.60
Total	318.1	79.5	248.6	62.1

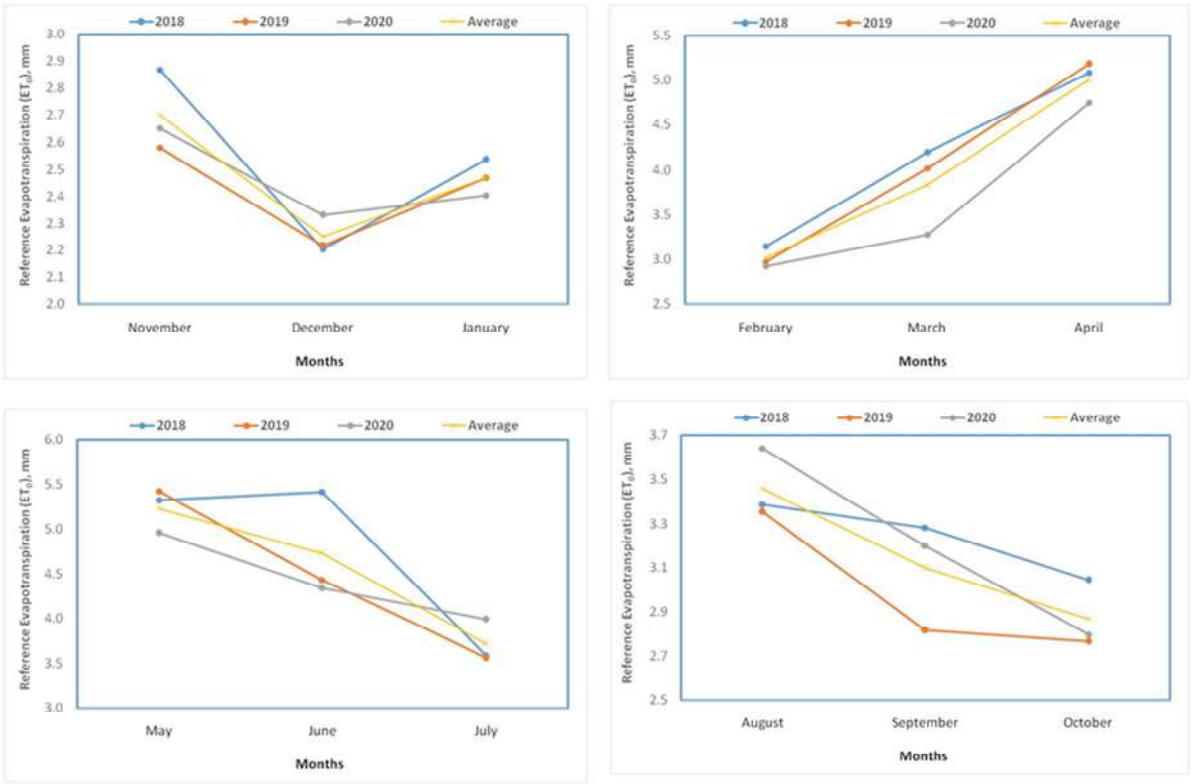

Fig 1. Monthly average daily reference evapotranspiration (ET_0) of different Zucchini crop duration (November-January, February-April, May-July and August-October)

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RESEARCH ARTICLE

Soil Moisture Conservation Techniques for Dry land and Rainfed Agriculture

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ABSTRACT

In the present agriculture scenario, the focus of feeding the increased population revolves around optimizing the water productivity within the land use. One of the important aspects of improving water productivity is soil moisture conservation. Mainly in dry land and rainfed agriculture the conservation of soil moisture is an essential aspect to avoid moisture deficient in the soil. In the arid and semi-arid regions though the rainfall is adequate for crop growth, the dry spells with unusual rainfall distribution during the critical growth stages of the crop can hamper the yield by 50-60%. Hence to avoid the yield loss and to maintain crop stand with optimum yield, some effective soil moisture conservation techniques like mulching, deep tillage, compartmental bunding and basin listing are to be adopted for successful crop production. These soil moisture conservation techniques can also improve the soil properties and reduces soil erosion and degradation.

Keywords: Soil moisture conservation, tillage, rotation, cropping systems, Rainfed

INTRODUCTION

Dry land and rainfed agriculture has a distinct place in Indian agriculture, occupying around 108 m ha area (75%) out of 143 m ha net cultivated area. Despite considerable progress in irrigation development since 1950s, 85.6% of coarse cereals, 83.8% of pulses, 42%of rice, 74.1% of oilseeds and 64.7%of cotton are still cultivated as rainfed. Dry land and rainfed agriculture contributes 40% of food grains production and supports half of the human and two-third of the livestock population. Dry land farming is characterized by low crop productivity and high variation in yields from year to year which are caused due to various factors including water availability(Patil *et al.,* 2014). Due to the increased population in India and increased inter-sectorial competition for fresh water, there is a push to use less water in agriculture (Zaman *et al*., 2017; Maitra and Pine, 2020). The water demands for urban people are also increasing day by day. Under this circumstances soil moisture conservation is one of the major approaches for improving agricultural productivity (Namirembe *et al*., 2015). Soil moisture conservation can be further

described as the method for prevention of moisture loss from the soil to attain high agriculture productivity. The primary goal of soil moisture conservation is to reduce the quantity of water lost from plants via transpiration and from the soil through evaporation or combined evapotranspiration (FAO, 2003). Soil moisture conservation is the primary method for reducing crop irrigation requirements and there by maintaining the essential water for agricultural output (FAO, 2003). To increase the moisture availability to the soil and increase the water infiltration rate, it was recommended to follow some soil moisture conservation techniques that are further discussed in the topic. In addition to different soil moisture conservation measures many agronomic practices like SRI (system of rice intensification), DSR (direct seeded rice), aerobic rice etc. are also promoted to improve water productivity and water use efficiency (Mohanta *et al*., 2021; Midya *et al*., 1021a, b).

TECHNIQUES OF SOIL MOISTURE CONSERVATION

Out of many different techniques, only a few techniques which conserve soil moisture have been discussed below (Figure 1).

Deep tillage

Tillage is one of the important conservation practices in which soil become porous and allows maximum water to infiltrate in to the deeper layers by minimizing the runoffs, nutrient loss and improves the crop productivity (Das et al., 2010). Deep tillage is often followed in heavy soils to break the hard pan which further improves the soil physical properties. The deep tillage is generally carried out with a depth of 25to 30 cm for every two to three years interval. In dry land and rainfed agriculture the soil get hardened when it is kept fallow during off season and summer season. In this instance the occurrence of rainfall can increase the surface runoffs and leads to soil erosion. In this regard, the deep ploughing can help to absorb maximum rain water to the soil during the monsoon period and retains the soil moisture for the crop growth (Philips, 1984). The deep furrow reduces velocity of runoff and increases the infiltration time there by incorporating maximum amount of water in to the soil.

Conservation tillage

In conservation tillage the tillage operations are limited to the single line where the crop is sown. In this practice the leftover stubbles of the previous crop is left on the field and with minimum disturbance to the soil the field is ploughed only at the sowing zone (Reddy, 2019). Tillage should be reduced or, in severe cases, eliminated to maintain healthy soil organic levels, to conserve the resources and deduces the evaporation loss by not disturbing the stubbles of the harvested crop. Conservation agriculture can improve the soil's ability to absorb and retain water (Sunday *et. al.,* 2011). Some studies says that conservation tillage especially without any tillage operations serve as an effective tool for retaining soil moisture content(Gosai, *et. al.,* 2009).

Compartmental bunding

This method is one of the cost effective rainwater conservation practice suitable for rainfed vertisols having slope of less than 1% (Chaudhry *et al.,* 2004). In this method the entire field has been divided into small predetermined-size compartments to retain rainwater where it falls and avoid soil erosion. In compartmental bunding as the water flow is limited to small areas the runoffs are avoided and Bund formers are used to create compartmental bunds (Reddy, J. 2019)**.**this practice enhances enough rain water moment in the soil profile for raising *rabi* crops on the conserved moisture Several studies have found that compartmental bunding is beneficial for in-situ moisture conservation and enhancing vertisol profile moisture (Patil*et. al.,* 2015). Further study says that compartmental bunding is more effective to increase the yield under rainfed and dry land farming conditions. (Kalhapure *et. al.,* 2014).

Mulching

Mulching is one of the important agronomic practice that has direct influence on hydro-thermal regimes of soils by suppressing the weeds, conserving the soil moisture, reducing water evaporation, modifying physical environment of soil and improving soil fertility (Yoo-Leong *et al*., 2003).It is a technique that promotes plant growth and

development by increasing nutrient availability, changing soil temperature, and improving moisture conservation (Kher *et al.,* 2010). Mulches can either be Organic- peat, wood chips, straw, or Inorganic mulch in the form of plastic sheeting (Figure 2). Some studies concluded that mulching improves the growth, flowering, fruit production, and minimal weed population in agronomic and vegetable crops (Table 1) (Iqbal *et. al.,* 2015). Organic mulch will also be more effective inimproving the water use efficiency by minimizing the evaporational loss from the soil (Danish *et. al.,* 2017).

Basin listing

It is a method of building soil and conservation basins with a unique tool known as a basin lister. These basins have been created across the slope. The listing of the basins provides the maximum time for rainfall to penetrate the soil **(**Reddy,2019**).**When the basin lister, broad bed former, and chisel plough were tested against the standard approach for moisture conservation in dry farming, the basin lister resulted in 11.0 percent increase in crop output when compared to the typical summer ploughing method. Basin listing increases surface depression storage of precipitation, thereby potentially reducing storm runoff and increasing soil water storage availability to crops (Jones *et. al.,* 1990).

Crop rotation

Crop rotation is the practice of growing different types of crops successively on the same plot of land over a year. Incorporation of legumes in cereal crops can result in reduced soil erosion, restore soil fertility and conserves soil moisture. It minimizes insect and weed infestation, optimizes soil nutrients, and promotes soil health. Rotating shallow-rooted crops and deep-rooted crops in cropping sequence can be effective for utilizing the soil moisture at different depths(Agriinfo, 2015). Some studies conclude that crop rotation will increase the moisture level in soil and increased yields in rainfed farming (Thierfielder*et. al.,* 2010).

Strip cropping

In this cropping system different crops are sown in alternate strips to prevent erosion and moisture loss of soil. The inter-tilled rows of close-growing segments slow down runoff and filter out the soil particles that are washed off the ground. Strip cropping is also a kind of agronomical practice in which ordinary crops are planted in the form of relatively narrow strips across the land slope. These strips are so arranged that the strip crops should always be separated by strips of close growing and erosion resistance crops. This runoff management also increases the possibility of water penetration, resulting in increased moisture in the soil. (Prem, *et. al*., 2017). Some studies suggest that strip cropping will Increase the yield, and due to less spacing, closer growth of crops, it will also increase the soil moisture more than other cropping systems. (Neduchezhiyan, *et. al.,* 2010).

Hydrogels

Hydrophilic gels/ hydrogels are physically or chemically cross-linked polymers that absorb significant amounts of water without dissolving in soil and making a large volume of an aqueous solution (Jakku ,*et al.*,2020). Hydrogels are hydrophilic in nature and contain carboxylic groups, enabling them to bind cations and adsorb water. Many factors affect the absorptive capacity of hydrogels for water which includes the tolerance of hydrogels to ionic solutions. Wichterle and Lim (1960) were the first to publish research on hydrogels (Table 2). These gels increase water retention capacity and WUE while minimizing soil erosion, agricultural run-off, and surface leaching. Some results revealed that seed coating with hydrogel resulted in the highest grain and stover production, as well as the highest water consumption and water usage efficiency (Singh, H.*et. al.,* 2012). (Yang,*et. al.,* 2018).

BENEFITS OF SOIL MOISTURE CONSERVATION

Soil moisture conservation practices plays a significant role in crop production under dry land and rainfed farming systems. Proper crop management practices along with adequate supply of water can improve the yields in rainfed crops. In rainfall deficit areas by proper conservation of soil moisture the *rabi* crops can be taken successfully with residue soil moisture from the previous *kharif* crop (Iqbal et al., 2015). Conservation techniques can also have other

beneficial techniques like weed management, protection of the soil surface from the inadequate consequences of heavy rain, runoffs, soil erosion and increased nutrient distribution to the soil, depending on the moisture-conserving method used (Manjeet *et al*., 2017). These methods can further involves in reducing water requirement and improving water use efficiency. It reduces waste management requirements by recycling organic waste and using plant and agricultural residues through decomposition in the soil.

BARRIERS OF SOIL MOISTURE CONSERVATION

Crop wastes are sometimes utilized as animal feed in some areas, providing extra investment for soil protection.Soil moisture conservation measures may not be used in all regions since growing new crops via mulch or other crop wastes may be challenging in non-mechanized agriculture (Gosai *et al*., 2009). The residue burning in the crop field is a barrier to use crop residues for in-situ soil moisture conservation. Some of the moisture conservation techniques like plastic mulching and strip cropping cannot applicable for all the crops. The techniques which involved high mechanization are not cost effective for small scale farmers. Less awareness about conservation agriculture practices among many farmers is also a key barrier for implementing moisture conservation practices.

CONCLUSION

Soil moisture conservation techniques are necessary to maintain optimum soil moisture for supplying the required quantity of water and essential nutrients to the crop plants and maintain physical, chemical and biological properties of soil. Different techniques are followed in different regions. The choice of appropriate technique depends on soil, topography, climate, and the farmer's choice. These techniques can also help in attaining agriculture sustainability in dry land and rainfed farming systems. However, all the moisture conserving techniques have some advantages and drawbacks. According to some experimental findings, the combination of different soil moisture conservation techniques is a better approach than following a single technique.

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Table 1. Mulching treatments in crops

Sl. no.	Crop	Types of mulch used	Outcomes
1	Wheat	Plastic and straw	Increases the yield
2	Maize	Legume mulching: sunhemp, Leucaena twig mulch, sunhemp+ Leucaena	Control's soil erosion, reduces weed population
3	Pigeonpea	Paddy straw mulching, sugarcane trash mulching	It increases the yield by 2.07tonnes per hectare
4	Sugarcane	straw and stubble mulching	It will reduce the weed occurrence

Source: (Chopra *et. al.,* 2020.)

Table 2.Soil application rates of hydrogel

Sl.No.	**Soil type**	**Dose of hydrogel**
1	Semi- arid and arid regions	3-6 g/kg of soil
2	All levels of water stress treatment and for improved irrigation period	2.5- 3.0 g/kg of soil
3	For improving Relative Water Content (RWC) and Leaf Water Use Efficiency (WUE)	0.5- 2 g/ pot
4	Delay Permanent Wilting Point (PWP) in Sandy soils	0.3- 0.5 g/kg or 0.8% of soil
5	For reducing 50% of irrigation water in loamy soils	3- 6 g/plant pit
6	For reducing drought stress	0.3- 0.6% of soil
7	In order to prohibit drought stress completely	230- 310 kg/ha of cultivated area
8	For decreasing water stress	4% by weight

Source: Hydrogel: innovative in situ moisture conservation (Jakku*et al.*, 2020).

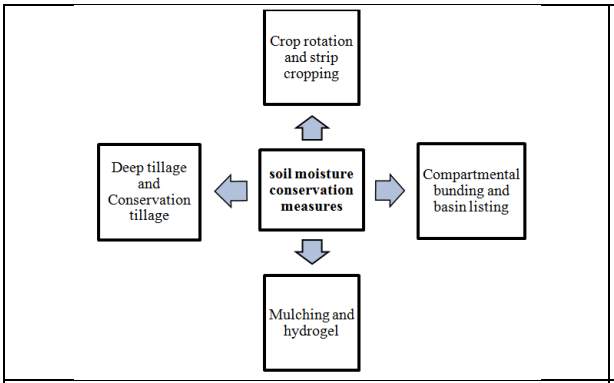

Figure 1. Soil conservation measures

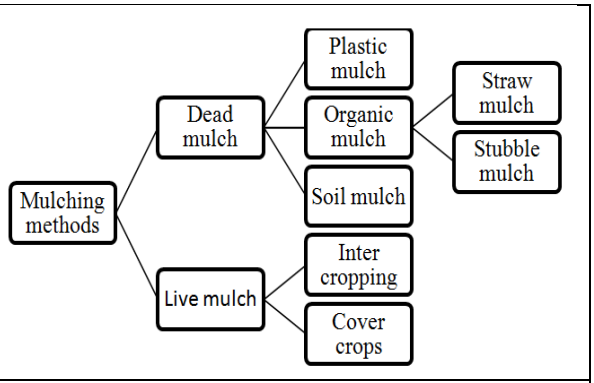

Figure 2. Types of mulches flow chat

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RESEARCH ARTICLE

Design and Implementation of an Efficient Multiplier for Fir Filter

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ABSTRACT

An infinite response filters (FIR) structure basically used for implementing any kind of frequency response digitally. FIR filters basically uses multipliers, delays and adders to obtain the filter output. While implementing theses filters the power, area and timing are main parameters which affect the overall performance of the design. In this paper ASIC implementation of an efficient multiplier for FIR filter is designed by adopting modified Booth encoding algorithm for reducing power consumption, area and delay. Simulation results are carried out in Xilinx ISE and presented to validate the design.

Keywords: FIR, Multiplier, Adder, Xilinx, Controller

INTRODUCTION

A finite impulse response (FIR) whose impulse response (or response to any finite length input) is of finite period, as a result of it settles to zero in finite time. This is often in distinction to infinite impulse response (IIR) filters, which can have internal feedback and will still respond indefinitely (usually decaying) [1]. An FIR filter with nth order has N number of delay stages with N+1 taps. By using convolving the input signal x[n] with impulse response LTI system output y[n] can be determined. Basically for FIR filters output is obtained by weighted sum of past input values. Mathematically it can be written as

$$y[n] = b_0 x[n] + b_1 x[n-1] + ...b_N x[n-N] = \sum_{i=0}^{N} b_i x[n-i] \quad (1)$$

The main limitation of FIR filter is that it uses considerably high computation power in processors as compared to infinite response filter with same characteristics such as selectivity with low cutoff frequency [2-4]. In this paper an attempt is taken to modify the hardware structure by modifying the multiplier structure by implementing a modified Booth encoding algorithm.

ALGORITHM OF PROPOSED MULTIPLIER:

In general multipliers have major role in implementing the filter structures as the performance parameters such as area, delay and power of the design depends on multiplier structure. The multiplications generally use the shift and add algorithms. The partial products numbers which are to be added are the main parameters which determine the multiplier performance. [6-8]. The proposed modified Booth multiplier is used to achieve reduction in number of partial products to be added. The technique of Wallace Tree is used to improve speed by reducing number of sequential adder stages [5]. Hence by combining Wallace tree technique with proposed modified Booth multiplier both speed and power performance can be improved.

The algorithm for N bit multiplicand and N bit multiplier is explained below.

Let,
$$Y = Y_{n-1}Y_{n-2}Y_{n-3} - - - - - - Y_2Y_1Y_0$$
$$X = X_{n-1}X_{n-2}X_{n-3} - - - - - - X_2X_1X_0$$

Where Y= Multiplicand and X=Multiplier.

Figure1 shows N-Bit Multiplier Operation for X and Y. Partial products (PP) are generated by using AND gates. If the multiplicand is having N number of bits and multiplier is having M number of bits there will be M×N number of partial products. The binary number multiplications can be now decomposed to additions [11],[12]. With A (Multiplicand) and B (Multiplier) having each 8-bit numbers generates products of 16 bit as shown in Fig.2. The generated products can be written in generalized mathematical form as shown in equation 2

$$P(m+n) = A(m) \times B(n) = \sum_{i-0}^{m-1} \sum_{j-0}^{n-1} a_j b_j 2^{i+j} \quad (2)$$

In the operation of multiplication if the multiplier LSB=1 the multiplicand is added to an accumulator and the multiplier is shifted right by one bit along with the multiplicand shifted left by 1 bit. The operation is stopped after all multiplier bits are zero. From the operation it can be observed that the numbers of multiplications are changed to addition of numbers.

ARCHITECTURE OF PROPOSED MULTIPLIER

Fig.3 shows the architecture diagram of implementation of the designed multiplier. From the diagram it can be noted that the multiplicand values are added and accumulated which depends on LSB value of multiplier. Multiplier value shifted right by 1-bit at start of individual clock cycles. Shift operation is done only when its value is zero else addition operation is done by adding multiplicand to accumulator and then shifted right by 1-bit. This process continues till all the bits are tested.

The size of the accumulator is 2×N× (M+N) and the LSB of the accumulator contains value of the multiplier. As the operation is done in each cycle, the maximum delay is N cycles. The controller of the multiplier to decide the required operation as per the algorithm is implemented by using the following state diagram shown in Fig.4 The controller generally take the decision of the basic operation i.e. either add or shift as per the algorithm described above.

CARRY SELECT ADDER

The Adder operation is implemented by using carry select adder. Fig. 5 shows the block diagram of carry select adder. This adder generally contains MUX and two ripple-carry adder. As addition operation is done twice, first time assuming carry being having zero value other time having value of one. Once the correct value of input carry is well known the correct value of sum and output carry is selected by the MUX [8]. The carry select block may contain either uniform or variable no. of bits. If its uniform then the delay is optimal for each block. In variable size case the delay is calculated form input of A and B for addition operation to output carry which is equal to MUX chain so that output carry is calculated at proper time [9].

From Fig. 5 it can be seen that it contains a block size of four. It has 2 four bit carry adder which is multiplexed together. MUX usually selects sum and carry bit by using the input carry. One for the carry adder assumes the input carry as zero where as other one assumes input carry of one as explained in the operation. The overall Block diagram of the complete design is shown in the figure 6.

METHODOLOGY

The proposed work is implemented using Verilog HDL Programming in Xilinx ISE. Verilog HDL code has been written for individual sub-blocks of the block diagram shown in the Fig.6 and functional simulation were obtained. For each block RTL design is obtained after doing the synthesis of the code. The performance parameters reports of individual blocks so as overall reports are obtained.

RESULTS AND DISCUSSION

Multiplicand

The multiplicand block is designed by using flip-flops (register) which store the value of multiplicand bits. It is loaded when the controller sends the Load command to load the multiplicand value. The register is designed by using behavioral programming of Verilog HDL. Figure7 shows the top level module of multiplicand and functional simulation result of multiplicand which shows the multiplicand output holds the value of Multiplicand.

Adder

The adder block is designed by taking eight bit carry select adder. It uses two eight bit ripple carry adder as per the operation explained above. It takes two 8-bit register values and C_{in} as input and produces sum which is 8 bit and C_{out}. The RTL block and functional simulation is shown in fig.8. From the functional simulation result it can be observed that it produces the output result as 0xF when 0xA and 0x5 is applied as input with Carry out is zero which validates the design.

Multiplier

The multiplier is designed and RTL is obtained as shown in the fig. 9. The multiplier value is loaded to the register when Load command is received. Fig. 9 shows the top module RTL block and simulation result of multiplier. The multiplier operation can be observed from simulation result

Controller

The controller is designed by using FSM state diagram which controls the operation of multiplier. Controller activated on arriving of positive edge of the clock. The load command is initiated when the initialization is done. Depending upon testing of LSB (1/0) ADD command or shift command is generated by the controller. After all the bits are tested finally the controller goes to the idle state and stop signal is generated as output. These operation can be seen form the functional simulation shown in fig. 10.

Integration of All Modules

After individual blocks are designed all the blocks are integrated as per the block diagram shown in Fig.6. The final RTL module after integration of all the sub-blocks and final simulation result is obtained as shown in the Fig.11. The simulation is carried out by taking the test vectors of A as 0xF4240 and B as 0x1E8480 and the final output is obtained as 0x1D1A94A2000 at the end of cycle when stop signal is asserted. The power and timing reports were obtained and presented in Fig. 11. The total power is found to be 56mW and the maximum net delay of 2.985ns which is better as compared to other multipliers.

CONCLUSION

An efficient multiplier is implemented by using modified Booth multiplier algorithm in ASIC using Xilinx ISE tool. The presented work implements different modules of the design separately and then integrated all the modules for performing the required operation that is meant for finite response filter operation. Simulation results presented in results and discussion section validated the design of individual blocks and final module. The power and delay values obtained for the final module indicates the proposed design having better performance in terms of delay and power as compared to existing designs.

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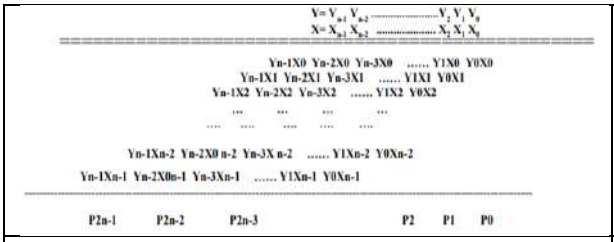

Fig.1 N-Bit Multiplier Operation

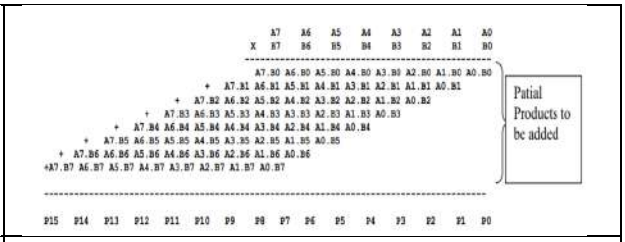

Fig.2 8×8 Bit Multiplier Operations

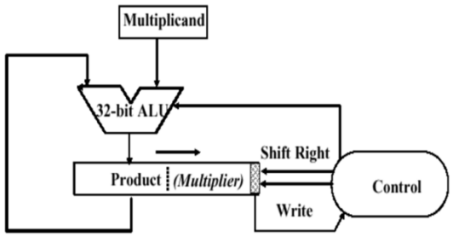

Fig.3 Architecture of Proposed Multiplier

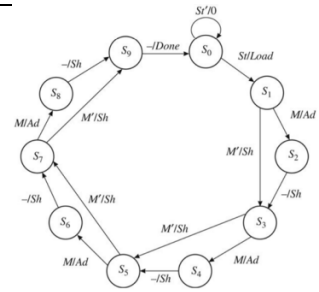

Fig.4 State Diagram of Controller

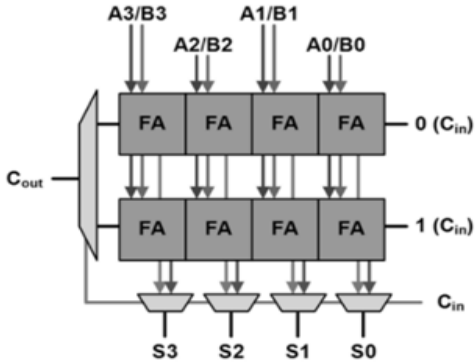

Fig.5 Block Diagram of CSA

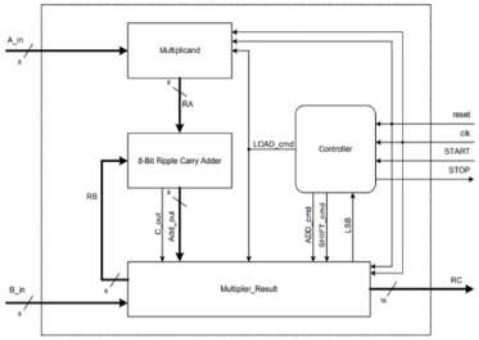

Fig.6 Overall Block Diagram

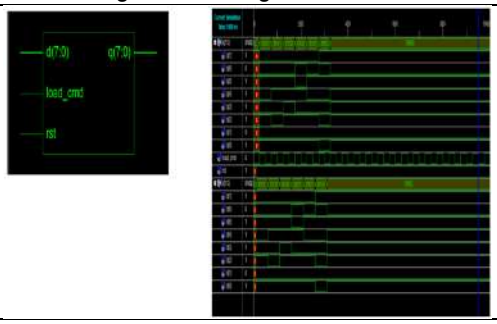

Fig.7. RTL Block and Functional Simulation of Multiplicand

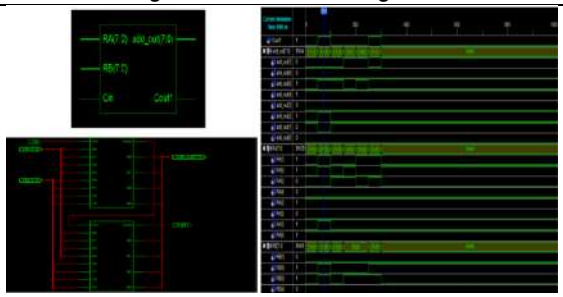

Fig.8. Top Module and RTL block with Functional Simulation of Adder

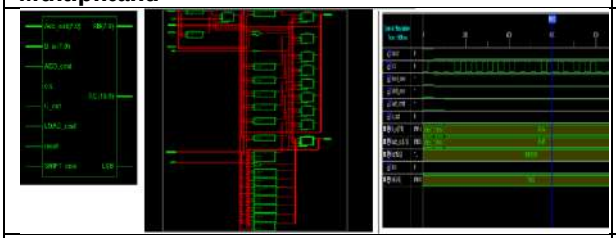

Fig. 9. Top Module, RTL Design and Simulation of Multiplier

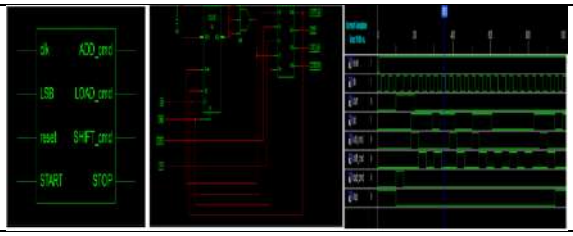

Fig.10a. Top Module, RTL Design and Simulation Result of Controller

Fig.10b. Top Module, RTL Design and Simulation Result of Final Module

Max Delay	Netname
2.965	reset_IBUF
2.564	LOAD_Cmd
2.494	inst_Controller/state_FFd3
2.409	inst_Multiplier_Result/temp_register<12>
2.376	inst_Multiplier_Results/temp_register<11>
2.012	inst_Controller/temp_count<2>
1.974	inst_Multiplicand/DFFs[0].DFFReg/Q
1.925	inst_Controller/state_FFd3_1
1.889	inst_Multiplier_Result/temp_register<10>
1.859	inst_Multiplier_Result/temp_register<14>
1.839	inst_Controller/state_FFd2
1.714	inst_Multiplier_Result/temp_register<5>

Power summary: I(mA) P(mW)

Total estimated power consumption: 34

Vccint 1.20V:	15	19
Vccaux 2.50V:	15	38
Vcco25 2.50V:	0	0
Clocks:	0	0
Inputs:	0	0
Logic:	0	0
Outputs:		
Vcco25	0	0
Signals:	0	0
Quiescent Vccint	1.20V:	15	19
Quiescent Vccaux	2.50V:	15	38

Fig.11. Delay and Power Report of Final Module

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RESEARCH ARTICLE

Biology and Lifecycle of *Henosepilachna vigitioctopunctata* FABRICIUS, a Serious Defoliator of Bitter gourd in Gajapati District of Odisha

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ABSTRACT

Hensoepilachna vigintioctopunctata (Fabricius), is also known as Hadda beetle. It is a polyphagous pest and holds great significance to Indian agriculture. Hadda beetle is considered as one of the pernicious pests different crops of Cucurbitaceous family, especially, bitter gourd. The present study was carried out during June-July, 2019 and its biological studies revealed that a gravid female laid 182 to 356 eggs in 6-7 batches during its life cycle. The duration of various stages of life cycle, viz., eggs, larvae and pupa lasted for an average of 4.1 ± 0.33 days, 20.8 ± 3.35days and 3.2 ± 0.72 days, respectively. The life span of an adult male was 29.8 ± 3.10days and female was34.4 ± 2.37days. The entire body of an adult insect is covered with fine short hairs. The present study can help the researchers in understanding the biology and behaviour of this particular pest, which can help in taking proper management strategies.

Keywords: *Hensoepilachna vigintioctopunctata*, Gajapati district, life cycle, nature of damage.

INTRODUCTION

Cucurbits, belong to form the major category of summer vegetables cultivated across the globe. Bitter gourd is one of the important crops belongs to the family. Bitter gourd has great significance in human diet because of their medicinal property and because of their ability to treat acidity, indigestion, ulcer etc. Bitter gourd is insected with many different pests starting from primordial stages to the harvest stage. The pests not only damage the crops directly, they also damage the crops indirectly as they are the vector of other pathogens especially virus, which further damages the crop. Some important harmful insects of cucurbitaceous familyare red pumpkin beetle, melon ladybird beetles and fruit flies (Gupta, 2004). *Hensoepilachna vigintioctopunctata* (Fabricius) is also known as melon ladybird beetle, belonging to Coccinellidae family of Coleoptera order. The melon ladybird beetleis multivoltine, oligophagus, coccinellid beetle that infests plants grown in the mid-hills as well as in plains in our country (Kumar and Kumar 1998). Both grubs and adultsare harmful to the crop as they feed voraciously on the leaves and and make the leaves skeletonized (Rath *et al.,* 2002; Mohasin and De, 1994).

MATERIALS AND METHODS

For studying melon ladybird life cycle, the adult beetles were brought from the infested crops of cucurbitaceous family in the month of June to July, and were nurtured in rearing cage, in laboratory for further study. They were fed on the host leaves (bitter gourd). The adults were allowed for sexual mating and the behaviour of each pair was recorded for pre-mating, mating and oviposition duration for each. The adult females laid eggs in batches. Eggs were counted and after hatching the grubs were reared and the morphometric measurements for each in star were recorded. Observations regarding the pre-pupal and pupal stages and adult longevity were also recorded. Data recorded were analysed statistically and presented below.

RESULTS AND DISCUSSION

The results of present investigation presented in table1 revealed that the incubation period ranged from 3 to 4 days with mean duration of 4.1 ± 0.33 days. showed complete metamorphosis with four different stages; Egg, Grub (Larva), Pupa and Adult (Beetle). The female laid as many as 182 to 356 eggs during the life span with an average of 265.8 ± 52.49 eggs. Hatching % of the eggs were 56.60 %.Similar results were obtained by Indu and Chatterjee (2006). While 272.32 eggs were reported by Verma and Anandhi (2008) and 302.5 by Qamar *et al.,* (2009). Newly hatched first instar larvae were yellowish in color and had six rows of long branched spines. The duration of first instar larva ranged from 3-5 with a mean of 5.8 ± 0.62 days. The range of second instar period varied from 4-6 days with mean duration 5.6 ± 0.41 days. The third instar larva was 4-5 days ranged with a mean of 4.3 ± 0.45 days. The fourth instar larva was observed 6-7 days ranged with the mean of 7.2 ± 0.63 days. The total larval period ranged from17 to 23 days with a mean of 20.8 ± 3.35 days. The full-grown fourth in star grub stopped feeding and roaming for 10-15 min to locate suitable site for pupation. The colour of the grub gradually fainted and the body shrank. Verma and Anandhi (2008) observed the larval period as 15.1 ± 4.90 days while 14.9 ± 0.43 days was reported by Qamar *et al.,* (2009). The full fed grubs spent 1 -3 days in the per-pupal stage with an average of 1.6 ± 0.89 days. The average pre-pupal length was 5.32 ± 0.42 mm and breadth was 3.26 ± 0.32 mm where as the mean pupal period was 3.2 ± 0.72 days and ranged from 2 to 5days.Similar results were obtained by Verma and Anandhi (2008) and Qamar *et al.,* (2009). The average pupal length was 6.15 ± 0.24 mm and breath was 3.73 ± 0.38 mm. The mean adult male beetle longevity was 29.8 ± 3.10 with the range from 27 to 32 days. The mean lifespan of female beetle of *E. vigintioctopunctata* was observed 34.4 ± 2.37 with the range from 31-39 days. The results further showed that the duration of life cycle varied from 49 to 75 days with mean duration of 65 ± 6.23 days. The mean fecundity was recorded as 56.8 ± 6.62 eggs/female with a range of 38 to 69 eggs/ female.

Nature of damage

Henosepilachna vigintioctopunctata is one of the harmful pests of bitter gourd (*Momordica charantia*). Both adults and larvae(grubs) stage of the beetlefeed on the lower surfaces of the leaves, scrapping and feeding voraciously on the parenchyma and the lower epidermis between the veins and skeletonize it in a characteristic manner leaving intact the upper epidermis as well as the tougher tissues (veins, etc.) in the form of "window". The leaves damaged by the pest appear as translucid, grey in colour and dry. In case of severe attack, the young plants are dried and can die. Adults can fly and damage largeer crop fields in sever attack (Nagia *et al.,* 1992).But the larvae damage more than adults.

Considering the significant damage brought about by this beetle care must be taken for their ready control to avoid damage to the plants especially in crops like bitter gourd where the damage is very severe.

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Table-1. Life cycle of *E. vigintioctopunctata*

BIOLOGICAL EVENTS	RANGE	MEAN±SE
Fecundity	38- 69	56.8 ± 6.62
Incubation Period (days)	3-4	4.1 ± 0.33
Larval period (days)		
Instar-I	3-5	5.8 ± 0.62
Instar-II	4-6	5.6 ± 0.41
Instar-III	4-5	4.3 ± 0.45
Instar-IV	6-7	7.2 ± 0.63
Total larval period(days)	17-23	20.8 ± 3.35
Total pupal period(days)	2-5	3.2 ± 0.72
Adult longivity		
Male	27-32	29.8 ± 3.10
Female	31-39	34.4 ± 2.37
Total life cycle(days)	38-69	56.8 ± 6.62

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RESEARCH ARTICLE

Osmotic Dehydration of Grapes

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ABSTRACT

The process of osmotic dehydration followed by tray drying was studied on grapes for raisin preparation. Grapes were dried out by osmosis using sugar syrup at grapes to sugar syrup ratio of 1:4, which were than dried in a commercial tray dryer maintained at 60°C temperature to obtained raisin. The grapes were dipped in sugar syrup of 40°Brix, 50°Brix, 60°Brix and 70°Brix concentration in beakers having fruit to syrup ratio 1:4 at 60°C temperature and time of immersion was 30 minutes and one hour for osmotic dehydration. From this it was concluded that, acidity and ascorbic acid decreases with increase in syrup concentration, temperature of solution and time of concentration and total, reducing and non-reducing sugar increases with increase in syrup concentration, temperature of solution and time of concentration.

Key words: Osmotic dehydration, Chemical composition, Raisins, Reducing and non-reducing sugars.

INTRODUCTION

India has been a predominantly agrarian economy since time immemorial. Raisins are formed from grapes made by osmotic dehydration followed by drying. The affinity of water to pass through a semi-permeable membrane into a solution where the solvent concentration is higher to equalize the concentration of materials on either side of the membrane is known as osmosis. The dehydration means loss or removal of water. Osmotic dehydration (OD) is a technique used to reduce water activity (a_w) in foods in order to improve nutritional, sensorial and functional properties of food. It consists of an immersion of the product into concentrated solution A grape is a small, sweet fruit that grows in clusters on a vine and botanically it is termed as 'berry'. It can be eaten fresh as table grapes or they can be used for making wine, grape juice, jam, jelly, raisins etc. Grapes contain 81% water, 18% carbohydrates, 1% protein and have negligible amount of fat. Raisins originated in the Middle East before making their way to Europe, where they were especially popular among the Greeks and Romans. A 100g of raisins contains,108 calories of energy,1 gram of protein, 0 of gram fat, 29 grams of carbohydrates,1 gram of fibres,21 grams of sugar and these are

a good source of iron (14%), potassium (16%), copper (14%) and vitamin B6 (13%). Raisins also contain boron this mineral helps maintain good bone and joint health, can improve wound healing, and may improve cognitive performance. So, the present study taken up to find out the dehydration of grapes for raisins. This project is taken up with following objectives

REVIEW OF LITERATURE

Anonymous *et al* 2003 Reported that in conventional raisin preparation on includes the pre-treatment of the grapes followed by shed drying in order to bring the moisture content of the final product up to 15-18 per cent from initial moisture content of grapes i.e. 70-85 per cent. This process requires very long period for drying that is about 15-21 days depending upon different weather condition. Diletta *et al* 2016 Conducted experiments without air drying is also known as tray drying, which uses hot air as a medium to make temperature gradient between grapes and air with simultaneous removal of moisture. The temperature of air decides not only the drying rate but also the quality of the end product. However, the part closer to the fruit surface dries up earlier, and the interior part is still left with some moisture. Caglar *et al* 2009 Found that the thermal energy consumes more time to reach that part and the vapour generated in the process also takes more time to reach the surface, resulting in higher drying time Lokhande *et al* 2007Reported hot air drying took 180 min to remove 65% of moisture from grapes. The quality characteristics of grapes, such phenolic content, anthocyanins, and antioxidant activity, also reduced during hot air drying treatment compared to nonconventional treatment. Collar *et al* 2007 Reroted hot air is considered to be a good alternative compared to sun drying of grapes, due to higher retention of phenolic content, anthocyanins, antioxidant activity, and other quality attributes along with less drying time. Tiara *et al* 2005 Found that the osmotic dehydration is the phenomenon of removal of water from lower concentration of solute to higher concentration through semi permeable membrane results in the equilibrium condition in both sides of membrane.

MATERIALS AND METHODS

Grapes were collected from the local market and then washed properly, Clean grapes were dipped in the different concentrations of sugar solutions and then kept for drying in the tray dryer for formation of raisins.

Raw Materials

Fresh green grapes (Thompson seedless) were collected from the local market. These table grapes were mainly grow for preparation of raisins and wine.

Quality of raw materials: The Thompson seedless grapes were neither fully ripen nor raw they were perfectly ripened which was good for this experiment.

Shape and size of the fruits: The shape of the grapes was round and oval they were not distributed uniformly, the size was varying from 3-5cm and whole grapes were used.

Equipments

(i) Tray dryer – It was a convectional drying equipment with enclosed insulated chambers and trays placed on top of each other in a trolley. Grapes were dried using tray dryer.

(ii) Hot air oven – It was an electrical device used for rapid evaporation, rapid drying. By using this equipment moisture in grapes were determined.

(iii) Weighing machine – It was a measuring instrument used to measure samples.

Other materials required to conduct the experiment

(i) Beaker – It was used for soaking of grapes in sugar solution.

(ii) Sugar – It was used for making sugar solution.

(iii) Water – It act as solvent in sugar solution.

(iv) Petridish – It was used for keeping grapes in it for weighing.
(v) Tray – Grapes were placed on it and kept for drying in tray dryer.
(vi) Spatula – It was used for mixing the sugar solution.
(vii) Tissue paper – It was used to remove water from surface of grapes.

Experimental procedures

Grapes were collected from the market
⇓
Cleaned
⇓
Grapes were wiped off by tissue paper
⇓
Sugar solutions of different concentrations were prepared
⇓
Grapes were dipped in the sugar solutions
⇓
Temperature was fixed in tray dryer
⇓
Grapes were kept inside the tray dryer for moisture loss
⇓
After 4 days of subsequent drying
⇓
Final product (raisins) was formed

Sample preparation

Green grapes were washed thoroughly in tap water to remove pesticides like carbaryl, pyre thin etc that were spread on grapes to protect it from pests and other organisms those cause damage to the fruit. After thorough washing of the grapes, they were dried by the help of tissue paper which remove water from the surface of the grapes.

Preparation of brix solution

For preparation of 4 brix solution of different concentration we took 4 beakers and labelled them as 40^{o}Bx, 50^{o}Bx, 60^{o}Bx and 70^{o}Bx. As we know every solution had a solute and solvent so here the solute was sugar and the solvent was water. 120ml of water was taken in 4 beakers. The weight of sugar was differed in each of the brix solution. Plate no.3

For 40^{o}Bx solution, the weight of sugar was 40/100* 120=48g. So in 1st beaker 48g of sugar which was measured by weighing machine was added to 120ml of water and stirred well by spatula to make a solution.

For 50^{o}Bx solution, the weight of sugar was 50/100*120=60g.

For 60^{o}Bx solution, the weight of sugar was 60/100*120=72g.

For 70^{o}Bx solution, the weight of sugar was 70/100*120=84g. Simultaneously 60g, 72g, and 84g of sugar was added to 120ml of water present in 2nd , 3rd and 4th beaker for preparation of a solution. After the preparation of all 4 brix solutions 100g of green grapes which were washed thoroughly and dried by tissue paper was added to each of the brix solutions for soaking in sugar water. Plate no.4

These were kept aside for 41hour for the process of osmosis. Grapes were placed in sugar solution i.e hypertonic solution due to which exosmosis occurred in which outward movement of the water molecules from the lower solute concentration to higher solute concentrations takes place through grapes covering resulted in shrinkage.

Measurement of initial and final moisture content of grapes

The standard method to determine moisture in fruits and seeds were bone drying by hot air oven. Moisture present in grapes was also determined by this method. Two petridishes were taken and its empty weight was noted down then few fresh grapes samples cut in to slices were taken along the petridishes and the weight was noted down. The initial weight was noted and then it was placed in a preheated hot air oven. After 24hour the petridishes were taken out from the oven and final weight was noted down.

Weight of Petridis 1 = 28.2g

Weight of grapes in Petridis 1 = 9.4g

Weight of Petridis 2 = 28g

Weight of grapes in Petridis 2 = 9g

PETRIDIS	INITIAL WEIGHT	FINAL WEIGHT	MOISTURE LOSS
1	37.6	30.2	7.4
2	37	30	7

% of peridish 1 = 7.4/9.4*100 = 78.72%

% of Petridish 2 = 7/9*100 = 77.77%

Total percentage = 78.72+77.77/2= 78.25

After 41hour soaked grapes present in different brix solution was taken out from the beaker and spreaded over 4 trays named as 40^{o}Bx, 50^{o}Bx, 60^{o}Bx and 70^{o}Bx. The tray dryer was pre-heated to reach the temperature of 60^{o}C and then these 4 trays were kept inside it. Plate no.5. On 1st day, few readings were taken in 30minutes interval and after that the readings were taken in 1hour interval to know the moisture loss in grapes. On 2nd day (after 16hour) readings were taken in 1hour interval. On 3rd day (after16hour 20minutes) readings were taken in 1hour interval. Plate no.6 On 4th day (after 16hour 30minutes) readings were taken in 1 hour interval and then final products i.e raisins were formed.

RESULTS AND DISCUSSION

When grapes were immersed in sugar solution of different ^{0}Bx values, weight loss of grapes was minimum in 50^{0}Bx solution i.e; 66.11% whereas weight loss of grapes was maximum in 40^{0}Bx solution i.e ; 70.65 %. 100g of grapes were immersed in sugar solution of different ^{0}Bx i.e: 40^{0}Bx, 50^{0}Bx, 60^{0}Bx, 70^{0}Bx for about 41 hours. After 41 hours, the grapes which were immersed in 50^{0}Bx, 60^{0}Bx and 70^{0}Bx have lost some weight whereas the grapes which were immersed in 40^{0}Bx gained 0.2g of weight. Table 1 shows the percentage of total weight loss of grapes after osmotic dehydration.

Initially, the readings were taken at the interval of 30 min. According to 1st reading, the highest moisture loss was occurred in the grapes which were immersed in 70^{0}Bx i.e; 1 % whereas lowest moisture loss was occurred in grapes which were immersed in 50^{0}Bx i.e; 0.61%. Five readings were taken at 30 min of interval in 1st day. After that four readings were taken at 60 min of interval in that day. In 1st day , highest moisture loss was observed after 150 min of tray drying i.e 3.55% (40^{0}Bx) whereas lowest moisture loss was observed after 30 min of interval i.e; 0.61 % (50^{0}Bx).Table 2 shows the percentage of weight loss occurred in 1st day after tray drying. Table 2 shows the percentage of moisture loss of grapes after tray drying in day 1. On 2nd day, it was observed that the colour of grapes was changed from green to brownish colour . On 2nd day, grapes were observed at the interval of each 60 min of tray drying. 1st observation indicated that the highest and lowest moisture loss was 5.01% (70^{0}Bx) and 4.48% (60^{0}Bx) respectively. Seven readings were taken in 2nd day at the interval of 60 min. In 2nd day the highest moisture loss was observed after 60 min of drying operation i.e; 5.01% (70^{0}Bx) and the lowest moisture loss was recorded after 240 min of tray drying i.e; 1.43% (40^{0}Bx) .Table 3 shows the percentage of weight loss occurred in day 2.

On 3rd day, it was observed that the colour of grapes was changed from brownish to dark brown. Table 4 represents the percentage of moisture loss occurred in 3rd day with respect to time. On 3rd day, 7 observations were taken. Among them highest moisture loss was observed after 360min of drying operation i.e; 5.91% (40^{0}Brix) whereas lowest moisture loss was observed after 180 min of drying operation i.e; 0.39% (50^{0}Brix). On that same day, after 300 min of drying operation, it was observed that there are some changes occurred in the shape and size of the grapes. The grapes were quizzed a little and there was reduction of the length of its diameter.Table 4 shows the osmotic dehydration of grapes after tray drying in day 3.

On 4th day it was observed that the grapes were dark brown in colour and some changes in the shape and size of the grapes were clearly observed. On 4th day, four observations were recorded at the interval of 60 min. Table 5 represents the percentage of moisture loss on 4th day. It was the last day of our experimentation. On that day three observations were taken at the interval of each 60 min. On that day highest moisture loss was observed after 180 min of drying operation (3rd observation) i.e; 12.80 %(60^{0}Bx) whereas lowest moisture loss was observed after 180 min of drying operation i.e; 2.0% (40^{0}Bx). On 4th day, after 180 min of drying operations, finally grapes are converted into raisins. Plate no. 6 and 7 represent the picture of raisins. The raisins had nice texture, appearance and good flavour.
Table 5 : Osmotic dehydration of grapes after tray drying in day 4.

Calculation

Percentage of total moisture loss =

$$\frac{\text{(Initial weight of grapes – Final weight of grapes)}}{\text{Initial weight of grapes}} * 100$$

Percentage of total moisture loss(40^{0}Bx) = $\frac{(100.2 - 29.4)}{100.2} * 100$ = 70.65%

Percentage of total moisture loss (50^{0}Bx) = $\frac{(97.4 - 33)}{97.4} * 100$ = 66.11 %

Percentage of total moisture loss (60^{0}Bx) = $\frac{(95.8 - 28.6)}{95.8} * 100$ = 70.14 %

Percentage of total moisture loss (70^{0}Bx) = $\frac{(99.2 - 33.4)}{99.} * 100$ = 66.33 %

Based on the above calculations,highest moisture loss occurred from those grapes which were emmersed in 40^{0}Bx sugar solution; i.e: 70.65 % whereas lowest moisture loss occurred from those grapes which were emmersed in 50^{0}Bx sugar solution; i.e: 66.11 %.

CONCLUSIONS

The process of osmotic dehydration was followed by tray drying was studied on grapes for raisin preparation. Raisins are basically grapes which are dried. Here, the grapes were immersed in different concentration of sugar solution i.e.; 40^{0}Bx, 50^{0}Bx, 60^{0}Bx, 70^{0}Bx for 41 hours. After 41 hours, the grapes were taken out from the sugar solution and spreaded over 4 trays. The tray dryer was pre-heated to reach the temperature at 60^{0}C and then these trays were kept inside. On 1st day five readings were taken at the interval of 30 min. and four readings were taken at the interval of 60 min.
On 2nd and 3rd day, subsequently 7 readings were taken at the interval of 60 min.

On 4th day, three readings were taken at the interval of 60 min. Among them highest moisture loss was occurred on 4th day, after 180 min (3rd observation) i.e.; 12.80% (60⁰Bx) and minimum moisture loss was recorded on 1st day after 30 min (1st observation) i.e.; 0.61%(50⁰Bx). After 4 days of drying operation , it was recorded that maximum moisture loss was occurred from the grapes which were immersed in 40⁰B sugar solution i.e.; 70.65% whereas minimum moisture loss was occurred from the grapes which were immersed in 50⁰B sugar solution i.e.; 66.11%. On 2nd day, it was noticed that the colour of the grapes was changed from green to brownish . On 3rd day, it was observed that the colour of the grapes was changed from brownish to dark brown and a little shrinkage was noticed on the grapes. On 4th day, after 180 min of drying operation, it was observed that the grapes are converted into dark brown colour as well as changes had been noticed in the shape and size of the grapes. There was huge reduction of the length of their diameter. Those dried grapes are so called raisins . Those raisins had nice texture, nice appearance as well as good flavour.

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Table 1: The percentage of moisture loss of grapes after osmotic dehydration.

Sl no.	Sugar conc.	Initial weight of grapes(g)	Final weight of grapes(g)	Moistureloss (%)	Moisture loss(%)
1.	40°Bx	100.2	29.4	70.8	70.65%
2	50°Bx	97.4	33	64.4	66.11%
3	60°Bx	95.8	28.6	67.2	70.14%
4	70°Bx	99.2	33.4	65.8	66.33%

Table 2 : Percentage of moisture loss of grapes after tray drying (1st day)

1st day						
Sl no.	Temp °C	Time (min)	°Bx	Initial wt.(W_1)	Final wt.(W_2)	Moisture loss (%)
1	50°C	30min	40°Bx	100.2	99.4	0.79
			50°Bx	97.4	96.8	0.61
			60°Bx	95.8	95.2	0.62
			70°Bx	99.2	98.2	1.00
			40°Bx	99.4	98	1.40

2	50°C	60min	50°Bx	96.8	95.4	1.44
			60°Bx	95.2	93.8	1.47
			70°Bx	98.2	97.2	1.01
3	50°C	90min	40°Bx	98	97	1.02
			50°Bx	95.4	94.2	1.25
			60°Bx	93.8	92.6	1.27
			70°Bx	97.2	95.8	1.44
4	50°C	120min	40°Bx	97	95.6	1.44
			50°Bx	94.2	93.2	1.06
			60°Bx	92.6	91.6	1.07
			70°Bx	95.8	94.8	1.04
5	50°C	150min	40°Bx	95.6	92.2	3.55
			50°Bx	93.2	92	1.28
			60°Bx	91.6	90.8	0.87
			70°Bx	94.8	93.6	1.26
6	50°C	210min	40°Bx	92.2	90	2.38
			50°Bx	92	90	2.17
			60°Bx	90.8	88.2	2.86
			70°Bx	93.6	91.4	2.35
7	50°C	270min	40°Bx	90	88	2.22
			50°Bx	90	87.8	2.44
			60°Bx	88.2	86	2.49
			70°Bx	91.4	89.4	2.18
8	50°C	330min	40°Bx	88	86.2	2.04
			50°Bx	87.8	86	2.05
			60°Bx	86	84	2.82
			70°Bx	89.4	87.6	2.01
9	50°C	390min	40°Bx	86.2	84.4	2.08
			50°Bx	86	84.4	1.86
			60°Bx	84	82.4	1.90
			70°Bx	87.6	86.2	1.59

Table 3 : Percentage of moisture loss of grapes after tray drying (2nd day)

2nd day						
Slno.	Temp °C	Time (min)	°Bx	Initial wt.(W_1)	Final wt.(W_2)	Moisture loss (%)
1	60°C	60min	40°Bx	79	75.2	4.81
			50°Bx	78.2	74.4	4.85
			60°Bx	75.8	72.4	4.48
			70°Bx	79.8	75.8	5.01
2	60°C	120min	40°Bx	75.2	71.6	4.78
			50°Bx	74.4	71.2	4.30
			60°Bx	72.4	69.4	4.14
			70°Bx	75.8	72.8	3.95
3	60°C	180min	40°Bx	71.6	69.8	2.51
			50°Bx	71.2	69.2	2.80
			60°Bx	69.4	67.8	2.30
			70°Bx	72.8	71.2	2.19

4	60°C	240min	40°Bx	69.8	68.8	1.43
			50°Bx	69.2	68.0	1.73
			60°Bx	67.8	66.6	1.76
			70°Bx	71.2	70.0	1.68
5	60°C	300min	40°Bx	68.8	65.6	4.65
			50°Bx	68.0	65.8	3.23
			60°Bx	66.6	64.2	3.60
			70°Bx	70.0	67.2	4.00
6	60°C	360min	40°Bx	65.6	62.6	4.57
			50°Bx	65.8	63.8	3.03
			60°Bx	64.2	61.8	3.73
			70°Bx	67.2	65.2	2.97
7	60°C	420min	40°Bx	62.6	59.6	4.79
			50°Bx	63.8	61	4.38
			60°Bx	61.8	59.2	4.20
			70°Bx	65.2	62.6	3.98

Table 4 : Osmotic dehydration of grapes after tray drying (3rd day)

3rd day						
Sl no.	Temp °C	Time (min)	°Bx	Initial wt.(W_1)	Final wt(W_2)	Moisture loss (%)
1	60°C	60min	40°Bx	53.4	51	4.49
			50°Bx	55.8	53.4	4.30
			60°Bx	53.8	52.2	2.97
			70°Bx	57.2	55.4	3.14
2	60°C	120min	40°Bx	51	48.4	5.09
			50°Bx	53.4	50.8	4.86
			60°Bx	52.2	50	4.21
			70°Bx	55.4	53.4	3.61
3	60°C	180min	40°Bx	48.4	46	4.95
			50°Bx	50.8	50.6	0.39
			60°Bx	50.0	47.8	4.40
			70°Bx	53.4	48.8	8.61
4	60°C	240min	40°Bx	46.0	43	6.52
			50°Bx	50.6	47.8	5.53
			60°Bx	47.8	45	5.85
			70°Bx	48.8	46.6	4.50
5	60°C	300min	40°Bx	43.0	40.6	5.58
			50°Bx	47.8	45.6	4.60
			60°Bx	45.0	43.2	4.0
			70°Bx	46.6	44.4	4.72
6	60°C	360min	40°Bx	40.6	38.2	5.91
			50°Bx	45.6	43.4	4.82
			60°Bx	43.2	41.4	4.16
			70°Bx	44.4	42.6	4.05

7	60°C	420min	40°Bx	38.2	36.4	4.71
			50°Bx	43.4	41.2	5.06
			60°Bx	41.4	39.6	4.34
			70°Bx	42.6	40.8	4.22

Table 5 : Osmotic dehydration of grapes after tray drying (4th day)

4th day						
Sl no.	Temp °C	Time (min)	°Bx	Initial wt(W1)	Final wt(w2)	Moisture loss (%)
1	60°C	60min	40°Bx	33	31.4	4.84
			50°Bx	36.8	35.8	2.71
			60°Bx	35	33.8	3.42
			70°Bx	37.2	36.2	2.68
2	60°C	120min	40°Bx	31.4	30.0	4.45
			50°Bx	35.4	34.4	3.91
			60°Bx	33.8	32.8	2.95
			70°Bx	36.2	35.2	2.76
3	60°C	180min	40°Bx	30	29.4	2.0
			50°Bx	34.4	33.0	4.06
			60°Bx	32.8	28.6	12.80
			70°Bx	35.2	33.4	5.11

Plate no. 1 Fresh grapes after cleaning

Plate no. 2 Grapes were laid on the tissue paper to dry

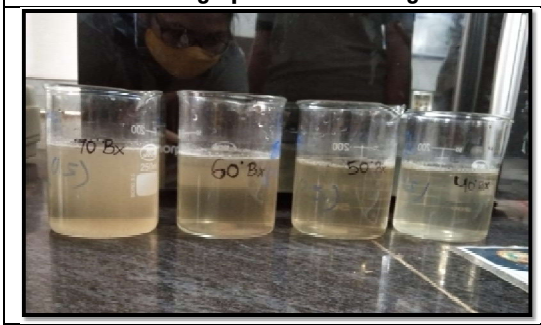

Plate no.3 Prepared sugar solution of different concentrations of sugar

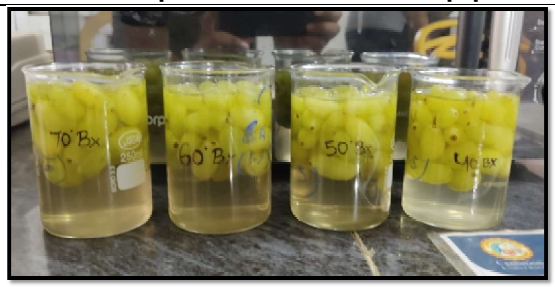

Plate no. 4 Grapes were dipped in the prepared solutions of 40^0, 50^0,60^0 and 70^0 Bx

Plate no.5 Grapes were kept inside the tray dryer for drying for 8h

Plate no. 6 Grapes after 4 days of continual drying 8h each day

Plate no. 7 Final product obtained as raisins

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RESEARCH ARTICLE

Assessing Sensitivity Analysis of Weather Parameters on Reference Evapotranspiration (ET_0) Calculated using FAO Penman-Monteith Equation

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ABSTRACT

The sensitivity of reference evapotranspiration calculated using FAO-56 Penman Monteith model to climate variables in Gajapti District of Odisha has been investigated. Reference evapotranspiration (ET_0) was computed using FAO-56 PM method. This method requires temperature, relative humidity, wind speed and solar radiation data on daily basis to compute the ET_0.Sensitivity analyses of ET0 to different climatic parameters were conducted using Excel. The sensitivity coefficients were derived for each variable on a monthly basis. In the present study, an attempt has been made to assess change and sensitivity of each climatic parameter on reference evapotranspiration (ET_0) using data from 2011 to 2020. The sensitivity coefficient values from study shows thatET_0 changes positively with a corresponding change in the value of solar radiation (SR_n), mean temperature (ST_{mean}), and wind speed (SU_2) while inversely with relative humidity (SRh).Results also show that the sensitivity of evapotranspiration to climatic variables has been more significant during the dry season from November to June.

Keywords: reference evapotranspiration, sensitivity coefficients, maximum temperature, relative humidity

INTRODUCTION

The evaporation component of the crop water requirement was studied using reference crop type, crop development and management practices (Zotarelli et al., 2009). Climatic parameters can be estimated using locally measured and recorded climatic data and it is the only factor that can affect calculation of reference evapotranspiration, represented by vegetated surface and shows the effect of climatic parameters in crop water requirement (Allen et al., 1998). There are several methods available to compute reference evapotranspiration, FAO-56 PM was most popular and considered as standard method to estimate reference evapotranspiration. This method needs daily maximum temperature, minimum temperature, relative humidity, sun shine hours, solar radiation, and wind speed data measured and recorded on daily basis (Allen et al., 1998). The FAO56-PM model has proved to be a relatively accurate method in both humid and arid climate because of incorporating thermodynamic and aerodynamic considerations.

Among the climatic parameters used to estimate the ET_0, some parameters significantly affect the output values in comparison to other climatic parameter, depending on the local weather (Debnath *et al.* 2015). Within restrictive circumstances, Koudahe *et al.* (2018) stated that finding more sensitive climatic parameter with influences the ET_0 is most important thing so can adopt the ET_0 models to local climatic conditions with small modifications (Nouri *et al.* 2017). Considering this current study aims to determine the magnitude of changes in ET_0 values with respect to change in climatic parameters under different scenario.

MATERIAL AND METHODS

A study was conducted to determine the sensitivity of climatic parameters on ET_0 using data measured and recorded from Meteorological Department, Centurion University of Technology and Management (CUTM) Paralakhemundi. The study area is located on the flat land at18°47' N latitude, 84°06' E longitude and altitude of 116 m above mean sea level.TheParalakhemundi climate is classified as subtropical with high humidity. The temperaturevaries between 18^{o}C -48 ^{o}C. The average climatic data i.e. rainfall, maximum and minimum temperature, relative humidity and wind speed of 10 years (2011-2020) were collected Meteorological Department, CUTM, Paralakhemundi.

The Modified Penman-Monteith method suggested by Allen et al. (1998) is the most reliable method to compute ET_0.The FAO-56 Modified Penman-Monteith equation for estimation of ET_0 is mentioned below

$$ET_0 = \frac{0.408\Delta(R_n - G) + \frac{\gamma(900)}{T+273}u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

Where,

ET_0 reference evapotranspiration [mm day^{-1}]

R_n net radiation at the crop surface [mj m^{-2} day^{-1}],

G soil heat flux density [mj m^{-2} day^{-1}],

e_s - e_a saturation vapor pressure deficit [kPa],

e_s saturation vapor pressure at T_c[kPa],

e_a actual vapor pressure [kPa],

Δ slope of the saturation vapor pressure temperature relationship [kPa^{0}C^{-1}]

γ psychrometric constant [kPa^{0}C^{-1}] and

u_2 wind speed at 2 m height [m s^{-1}]

Sensitivity analysis was performed to determine the most sensitive climatic variable to ET_0 in a given location and to determine the extent to which changes in weather variable affects ET_0. A simple technique as used in this study and adopted by numerous hydrological studies involves plotting the relative change in dependent variables (ET_0) against the relative change in independent variables (solar radiation, minimum temperature, maximum temperature, wind speed, and relative humidity).Partial derivatives have been used to compute the sensitivity coefficient (Saxton 1975). Since the FAO equation is a multivariable equation, the sensitivity coefficient transforms the partial derivatives into a dimensionless form (Nouri *et al.* 2017). The sensitivity coefficient (SC) is simply defined as the ratio of the changes in the ET_0 with respect to changes in a climatic variable (Irmak *et al.* 2006).

RESULTS AND DISCUSSION

The modified Penman-Monteith method proposed by Allen et al. (1998) was used to compute reference evapotranspiration (ET_0).The monthly average of estimated daily ET_0 of ten years (2011-2020) presented in Table 1. Maximum daily average ET_0 value found in May and the lowest in December, which was the trend that got followed during all the ten years. May coincides with the dry season characterized by dry wind and high temperature. On the other hand, during the winter season, reference evapotranspiration (ET_0) tends to fall to a minimum value of 2.45 mm day^{-1} in January. This decrease in ET_0 is due to lower temperature and reduced solar radiation during this period. Average ET_0values are gradually increasing from January to May (2.45 mm to 5.25 mm) further, which was decreasing in trend from June to December (4.81 mm to 2.48 mm).

Relative change in annual ET_0 values due to the percent change in each associated climatic parameters shown in Table 2. Change in values of ET_0 due to increase or decrease in climatic parameters were compared with the meanET_0 values. The relative change ineach climatic variable has a different influence on annual ET_0.The mean of ten years annual $ET_0$was found as 1372 mm. The changes in ET_0valuedue to the percent change in each associated climate parameters are graphically presentedin Figure 1. Six separate lines shown in Figure 1 denotes the change in ET_0 for percent increase or decrease in each climate parameter annually. It is observed thatET_0 response is linear with percent change in all climate parameters.

It can be seen from the results depicted in Figure 1 and Table 2 that the effect of a change in net solar radiation on ET_0 had more prominent in comparison to other parameters. Increase or decrease of ±20% annual ET_0value accounted due to ±25% change in net solar radiation. Annual ET_0got increased from 1372 to1640 mm for 25% increase in net solar radiations. However, if solar radiations are decreased to 25%, ET_0value decreased to 1104 mm. The temperature is the second climatic parameter, which has more influence on the change in ET_0.The decrease in mean maximum temperature by 25% caused a decrease in theET_0 by 11% (from 1372 to 1227 mm). If the mean maximum temperature is increased by 25%, the ET_0increased by 11% (from 1372 to 1522 mm). Increase in minimum temperature by 25%, causedan increase in the ET_0 by 7%. The decrease in mean minimum temperature by 25% decreased the $ET_0$by 7%. The ET_0alsofound to be sensitive to wind speed. The maximum change of ±5% observed in summer ET_0accounted for a change of±25% in wind speed. Annual ET_0got increased from 1372 to 1447 mm for 25% increase in wind speed. However, if the wind speed decreases to 25%, ET_0 decreased to 1296 mm. Change in mean relative humidity also influences ET_0value,but the effect is less than the mean temperature. The maximum change of ET_0 was ±3% for±25% change in mean relative humidity. Annual ET_0got increased from 1372to 1406 mm for a 25% decrease in relative humidity. Reference evapotranspiration was less sensitive to relative humidity. However, if relative humidity increased to 25%, ET_0 decreased to 1329 mm.

These results indicate that annual $ET_0$was more sensitive to mean solar radiation followed by mean maximum temperature. Thisanalysis suggests that the net solar radiation and maximum temperature are major climatic parameters that influence the ET_0ofthe study region. However, change in wind speed, relative humidity, and the minimum temperature had a varying effect on the relative change in ET_0. After solar radiation and maximum

temperature, ET_0 was observed to be more sensitive to minimum temperature followed by wind speed and relative humidity. The results of this study are also in agreement with similar studies conducted in different countries. In the Delta of the Senegal River, Djaman et al. (2016)reported that evapotranspiration was more sensitive to maximum temperature and wind speed. By studying the sensitivity of ET_0 computed by five methods (FAO 56 Penman-Monteith, FAO 24 Penman, FAO 24 Blaney-Criddle, FAO 24 Making and Hargreaves), Ambas and Batas, (2012) concluded that solar radiation and temperature have more influence on reference evapotranspiration (ET_0) than wind speed and relative humidity. Vicente-Serrano et al. (2014) analyzed the sensitivity of maximum temperature, minimum temperature, wind speed and sunshine in Spain and found that ET_0 values were more sensitive to variation in maximum temperature and wind speed. However, contrary to our results where wind velocity and relative humidity as variables that have less influence on ET_0. Aydin et al. (2015) noted that evapotranspiration is more sensitive to changes in relative humidity, followed by radiation, temperature, and wind speed in coastal and mountain regions of West Korea. Goyal (2004) reported that in the arid zone of Rajasthan in India, evapotranspiration was more sensitive to temperature followed by wind speed, solar radiation, and relative humidity. Whereas, in the present study area (sub-humid), after solar radiation, the temperature has more effect on ET_0.Thisis because the aridzone of Rajasthan falls in the famous Thar Desert and, therefore, temperature and wind speed are relatively higher as compared to the sub-humid region of India. Vapour pressure, which represents relative humidity had the least effect on ET_0in Rajasthan in contrast to sub-humid regions of India where wind velocity had the least effect.

Figure 2 presents the mean of daily changes in sensitivity coefficients. The ratio of changein ET_0 to changes in climate variables expressed as sensitivity coefficient. The sensitivity coefficient values indicated thatET_0 changes positively with a corresponding change in the value of solar radiation (SR_n), mean temperature (ST_{mean}), and wind speed (SU_2) while inversely with relative humidity (SRh). The sensitivity coefficients of all climatic variables varied between −0.05 and 0.35.The sensitivity coefficient value of ET_0had a significantly great influence of net solar radiation (R_n) and became almost constant values of coefficient throughout the year. The magnitude of the sensitivity coefficients ranked as SR_n> ST_{mean}> SU_2> SRh in a year, which, S stands for sensitivity coefficient, and R_n is net solar radiation, T_{mean} is mean temperature, U_2 is average wind speed at 2 m height, and Rh is relative humidity.

Overall, the sensitivity of evapotranspiration to climatic variables has been more significant during the dry season from November to June. The importance of measuring climatic variables over a specific period has been pointed out by some researchers. Estevez et al. (2009) reported that it is important in semi-arid regions to measure temperature or solar radiation during the summer than during the winter months for a better estimate of evapotranspiration. Sharifi and Dinpashah (2014) also noted thatET_0 is more sensitive to the mean air temperature, and this sensitivity is higher during summer than during winter season.

CONCLUSIONS

In this study, the change in ET_0 due to change in climatic variable is determined using sensitivity analysis. Short-term (10 years) climate variables (solar radiation, minimum temperature, maximum temperature, relative humidity, and wind speed) were subjected to a ±5 to ±25% increase and decrease. The effects of the change of each variable on ET_0 and the sensitivity coefficients were determined. The result shows that the ET_0 changes positively with a corresponding change in the value of solar radiation (SR_n), mean temperature (ST_{mean}), and wind speed (SU_2) while inversely with relative humidity (SRh).

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Table 1. Monthly average of daily Evapotranspiration (ET_0), mm values

Months	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Mean
January	2.59	2.63	2.63	2.62	2.27	2.37	2.56	2.39	2.42	2.32	2.45
February	3.28	3.64	3.60	3.64	2.77	2.74	3.29	3.16	3.16	3.09	3.24
March	4.54	4.87	4.50	4.62	3.59	4.00	3.87	4.57	4.19	4.17	4.29
April	5.91	5.64	5.46	5.40	4.79	4.65	4.49	5.68	4.85	4.76	5.16
May	5.67	5.59	5.33	5.25	4.91	5.00	5.21	4.97	5.11	5.49	5.25
June	5.46	4.92	5.44	5.05	4.27	3.71	4.67	4.66	4.63	5.27	4.81
July	4.22	4.14	3.64	3.78	3.43	3.21	3.59	3.71	4.19	4.05	3.80

August	4.16	3.40	3.88	3.88	3.36	3.14	3.57	3.76	3.91	3.67	3.67
September	3.93	3.39	3.56	3.92	2.97	2.98	3.49	3.44	3.60	3.60	3.49
October	3.46	3.52	3.13	3.75	3.12	3.06	3.51	3.13	3.46	3.36	3.35
November	3.53	3.41	3.13	3.30	2.79	3.00	2.90	2.88	3.21	2.92	3.11
December	2.70	2.75	2.54	2.65	2.29	2.26	2.47	2.24	2.33	2.25	2.48

Table 2. Change in annual Reference evapotranspiration due to change in principal climatic parameters

Climatic parameter / Percent	ET_0 (mm) (Percent ET_0)										
	-25%	**-20%**	**-15%**	**-10%**	**-5%**	**Mean**	**+5%**	**+10%**	**+15%**	**+20%**	**+25%**
T_{max},^{0}C	1227 (-11)	1256 (-8)	1285 (-6)	1314 (-4)	1343 (-2)	1372	1399 (2)	1426 (4)	1454 (6)	1481 (8)	1522 (11)
T_{min}, ^{0}C	1277 (-7)	1295 (-6)	1314 (-4)	1333 (-3)	1353 (-1)	1372	1391 (1)	1410 (3)	1429 (4)	1449 (6)	1468 (7)
T_{mean}, ^{0}C	1134 (-17)	1181 (-14)	1228 (-10)	1276 (-7)	1323 (-4)	1372	1420 (4)	1469 (7)	1518 (11)	1568 (14)	1618 (18)
R_n,	1104 (-20)	1157 (-16)	1211 (-12)	1264 (-8)	1318 (-4)	1372	1425 (4)	1479 (8)	1532 (12)	1586 (16)	1640 (20)
U_2, m s^{-1}	1296 (-5)	1312 (-4)	1327 (-3)	1342 (-2)	1357 (-1)	1372	1387 (1)	1402 (2)	1417 (3)	1432 (4)	1447 (5)
Rh, %	1406 (3)	1400 (2)	1394 (2)	1387 (1)	1379 (1)	1372	1364 (-1)	1355 (-1)	1347 (-2)	1338 (-2)	1329 (-3)

(Values in parentheses show the percent change)

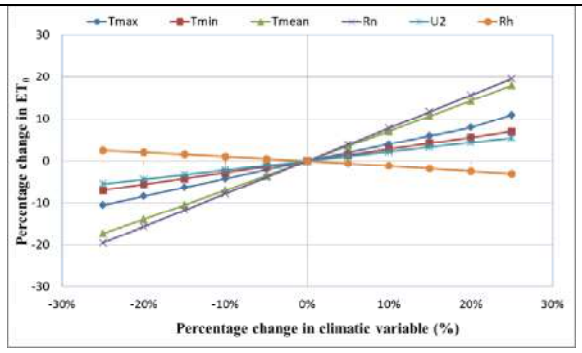

Fig. 1. Percent change in ET_0 to percent change in climatic parameter

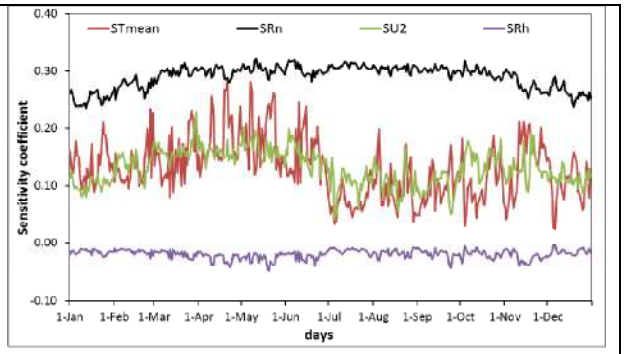

Fig. 2. Variation of daily average sensitivity coefficients.

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RESEARCH ARTICLE

Preparation of Ragi Biscuits

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ABSTRACT

The primary ingredients of biscuits are mainly wheat flour, water, sugar and fat. The white flour used for the preparation of biscuits is deficient in several nutrients including protein, vitamins, minerals and dietary fibre. So use of composite flour for preparing biscuits can enhance the nutritional and functional properties of biscuits. Finger millet (*Eleusine coracana*) or Ragi belongs to the family Gramineae. Ragi is a healthy choice over white flour as it is gluten free, rich source of calcium, iron, some essential amino acids such as leucine, isoleucine, phenylalanine, methionine etc. Finger millet has also many health promoting benefits such as hypoglycemic, hypocholesterolemic and anti ulcerative effect. So the objective of the study is to prepare biscuits with 50% ragi flour with wheat flour and to study what are the different types of factors affect the processing and shelf life of biscuits. The biscuits were prepared with ragi flour (125 g), wheat flour (125 g), powder sugar (100 g), butter (110g), a pinch of salt and milk was added as required to make the dough. Prepared biscuits were baked in a preheated oven at 180°C for 15 minute. After preparation the sensory evaluation(qualities like colour, appearance, taste, aroma, texture and overall acceptability) of prepared biscuits was done by 7 no. of students using 9 point hedonic scale and it was found that ragi biscuits with 50% ragi flour are highly acceptable. There are mainly two types of factors affect the processing and shelf life of biscuits: internal factors and external factors.

Key words: Bakery product, Ragi, Wheat, Baking, Shelf life,Deficiency, Sensory evaluation

INTRODUCTION

Finger millet (*Eleusine coracana*) commonly known as Ragi belongs to the family Gramineae. It is mostly preferred as staple food by people of arid and semi-arid region. The grain ragi is similar to reddish mustard. It is harvested during December to January.(Taynath et al.,2018).It is an important staple food for traditional consumers and the people belonging to the lower socio-economic strata in the Indian subcontinent and also in some of the African

countries. (Saha et al., 2010).Finger millet or Ragi or Manduais extensively cultivated in various regions of India and in the entire world. India is the major producer of finger millet contributing nearly 60% of the global production. Finger millet is grown in higher rainfall areas, particularly in acid soils and mature within 100-130 days. In India finger millet is widely grown in the states of Karnataka, Tamilnadu, Andhrapradesh and some parts of North India.(Gull et al., 2014. Common bakery products include breads, cookies, pastries, cakes, bread etc. The word biscuit derived from latin word "Bisococtum" means twice baked.(Muktar et al.,2018).. Also, with good taste biscuits provide substantial energy.. The protein or mineral content of biscuits for quality and availability can be achieved by increasing the ratio of whole grain raw materials other than wheat. Whole grain flour like finger millet or ragi (*Eleusine coracana*), which is full of vitamins, mineral and dietary fibre.

Structure of finger millet

The grain is globular in shape and the diameter varies from 1.0 to 1.5 mm (Taylor et al., 2019)..he grain pericarp consists of three layers with varying thickness: the epicarp (outermost layer), mesocarp (middle layer) and endocarp (inner layer).Prior to further processing, the pericarp is removed from the kernel because it is a non-edible tissue (Patel and Varma ,2015). The endosperm forms the largest anatomical component of the kernel. Endosperm is attached to the seed coat and is used in the production of flour. (Tayler et al.,2019).

Nutritional composition and health benefits of finger millet:

Finger millet grains are said to contain essential minerals such as calcium (Ca) and phosphorus (P). The grains contain the highest amount of Ca, ranging from 162.0-358.0 mg/100 g when compared to other millet species. Calcium which is predominantly present in finger millet, plays an essential role in growing children, pregnant women, the elderly as well as in people suffering from obesity, diabetes and malnutrition. (Tayler et al., 2019). The calcium content is higher than all cereals and iodine content is considered to be highest among all the food grains. Finger millet contains higher proportion of carbohydrate which is the form of non-starchy polysaccharide and dietary fibre, which provides several nutritional and physiological benefits (Kokani et al., 2018).This could be one of the possible reasons for the higher dietary fibre content in finger millet.(Sharma et al., 2016). The seeds are abundant source of essential amino acids such as leucine, isoleucine, methionine, phenylalanine, tryps in inhibitory factors and also gluten free. Finger millet has also many health promoting benefits such as hypoglycemic, hypocholesterolemic and anti- ulcerative effects (Maharajan et al., 2018).

Mamat et al., 2017 studied on structural and functional properties of major ingredients of biscuits.. Starch is the major structural element in many foods, with the fat or sugar also playing key roles. Sugar gives sweetness, colour, add volumes and influence the texture of a biscuit. Fat plays an important role in biscuit production and the type of fat used determines the quality of the final product. Singh and Raghuvanshi, 2011 studied onFinger millet for food and nutritional security.According to the chemical compositiontotal carbohydrate content of finger millet has beenreported to be in the range of 72 to 79.5%. Finger millet has nearly 7% protein in diet.

Arora et al.,2016studied on the health benefitting nutrients of Finger millet. In India, finger millet (Eleusine coracana (L.) Gaertn) occupy the highest area under cultivation among the small millets..It is one of the important minor millet, a rich source of calcium (344mg/100g), dietary fibre (18%), phytates (0.48%) and phenolics (0.3–3%). Apart from this it is also a rich source of thiamine (0.42mg), riboflavin (0.19mg), iron (3.9mg), Kokani et al., 2018 studied on utilization of Ragi for preparation of malted Ragi cookies. Cookies are one of the most popular bakery products. It is a rich source of protein, fat and carbohydrates but limiting in minerals and dietary fibres.

MATERIALS AND METHODS

Ragi is rich in calcium and iron other than all food grains. It is also rich in protein, dietary fibre, phosphorous and vitamins. So, implementation of ragi flour in biscuits making can combact the deficiency of Calcium and Iron among people.Ragi is gluten free so it helps to control blood glucose levels in diabetic patients very efficiently.

Ingredients required

Ragi flour (125 g), Wheat flour (125g), Powder sugar (100 g), Butter (110g), Salt (1pinch), Milk(As required to make the dough)

Ragi flour: Ragi flour is highly nutritious as it is gluten free, rich in protein, dietary fibre, calcium and iron. It has rusted flavour.

Wheat flour: Wheat flour helps in binding the mixtures as there is gluten and also helps in balancing the flavours of the ragi flour.

Powder sugar: Sugar is used for sweetness. Powder sugar is used so that it mix easily with all the ingredients. Dissolved sugar inhibit starch gelatinisation and gluten formation and make the biscuits more tender in texture.

Butter: Butter is used to enhance the softness of the biscuits.

Salt: Salt modifies the flavours and increases the crust colour. With salt present gluten holds more water and carbon dioxide, allowing the dough to expand without tearing.

Milk: Milk is used to bind all the ingredients to make the dough.

Equipment required

Rotary convection oven, Refrigerator, Weighing balance, Knife, Spatula, Plastic wrapper, Bowl

1.Rotary convection oven: It is used for baking.

2. Refrigerator: It is used to set the dough so that the dough cut into small pieces.

3. Weighing balance: It is used to weigh all the required ingredients.

Procedure

All the ingredients were received and weighed by using weighing balance. 125g of Ragi flour, 125 g of wheat flour and 100g of powder sugar were sieved into a mixing bowl. Then a pinch of salt and110g of melted butter were added to the mixture. Milk was added to the mixture as required. Then all the ingredients were mixed well and a cylindrical dough was made. The dough was wrapped in a plastic wrapper and refrigerated for about 20 minutes. Then the dough was cut into square pieces and baked at 180°C for 15 minutes in a preheated oven.

Receive the ingredients

↓

Mixall the ingredients with milk and make a cylindrical dough

↓

Wrap the dough in a plastic wrapper

↓

Keep the dough in refrigerator for 20 minutes

↓

Take it out and cut it into square pieces

↓

Bake at 180° C for 15-20 minutes in preheated oven

↓

Cool down to room temperature

↓

Packaging and storage

RESULTS AND DISCUSSION

Sensory evaluation

The sensory evaluation (qualities like colour, appearance, taste, aroma, texture and overall acceptability) of prepared biscuits was carried out by 7 no. of students using 9 point hedonic scale. The result in table no. 2shows the sensory parameters used in accessing the product and deciding preference for the final product.

The brown colour of biscuits occurs as a results of sugar caramelization, starch dextrinisation and millard reaction (Non enzymatic browning) in which amino acids of protein react with reducing sugar during baking. That help to enhances the appearance. The ragi flour also provide a good flovour or aroma to the ragi biscuits. Taste is the main factor that determines the acceptability of the product by consumers, which has the most impact in measuring the success of the product on market. The cooking conditions, the amount of components such as fibre, starch and water absorbed during kneading of biscuits, contribute to the final texture of the product. The biscuits were crunchier and soft to break in comparison to all refined flour biscuits, which were in comparison more compact and harder to break in texture. The difference in the texture may be due to extra fibre and protein of ragi flour. Overall acceptability is the main factor to determine the acceptability of the product by the consumer was mainly based on hardness, mouth feel and taste of ragi biscuits. (Taynath et al.,2018). From the results of sensory evaluation on9 point hedonic scale, it was concluded that the biscuits prepared by incorporating 50% Ragi flour got high acceptability on hedonic scale without affecting the sensory quality. It was reflected by the score given for colour, appearance, taste, aroma and texture of ragi biscuits.

CONCLUSIONS

- Ragi is gluten free, rich source of protein, B vitamins, minerals such as calcium, iron, phosphorus, rich source of some essential amino acids such as leucine, isoleucine, methionine and phenylalanine. Ragi has higher dietary fibre and antioxidant property. So, the product was having good sensory attributes and nutritional value as well.
- Ragi biscuits made with 50% ragi flour are highly acceptable as it positively influence the sensory quality with greater crispness and mouthfeel.
- Ragi implementation significantly improved the nutritional and functional value of biscuits as it has high calcium and iron content. Hence, utilization of ragi will improve the nutritional status of consumer.

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Table 1: Nutritional composition of finger millet

Nutrient	Per 100 gm
Carbohydrate	72.6
Protein	7.7
Fibre	3.6
Fat	1.5
Calcium	344 (mg)
Iron	3.9 (mg)
Niacin	1.1 (mg)
Thiamin	0.42 (mg)
Riboflavin	0.41 (mg)

(Source: USDA nutrient database, 2018)

Table :2- Sensory evaluation of ragi biscuits by 9 point hedonic scale

Individuals	Colour	Appearance	Taste	Aroma	Texture	Overall acceptability
Person 1	9	9	8	8	8	9
Person 2	8	9	9	8	7	8
Person 3	8	8	7	8	8	8
Person 4	9	8	8	9	9	9
Person 5	8	9	8	8	9	8
Person 5	9	8	8	7	8	8
Person 7	9	9	8	8	9	9

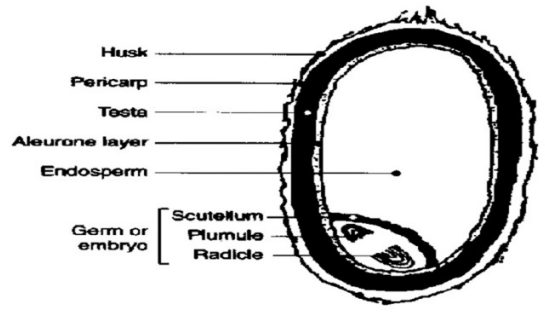

Fig.1. Structure of Finger millet (*Eleusine coracana*) grain. Source: Ramashia, 2018

Fig.2. Rotary convection oven

	
Fig.3. Mixture of ragi flour, wheat flour, Powder sugar, butter and salt	**Fig.4. Milk was added to the mixture**
	
Fig.5. Preparation of dough	**Fig.6. Prepared dough**
	
Fig.7. Prepared dough wrapped in a plastic wrapper	**Fig.8. Dough after 20 minutes refrigeration**
	
Fig.9. Dough cut into square pieces (Before baking)	**Fig.10. Cut pieces baked in**

Fig.11. Final product (Ragi biscuits)

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RESEARCH ARTICLE

System of Rice Intensification-A New Approach of Rice Cultivation

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ABSTRACT

Population of India as well as world continues to grow for the last few decades and with this growing population availability of per capita fresh water declines day by day. Rice is the most important staple food crop of India as well as world and growing rice with continuous flooding have been wasting large volumes of water form last several years. There is very limited scope of increase in rice cultivation area. Therefore, to meet the demand of present and future generation, it is need to be increased rice productivity by using less water with more efficient agronomic practices. As an alternate rice cultivation methodology, System of Rice Intensification (SRI), developed to increase rice productivity with less input use. SRI is the application of certain management practices those include changes in nursery management, time of transplanting, water and weed management which provide better growing condition in the root zone of rice plants compare to plants grown under conventional practices. As a result of the organic fertilizer application, SRI improves soil structure and increases nutritional value, as well as environmental benefits including reduced water usage due to intermittent flooding.

Keywords: SRI, principles, modified SRI, resource use efficiency, carbon sequestration.

INTRODUCTION

Food security is a big challenge for any developing country like India. Due to factors such as population growth, urbanization, and per capita increases in income, demand will undergo several changes and the natural resource will become increasingly stressed, based upon which agriculture depends. Global rice production must be increase at least30% by 2050 to meet demand of future generation (Shew *et al*., 2019).Producing more yield with less input use, while preserving and enhancing the livelihoods of small-scale and farming family, is a key challenge for the future. To meet the growing and changing food demand, globally it need to be achieved improvements in resource-use efficiency and gains in resource conservation which will halt and reverse environmental degradation (Tampubolon *et al*., 2021).

For more than half of the population of the world, Rice (*Oryza sativa* L.) is the staple food and an important target to provide food security and livelihoods for millions. Globally India ranks number one in rice area and stands next only to Chinain total paddy production (Duary and Pramanik, 2019).Different technologies are now used for reducing water requirement of rice in India. One such approach is SRI which has the potential to improve the water and land productivity using less water while it increases production (Anbarassan *et al*., 2013; Midya et al., 2021a, b).SRI an alternate rice cultivation methodology developed 25 years ago in Madagascar is gaining wider acceptance in many countries including India (Kumar *et al*., 2017; Mohanta et al., 2021).

WORKING PRINCIPLE OF SRI

SRI is the integration of different agronomical cultivation practices such as reduction of plant population, transplanting single young seedling per hill, wider square planting, mechanical weeding with cono weeder and use of the leaf colour chart (LCC) for better nitrogen management resulted higher yield (Anbarassan *et al*., 2013). One of the main principal factors in SRI for better crop growth and productivity is use of intermittent irrigation with alternate wet and dry intervals (AWD). Seedlings are transplanted quickly, within 15-30 min of gentle removal from nursery. They are gently transplanted at a depth of only 1-2cm. Unlike the traditional method, which transplants seedlings into flooded soil, the SRI method transplants seeds into appropriately moist soil. They are not subjected to shock, which slows their growth.

MODIFIED SRI

Modified SRI (MSRI) implies an intermediate practice between SRI and conventional transplanting system of rice which includes transplanting of 20-days-old seedlings in square planting at 20 cm X 20 cm spacing with using two seedlings $hill^{-1}$ as against transplanting of 10 to 12-days-old seedlings, one seedling $hill^{-1}$ and wider spacing of 25 cm X 25 cm under SRI. In this system of transplanting, medium aged seedlings have been found to withstand in extreme rainfall condition and maintain adequate plant population and enhances rice productivity (Das *et al*., 2012). Under MSRI the rice productivity is increased up to 39% and employment and net returns were enhanced by 15% and 61%respectively than that of conventional method.MSR I also resulted increase in water-use efficiency by 12% and water productivity by 59% compared to traditional method and rice crop matured 15 days earlier under MSRI (Das *et al*., 2018).

Comparative study between SRI and Conventional Methods

SRI (97.3%) recorded the highest establishment percent of seedling after transplanting that is significantly different from the conventional method (81.7 %) due to careful handling of seedlings as well as transplanting of very young seedlings which prevented transplanting shock of the roots. It also enhanced the days to 50 % flowering and took the longest (104.3) days to flower than the conventional methods (91.0), probably due to longer and higher accessibility of nitrogen by the SRI plants (Dzomeku *et al*., 2016). Compared to the traditional system, yield parameters including the number of fertile tillers per m^2, the number of spikes, the weight of the 1000 grains including yield is higher in SRI and is more productive and economically more profitable than the traditional system (Diedhiou *et al*., 2021).

Single plant in SRI showed greater root-pulling resistance (RPR) compared to clumps of multiple rice plants grown in the transplanted methods of rice cultivation. SRI cultivation method contributes better nutrient uptake by the rice plants due to greater root growth, quantified by measured differences in root-pulling resistance and in root length density (Barison and Uphoff, 2011). Though, SRI is little more costly but also more profitable due to higher yield brought on by the adoption of best agronomic practices associated with SRI. The promotion of the SRI method is possible by creating awareness, extension services and different training programme (Abankwah and Tutu, 2021).

Reduction in cost of nursery, seed, weeding, irrigation, plant protection chemicals and labour requirement resulted lower cost of production in SRI by 15.6% than conventional practices (Ali and Izhar, 2017).SRI creates a situation for agriculture which ensure climate security and food security. It increases rice production with a sustainable manner and also farmer incomes. SRI also strengthens crops' resilience to climate change and variability

Under SRI, plant roots are significantly longer and heavier than conventional method. Number of root hairs also increased by 60%, and root hairs were healthier and more vigorous in SRI method compared with conventional method.In SRI method, the rates of photosynthesis at the vegetative, flowering, grain filling, and mature grain phases were significantly different compared to the conventional method (Hidayati *et al*.,2016).As a result of aerobic and biologically rich soils and less competition for resources, SRI fields are better able to resist pest and disease attacks and withstand drought and severe wind or storms (Hayat *et al*., 2019).

SRI AND RESOURCE USE EFFICIENCY

For the conventional 15 kg/ha of rice cultivation is high because of huge quantity of seeds, fertilizers, plant protection chemicals, animal labour seeds is required but for SRI only 5 kg of seeds is required for nursery (Ali and Izhar, 2017).Use of fertilizer and agrochemical inputs decreased by 30-50%, and by 100% with organic SRI when relying on organic fertilization and higher nutrient uptake also resulted by larger root systems (Toriyama and Ando, 2011).The variable cost in conventional method and irrigation charges compare to SRI method and per hectare cost of cultivation is around five per cent lower than the conventional method (Anbarassan*et al*., 2013).

CARBON SEQUESTRATION AND RICE ECOSYSTEM

In SRI as soils are maintained under mostly in aerobic conditions as a result methane (CH_4) emission is reduced by between 22% and 64% (Suryavanshi *et al*., 2013). Cono weeding in SRI aerates the rhizospheric soil which in turn promotes microbial activities and proliferates root growth. Rhizosphere soils has a higher enzyme activity viz., dehydrogenase, urease, acid phosphate, alkaline phosphate and nitrogenase in SRI than conventional system. Weeding implement in SRI churns up the top 3-5 cm of soil and buries weeds and decomposing the weeds provides additional nutrients to the plants and beneficial aerobic microorganisms. SRI soils reported higher microbial biomass carbon (MBC) in the range of 2-41 %in all seasons (Rajkishore *et al*., 2015).

Nitrous oxide (N_2O) emission is reduced as use of N fertilizers is reduced in SRI, so global warming potential is also reduced (Choi *et al*., 2014). Total global warming potential (GWP) from flooded rice paddies is reduced 20-30% in SRI (Jain*et al*., 2014).Several environmental benefits are resulted by SRI production systems like reducing water and energy use by 60% and 74% per kg respectively, reducing GHG emissions by 40% per kg, reducing reliance on nutrient inputs and also improving farmer returns by more or less 400% through increasing yields while reducing costs (Gathorne-Hardy *et al*., 2016).

CONSTRAINTS

The main constraints for SRI production in farmers field are lack of skill labour required for transplanting, weed management with manual conoweeder, availability of FYM at proper time, lack of suitable irrigation and drainage facility(Channa and Syed, 2017).Apart from these other major constraints in SRI production are lack of awareness, scarcity of skilled labour, nursery management (Agarwal and Kumar, 2017).

CONCLUSION

To apply in the field SRI required a lot of technicalities and resulted a higher production cost. Difference field experiments shown that adoption of SRI particularly or fully has a great influence on yield and income. SRI creates a favourable soil-water-plant-atmosphere condition than traditional wetland rice production, which depends on constantly flooded fields and hypoxic soil conditions. Practices of SRI creates beneficial effect on soil micro-organism, as well as rice plant roots and canopies. In comparison to currently favoured farming methods with inundated rice paddies, SRI practises show positive impact on crop performance and water productivity, as well as an increase in land productivity (yield per unit of land).

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Table 1: Different cultivation practices of SRI and conventional method

Practices	SRI	Conventional
Seed rate (kg ha^{-1})	5 kg/ha	15 kg/ha
Seedling age (days)	8 to 12 days	15-30 days old seedling
Spacing	25 cm X 25 cm	30 cm X 10-15 cm
Seedling number $hill^{-1}$	1	4-7
Water management	Alternate wetting and drying	Continuous flooding throughout crops growing cycle
Weed management	Mechanical weeding (Mainly cono weeder)	Use of herbicide and sometimes hand weeding
Nutrient management	Organic fertilizer (chemical fertilizer added in need bases)	Depends only in chemical fertilizer
Yield	8 t/ha	3 t/ha

Table 2: Difference on plant growth parameter between SRI and conventional method of rice cultivation

Management practice	Plant height (cm)	Culm height (cm)	Average tiller number (hill^{-1})	Tiller number (m^{-2})	Average tiller perimeter(cm)
SRI	124.20	84.0	18.3	450.1	2.9
Conventional	101.40	67.5	8.9	441.2	2.1

[Source: Thakur *et al*., 2014]

Table3: Effect of cultivation methods on root characteristics of rice

Cultivation methods	Root aerenchyma area (%)	Root length (cm)	Root dry weight	Number of root hairs (number/mm^2)
Conventional	70.9	32	7.4	510
SRI	45.1	34	14.2	816

[Source: Hidayati *et al*., 2018]

Table 4: Difference on cost of cultivation between SRI and conventional method of rice cultivation

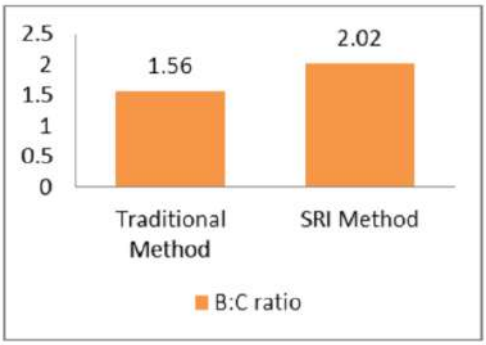
Sl.No	Particulars	SRI	Conventional
1	Total Fixed cost	11788	12462
2	Total variable cost	28863	30210
3	Total cost	40651	42672

[Anbarassan *et al*., 2013]

Table 5: Emissions of methane, nitrous oxide, and carbon equivalent emission from rice soils as influenced by different methods of rice cultivation

Method of planting	Methane	Nitrous oxide	Global warming potential ($kgCO_2eq.\ ha^{-1}$)	Carbon equivalent emission (CEE)
Transplanted rice	22.59	0.61	888.1	242.2
SRI	8.81	0.91	644.3	175.7
Modified SRI	8.16	0.89	620.4	169.2

[Soursce: Jain *et al*., 2013]



[Source: Kumar *et al*., 2020]

Fig.1.Climate change and variability

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RESEARCH ARTICLE

Crop Residue Burning in India: Causes, Impacts and Solutions

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ABSTRACT

India is one of the key producers of cereals, pulses, oilseeds and other agricultural crops. Currently, the majority of farmers in North India follows the rice-wheat cropping system which produces a bulk amount of uneconomical yield (Straw).With the increase in the crop production, the crop residues are also increasing and ultimately due to several factors, the farmers are compelled to burn the residues. In India on an average the total amount of crop residues generated is 516 million tonnes and the crop burned is 116 million tonnes for the year 2017-2018. The problem of on-farm burning of crop residues is increasing due to many reasons. The crop residues can be used for multiple beneficial uses and hence, residue burning causes wastage of resource potential and results in environmental pollution. Residue burning can decrease the soil fertility, degrade the air quality, imparts health issues, emits greenhouse gases, results in the imbalance of radiation and reducethe soil organic matter and evenproductivity of soil.The major constraints such as time, resource, financial and farm mechanization are responsible for crop residue burning. Alternative uses such as composting, residue incorporation, mulching etc. are few effective techniques that can help to minimize the negative impacts arising from residue burning.

Keywords: Crop residue, incorporation, sustainability, happy seeder, rice, wheat.

INTRODUCTION

Agriculture has a huge impact on the overall Indian economy (Maitra *et al*., 2021; Praharaj *et al*., 2021). In India, with diverse ecological and agricultural regions, a wide variety of plant species are cultivated in large tracts of land. The vast majority of crop residues are left in the field after harvest. After harvesting the economic parts of the crop, the rest of the crop parts, including the stems, leaves, seed pods and roots, is known as Crop residue (Bhatt *et al*., 2014). It is estimated that India produces about 500 Mt of plant residues annually (MNRE, 2009)with significant regional diversity. Unequal distribution and use of crop residues depends on cultivated crops, planting potential and production nationwide (Bhuvaneshwari *et al*. 2019). The highest amount crop residues are generated in Uttar Pradesh (60 Mt) as it has the highest agricultural land in India (Badarinath and Chandkiran, 2008). According to

current estimates, the burning of crop residues is prevalent in four states, namely Punjab, Haryana, West Bengal and Uttar Pradesh. Cereals, fibres, pulses, oilseeds and sugarcane contributes the majority of crop residue with production estimates of 352 Mt, 66 Mt, 13Mt, 29 Mt and 12 Mt respectively(Table 1)(NPMCR, 2014). Among cereal crops, rice, wheat, maize and millets together contributed most of crop residue and then followed by fiber crop (Table 2) (MOSPI, 2013-14). Oil seed residues were burned in Rajasthan and Gujarat at about 9.26 and 5.1 Mt residues respectively while burning fiber crop residues were prevalent in Gujarat (28.6 Mt) (NPMCR, 2014).The amount of crop residues produced can be calculated as the product of residue/straw to crop ratio, dry matter to crop biomass ratio and total crop production. The straw to grain ratio varies with the crops i.e., the ratio of 1.5 to 1.7 for cereal crops, 2.15 to 3.0 for fiber crops, 2.0 to 3.0 for oilseed crops and 0.4 for sugarcane(NPMCR, 2014). So proper utilization of these crop residues can improve the agriculture waste management, reducing the pollution contributed through agriculture and there by attaining sustainability (Singh *et al*., 2020).

CAUSES OF CROP RESIDUE BURNING

- Time factor forces the farmers to burn residue to clear the farms early to prepare the land for growing the succeeding crop.
- Farmers in the north western regions of India have a traditional belief that burning crop residue will restore nutrients to the soil and minimise weed growth.
- Employment guarantee schemes like MGNREGA (Mahatma Gandhi National Rural Employment Guarantee Act) have been introduced earlier and this leads to the shortage of farm labourers.
- Regarding Farm mechanization, crop residue management machines like Super SMS and happy seeder is insufficient (Dhaliwal *et al*., 2011). This Super SMS (straw management system) is attached with the combine harvester which chops the paddy straw into minute pieces and spreads it uniformly in the field which makes it easier and farmers are not required to burn the straw before sowing of next crop (Erenstein and Laxmi, 2008).

THE IMPACTS OF RESIDUE BURNING

The on-site impact of burning, which includes taking away of a large amount of nitrogen, phosphorus, organic matter and also other nutrients and even loss of useful microorganisms which are present in the upper layers of soil (Nair *et al*., 2020).The off-site impacts are basically related to health due to general air quality degradation leading to the problems of respiratory organs, cough, asthma, eye and also skin diseases. Cardiovascular diseases can also be the problem by those finest particles in smoke and may lead to death. In the open field, burning of crop stubble results in the emission of many harmful gases in the atmosphere i.e., for 1 tonne of straw, it releases carbon dioxide of 1460 kg, carbon monoxide of 60 kg, ash 199 kg and small amounts of nitrous oxide, nitric oxide, sulfur dioxide and methane along with particulate matter and hydrocarbons (Mandal *et al*., 2004)

Time constraint

In Rice-Wheat cropping system, usually Rice is sown in kharif season and the harvesting occurs during September - October. In North - West India, when the temperature becomes low i.e., after second week of November which leaves the farmers with a time period of 20- 30 days, within the months of October - November they will harvest the paddy and sow the next season crop i.e., Wheat by the 15th of November (mid - November). In the Northern states, the most important time for wheat crop to mature is mid - April that is when the temperature is above 35 degree C and the exact time for full maturity of wheat crop is about 140-150 days.(Vijayaprabhaka *et al*., 2017). Hence there is a very short period between paddy harvesting and wheat sowing. Hence, due to lack of time for field preparation and residue decomposition between *kharif* harvesting and *rabi* sowing, they usually go for burning of the residue.

Resource constraint

Labourers are required to remove the rice straw from the field and it is considered a labour-intensive process. For the harvesting of rice crop, the labourers from the states of Eastern Uttar Pradesh and Bihar are primarily employed in the North - west India. When the MGNREGA (Mahatma Gandhi National Rural Employment Guarantee Act) in 2006

was introduced, this time onwards it led to significant shortage of labour (Turmel *et al*., 2015). Due to lack of human resource and high cost of wages the residue conservation is highly difficult.

Financial constraint

Large farmers can afford the amount by purchasing the costly equipment for the fields as well as for other purposes, but the marginal and small farmers are not able to purchase the implements to plough back the stubbles to mix back into the soil particularly for the managing of crop residues as there is a lot of burden for those farmers to spend that much amount on these implements.

Farm Mechanization

Due to farm mechanisation, there is a major change in the entire system i.e., how farmers plant, how to irrigate the fields and even the harvesting of crops. With the introduction of combine harvester, large residue loads remained in the field, making sowing of succeeding crop relatively more difficult. By using sickle, the farmers cut the residues very close to the ground and whatever the residue left that is very minimum and will not hinder for sowing of next crop, but with combine harvester about 25-30 cm stubble remains above the ground level.

APPROACHES FOR RESIDUE MANAGEMENT

Various solutions have been developed for managing the crop residue burning but no single option can substitute the residue burning effectively. Therefore, these all approaches/solutions can be integrated to manage residues.

Composting

Composting is an ex-situ practice i.e., composting the material somewhere else and they are returning it to the soil. So, whatever the nutrients present those are coming back to the soil and this is considered as one of the sustainable practices (Naresh *et al*., 2013). As compost consists of rich organic matter, it plays an important role in sustaining the soil fertility. Higher potential for increased yields, eco-friendly, improvements in soil quality, enhances the structure of the soil and the nutrient value of soil are some of the beneficial effects of compost amended soil. The application of composts like FYM and vermicompost can enhance the yields of rice (Sairam *et al*., 2020). These techniques also help in uptake of nutrients, and active nutrient cycling occurs due to enhanced microbial activity in the soil. The problem is the amount of residue, time and the decomposability (Smitha *et al*., 2019). Recently the Indian Agricultural Research Institute (IARI) developed a bio-decomposer technique also called as 'Pusa Decomposer' which converts the crop stubbles into compost and it is alternative for the chemical fertilizers. It is basically a fungi-based liquid solution. It can soften these hard stubbles and easily mixes with the soil in the field as a compost. It can degrade the waste and it converts into nutrient rich compost. Low cost and an effective way of dealing this stubble burning problem.

***In-situ* incorporation**

The main reason for residue burning was the lack of choice to dispose the straw. Burning was considered as the easiest way. However; incorporation of stubbles into the field can be a relatively better option even though it increases the cost of stubble management. Plants contains some nutrients like N,P,K etc., so whatever the nutrients present inside the plant that is present within the straw also. If the farmers burn their residues, there are 5 nutrients which are actually lost i.e.,C,H,O,N. Even after burning the residues, the straw is rich in Potassium and Magnesium because they are volatile. So, the physical properties of soil are improved due to in-situ incorporation of straw that includes the infiltration rate, water holding capacity of soil, bulk density, cation exchange capacity (CEC) and even the soil structure. The availability of soil organic carbon improves the microbial growth and mobilization of nutrients. In the rice-wheat cropping system of North-western India, application of rice residues in the next season crop was found to be improved in wheat yields (Gupta *et al*., 2007).

Surface retention and mulching

The residue retention considers the crop remains which are left in the field rather than crop remains which are brought from somewhere else and then added to the soil. Simply, change and improve the properties of soil with the fresh plant material or crop residues. The main advantages of retention of crop remains on soil surface includes the decrease growth of weeds, saves the cost of using weedicide, by lowering the use of chemical fertilizers as it improves the physical, chemical, biological attributes of the soil, recycling of plant nutrients and it is a low-cost option. Direct drilling in surface mulched residues is a technique in which the residues from the preceding crop are left on the soil surface without being incorporated (Ghouse *et al*., 2020). It protects the surface soil from erosion by the increase of organic carbon and total nitrogen in the top 5-15 cm of soil (Maitra *et al*., 2018). As the residues decompose slowly on the surface because of that farmer choose to burn the residues.When compared to residue burning, farmers may leave residues on the soilsurface which increased the soil nitrate concentration by 46 %,uptake of Nitrogen by 29 %, and alsoyield by 37 %. This technology has become less popular with the farmers due to the labour shortage and high cost.

Paddy straw for mushroom cultivation

Paddy straw is the main ingredient to be used as a raw material for the mushroom culture, although farmers often use wheat straw or others. Paddy straw mushroom, more simply called as straw mushrooms, are the species of fungi mainly cultivated in rice straw. It may refer to various species of edible mushrooms grown on rice straw or other cereal straw-based substrates, including wheat. Other than these, cotton, sugarcane, or any corn husks and other organic substrates may also be used, either alone or in a combination with rice or any other cereal straw (Choudhary *et al*., 2009). In India, there is huge availability of these paddy straw which enhances the profitability of farmers and can be considered as one of the sustainable substrates for mushroom cultivation.

Happy seeder

If a farmer has less amount of straw, they may not require any intervention and the farmers may simply sow the next season crop. But in the regions of Punjab and Haryana the straw height is more and farmer cannot sow the next crop with just a ploughing operation and it is very difficult to plough in those conditions. In that case, they choose to burn the residue. If there is a machine such as Happy Seeder, a technique mainly used for sowing of wheat directly without any prior seed bed preparation rather than burning of crop residue (Sidhu *et al*., 2007). This technology is eco-friendly; improve health of soil and the sowing of wheat is also done on time. Farmers can also manage crop residues effectively by some machinery like

- **Paddy straw chopper:** This is a machine which can be used for cutting of paddy stubble into small pieces for easily mixing with the soil.
- **Baler:** This machine is used for collecting of the straw from the field and making like bales of those paddy stubble into compact bales.
- **Zero till seed drill:** mainly used for the preparation of land and makes it possible to sow the new crop in the withstanding previous crop stubble.
- **Reaper binder:** This is used for the harvesting of paddy straw and then this machine makes in the form of bundles.

Fodder for animals

The other option to necessitate the use of agricultural crop residues as feed for animals is one of the alternative solutions in northern regions of India for crop residue burning. Residues of rice or wheat can be used as a fodder for animals. But rice residues are not that much utilized among farmers in Punjab because the rice residue contains higher silica content as it is very difficult for the microorganisms to act upon (Singh *et al*., 2005). Even if the farmers could allow the rice straw to decompose, it will takes time as the rice residues are not easily decomposed (Pathak *et al*., 2020). Many farmers in northern state like Punjab, Haryana, Uttar Pradesh they use to separate the grains by threshing with some machines and it yields the straw pieces of 1-3 cm in length, ‘and it is used for feed and even the farmers realize the wheat straw can be more suitable for cattle feeding than paddy straw.

Bio gas using rice straw

Rice straw is one of the organic materials that can be used for continuous production of bioenergy and biofuels such as Biogas (about 50-75% methane and 25-50% carbon dioxide). Biogas is a renewable source of energy and its can be produced through various ways. As burning this crop waste considered to be the cause of air pollution, the government of India has been declared and pushes towards more sustainable use of organic waste to decrease the problem and convert paddy straw into biogas production. Generating this biogas from crop wastes could be an effective and environment friendly alternative to stubble burning (Sidhu and Beri, 2005). Biogas from agricultural wastes is produced by microorganisms during fermentation and it can be used as a fuel to replace fossil fuels. Agricultural crop residues, such as rice straw is a chief source of ligno-cellulose which is required for biogas production.

Crop residue usage in bio thermal power plants

Stubble burning is not only limited to the northern parts of the India and it becoming a serious issue in other areas as well due to the mechanised farming. Alternative to this, the government wants all thermal power plants to procure crop residues from the farmers and they use to utilise it for the power generation. Rice residues can be used for generation of electricity. Jalkheri, District Fatehgarh Sahib in Punjab is the first Plant in India based on the usage of biomass, i.e., source of renewable energy (Pathak, 2004). This Bio thermal plant can use rice husk, agricultural waste, the straw of various crops like paddy, wheat, etc. for the generation of electricity. The project is providing extra income to many farmers from the sale of agricultural waste.

Bio-char

Biochar is a fine-grained carbon rich porous product and it is highly stable, comprises of more than 65% obtained from the thermo-chemical conversion process called the pyrolysis which is done at low temperatures in an oxygen free environment (Amonette and Joseph, 2009). Biochar is considered to be the cost effective and environmentally friendly solution. The chemical composition is highly dependent on feedstock and pyrolysis conditions. It is used as a soil improver for both carbon sequestration and soil health benefits (Gaunt and Lehmann, 2008).When amended to soil, the biochar helps in improved water retention, increased pH of acidic soils and increased soil surface area (Gangil and Wakudkar, 2013). It mainly interacts with the soil microorganisms and plant roots, which helps in retention of nutrients like nitrogen and sulphur and improving the soil health, reduce soil emissions of greenhouse gases, along with 10 percent increase in the grain yield with a less risk of reduced crop yields. It also leads to the improvement in the porosity, soil aggregate ability, infiltration rate, increase in the earthworm population and even the increase of water-holding capacity of the soil (Lehmann and Joseph, 2009).

Educating through different ways

Educating the farming community is very necessary to bring them out and they should be educated about the advantage of reduced chemical fertilizers by implementing though various solutions of this crop residue burning (Goswami *et al*., 2020). As mentioned previously, it produces extremely high levels of toxic particulates, which affects the people health. Moreover, through kisan camps, training and workshops, the efforts have been and being made, apart from campaigns through various media, T.V. shows, and radio, in informing farmers about the possibility usage of crop residues.

CONCLUSION

Crop residues are of great economic value being used as feed for livestock, fuel usage and also used as raw material for industries. Depending upon different regions, the management practices of crop residues also change. Crop residues contains large quantities of nutrients and thus the return of crop residues to the soil can save a considerable quantity of fertilizer and to maintain the human, animal and soil health etc. The government should encourage and provide alternative support-based measures to the farmers to stop this residue burning. Another way to be

successful for farmers by providing credit to buy the machinery, equipment and a good interest rate through banks. Although, they have various programs, schemes, interventions, rules and regulations; however, this is still a major issue and there is a strong need for other innovative approaches for the effective substitution of crop residue burning.

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Table 1. Crop residue production estimate of major crops in India (NPMCR, 2014)

Crop	Estimate of residue production (Mt)
Rice	105
Wheat	94
Sugarcane	361
Oil seeds	30
Cotton	35
Jute	11
Pulses	17

Table 2. Crop residues produced by major crops (Turmel *et al.*, 2015)

Source	Composition
Rice	Husk, Straw
Wheat	Bran, straw
Maize	Stover, husk, skins
Millet	Stover
Sugarcane	Sugarcane tops, bagasse, molasses

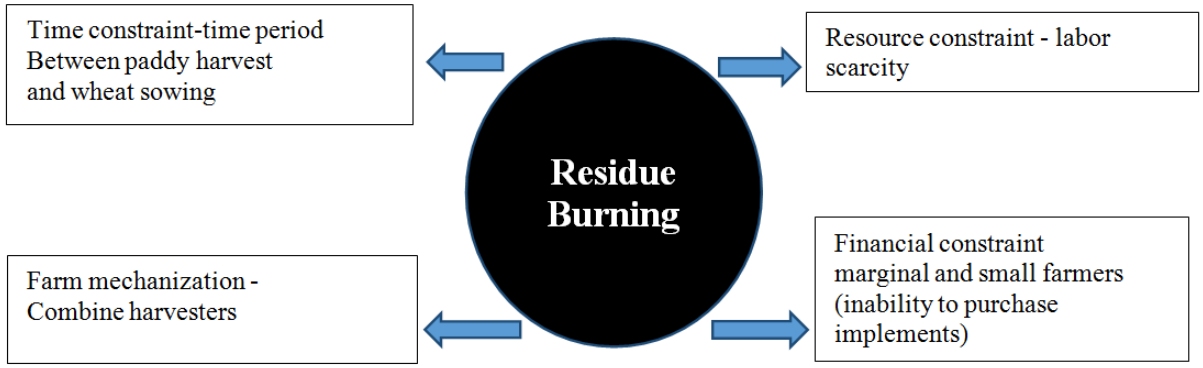

Fig. 1: Causes of residue burning

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RESEARCH ARTICLE

Bio-efficacy of Acephate 75% SP against Bollworm Complex and Jassids Infesting Cotton

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ABSTRACT

Cotton is a main commercial crop of vital importance, commercially grown for the fibre that is used as achief raw material for textile industry. India being largest cultivator of cotton area wise still the productivity is low. Amidall aspects contribute to low output, biotic limits are vital and are mainly leading to insect pests. With development pest resistance to the existing chemicals, it is essential to search for novel compounds to manage these pests. A study was conducted at University Experimental Farm, 'C' Unit, BCKV, West Bengal to evaluate the bio-effectiveness and phyto-toxicity of Acephate 75% SP Manufactured by M/s Krishi Rasayan Exports Pvt. Ltd. against bollworms and Jassid in cotton crop and also to observe the effect on natural enemies. It was concluded that Acephate 75% SP @ 292 g.a.i./ha for jassid management and @ 584 g a.i./ha for bollworms management, gave better results for protection to cotton crop against jassid, a sucking pest (96.25% reduction) and bollworm complex consisting of Spotted boll worm, *Earias insulana*, American bollworm, *Helicoverpa armigera* and pink boll worm *Pectinophora gossypiella* (8.50% fruiting bodes damaged/plant) along with significant increase in seeded cotton yield (30.45 q/ha).

Keywords: Cotton, Bio-efficacy, Acephate, Jassids, Bollworm complex.

INTRODUCTION

Cotton is a main commercial crop of vital importance. It is cultured in over 38 million hectares all over the world under different agro-climatic conditions, and India contributes to the 24 percent of those area. 29.8% to India's agricultural GDP is contributed by cotton production. The fiber which is a chief economic part provides the raw material to various clothing and textile industries. In India cotton is cultivated in 34% of agricultural lands (12 million ha) trailed by China (5.5 million ha). Cotton contributes to around 33% of agricultural exports and 65% of clothing raw materials (Mayee and Rao, 2002; Mayee, 2011). Although India has major area under cotton yet the productivity is low. Amongst numerous aspects attributing to low yield in cotton, biotic constraints are of essence. Among them, insect pests assume great up to 35-40% (Kannanand Uthamasamy, 2004). In India the use of insecticides is very high in cotton attributing to Rs. 2800 crores and is increasing every year (Ghosh, 2002). Over 1300 species borers, sucking pests and mite pests have been described to infest cotton across the globe (Hargreaves, 1948 and Atwal, 2002). However, in India the number is restricted to 130 to 200 species (Agarwal *et al*., 1984 and Balakrishnan *et al*., 2010). Among them, the boll worms viz., American boll worm, *Helicoverpa armigera* (Hubner), Spotted boll worm, *Earias vittella* (Fabricius), Spiny boll worm, *Earias insulana* (Boisduval) and Pink boll worm, *Pectinophora gossypiella* (Saunders) caused devastating damage to cotton during early 2000. Besides these, Green leaf hopper, Jassid, *Amrasca biguttula biguttula* (Ishida) is also known to cause a great loss (Gosh, 2001) and contributing up to 23% loss in production (Satpute *et al.,* 1990). Insecticides appraised at US$ 330 million are used annually on cotton in India (Manjunath, 2004). Cotton contributes to about 48 per cent of insecticides in India (Saiyed *et al*., 2005). The biological control measures have found to be less effective in controlling the pests (Rafee, 2010). With a view to find alternative chemicals a study was conducted to evaluate the bio-effectiveness of Acephate 75% SP Manufactured by M/s Krishi Rasayan Exports Pvt. Ltd. (KREPL) against bollworms and Jassid in cotton crop and also to observe the effect on natural enemies.

MATERIALS AND METHODS

A field experiment was conducted at University Experimental Farm, 'C' Unit, BCKV, Kalyani, Nadia, West Bengal to evaluate insecticide molecules i.e., Acephate 75% SP against Bollworm complex and Jassids in cotton variety "Sujata"comprising seven treatments including control and three replication in randomized block design during June-November of 2017-18. Three rounds of applications were given one at two stages i.e., after jassids and bollworms crossing ETL respectively. Spraying was carried out by using high volume Knapsack sprayer fitted with hollow cone nozzle using water @500 l/ha at first stage for Jassids appearance and @1000 l/ha at second stage at bollworm damage stage (depending upon the crop canopy). Assessment of jassids control, its average population per plant was recorded by counting no. of jassids (adult and nymphs) on ten randomly selected plants and three leaves per plant (Upper, Middle and Lower leaf) per replicate at before treatment and 1, 3, 5,7 and 10 days after each spray.

To study the bio-efficacy of the test insecticides against bollworm complex, percent damaged fruiting body by bollworms was counted on ten randomly selected plants per replicate 1 day before of second round of spray schedule and at 1,3,5,7 and 10 days after each spray and finally at harvest of crop. Number of Good open boll (GOB) and Bad open Boll (BOB) and total no. of bolls per plant were also counted at each picking as well as at harvest. The seeded Kapas yield was recorded in each plot at each picking and at the time of harvest of crop and converted to quintals per hectare. The data thus collected were converted into percent damage index. The data on pest incidence and yield were subjected to analysis of variance after making necessary transformations wherever needed.

RESULTS AND DISCUSSION

Jassids management

With the application of Acephate 75% WP (KREPL) @ 292 gm a.i./ha gave maximum control of jassids population and varied from 80.02%, 88.98% and 96.25% after 15 days of 1st, 2nd and 3rd spray and significantly superior to other treatments. Whereas Acephate 75% WP (Market sample) gave 73.17%, 78.90% and 82.15% control closely followed by Acephate 75% WP (KREPL) @ 225gm. a.i./ha with 68.21%. 76.11% and 84.13% for the same period and both the treatments were at par with each other. Whereas, the percent reduction in jassids population in the treatment of Fenvale rate 20% EC @ 30 gm a.i./ha was 58.51%, 65.80% and 70.13%, followed by Cypermethrin25% EC @ 30gm a.i./ha with 53.14%, 60.00% and 65.42% population control. Acephate 75% SP (KREPL) @ 152 gm. a.i./ha was least effective for managing jassids population in cotton crop.

Bollworm Complex management

The Experimental results showed that with the application of Acephate 75% WP (KREPL) @ 584 gm. a.i./ha, the damage of fruiting body (bolls) due to boll worm complex was 8.20% and statistically superior to Acephate 75% (KREPL) @ 450 gm.a.i./ha with 10.30% bolls damaged. Acephate 75% WP (Market sample) having 13.80% bolls damaged closely followed by Acephate 75% WP (KREPL) @ 350 gm. a.i./ha with 14.50% boll damaged and both the treatments were statistically at par in managing cotton boll worms,. Fenvalerate 20% EC @ 100 gm. a.i./ha and Cypermethrin25% EC@70g a.i/ha were least effective for managing boll worms damage having 18.90% and 17.00% cotton bolls damaged, respectively.

Yield

Maximum seeded cotton yield was recorded in the treatment of Acephate 75% SP KREPL@ 584 gm a.i./ha having 30.45 q/ha and the percent increase over control was 49.48%, closely followed by Acephate 75% SP (KREPL) @ 450 gm. a.i./ha with 29.12q/ha and 42.95% increase over control and at par with each other Acephate 75% SP (Market sample) @ 584 gm. a.i./ha gave 24.35 q/ha seeded cotton yield which was 19.53% increased over untreated control. Among all the treatments Cypermethrin 25% EC gave minimum cotton production to 24.21 q./ha and 18.85% increase over control.

CONCLUSION

The finding recorded in this observation that Acephate 75% SP (KREPL) applied @ 292 g.a.i./ha for jassid management and @ 584 g a.i./ha for bollworms management, gave better results for protection to cotton crop against jassid, a sucking pest and bollworm complex consisting of Spotted boll worm, *Earias insulana,* American bollworm, *Helicoverpa armigera* and pink boll worm *Pectinophora gossypiella* along with significant increase in seeded cotton yield. The product was safe to cotton plants, hence recommended for jassids management @ 292 gm a.i./ha and bollworm complex management @ 584 gm. a.i./ha respectively. The results of the experiment agreed with the findings of Nihal et al. 2021a; Nihal et al., 2021b; Jeer et al., 2017; Seni and Naik, 2017; Kumari et al.,2019; Patidar et al., 2018;Badariprasad et al., 2020; Subhash et al., 2017 which stated that Acephate is very effective in controlling both sucking and bollworm pests of cotton.

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Table 1: Bio-efficacy of Acephate 75% SP(KREPL)against jassids on cotton crop during 2017-18 at University Experimental Farm, 'C' Unit, BCKV, Kalyani, Nadia, West Bengal.

Sl. No.	Treatments	Dose (g a.i ./ha)	Percent reduction in Jassids population over control after days of spray (mean of three replications).															
			Pre - treatment	First Spray					Second Spray					Third Spray				
				1	3	5	7	10	1	3	5	7	10	1	3	5	7	10
T_1	Acephate 75% SP (KREPL)	150	4.23 (12.56)	43.40 (41.50)	45.15 (42.50)	48.20 (44.26)	48.50 (44.43)	51.12 (45.93)	52.00 (46.43)	54.10 (47.64)	55.35 (48.36)	55.00 (48.16)	55.84 (48.64)	56.29 (48.90)	57.38 (49.53)	58.90 (50.42)	60.40 (51.30)	62.13 (52.32)
T_2	Acephate 75% SP (KREPL)	225	4.20 (12.52)	65.25 (54.18)	67.26 (55.40)	67.80 (55.73)	66.20 (54.76)	68.21 (55.99)	72.30 (58.56)	74.10 (59.74)	75.00 (60.33)	75.10 (60.40)	76.11 (61.08)	77.00 (61.68)	79.00 (63.08)	80.20 (63.94)	81.24 (64.70)	84.33 (67.08)
T_3	Acephate 75% SP (KREPL)	292	4.26 (12.60)	75.86 (60.91)	76.32 (61.22)	76.80 (61.55)	78.00 (62.38)	80.02 (63.81)	80.00 (63.79)	81.46 (64.87)	82.00 (65.27)	88.40 (70.54)	88.98 (71.07)	89.00 (71.09)	91.00 (73.05)	92.86 (75.07)	95.48 (78.43)	96.25 (79.61)
T_4	Acephate 75% SP (Chemnova India Ltd)	292	4.06 (12.33)	69.20 (56.60)	70.10 (57.17)	72.00 (58.37)	71.48 (58.04)	73.17 (59.13)	75.00 (60.33)	75.00 (60.33)	76.20 (61.14)	76.48 (61.33)	77.48 (62.01)	78.90 (63.01)	80.00 (63.79)	80.40 (64.09)	81.42 (64.84)	82.15 (65.38)
T_5	Fenvalerate 20% EC	30	4.26 (12.60)	52.10 (46.49)	54.48 (47.86)	55.00 (48.16)	55.00 (48.16)	58.51 (50.19)	58.00 (49.89)	62.00 (52.24)	63.00 (52.83)	63.40 (53.07)	64.45 (53.70)	65.80 (54.51)	68.00 (55.86)	68.80 (56.35)	69.00 (56.48)	70.13 (57.18)
T_6	Cypermethrin25% EC	30	4.26 (12.60)	50.00 (45.29)	52.48 (46.71)	53.00 (47.01)	52.50 (46.72))	53.41 (47.24)	56.20 (48.85)	57.00 49.31)	57.40 (49.55)	56.80 (49.20)	58.64 (50.27)	60.00 (51.06)	61.80 52.12)	63.00 (53.31)	63.80 (53.31)	65.42 (54.28)
77	Control (Untreated)	-	4.26 (12.60)	-	-	-	-	-	-	-	-	-		-	-	-	-	-
	CD (5%)				0.162	0.135	0.174	0.195	0.0951	0.160	0.1357	0.157	0.237	0.129	0.147	0.144.	0.11	0.149

Table 2: Bio-efficacy of Acephate 75% SP (KREPL)against Bollworm complex in cotton crop during 2017-18 at University Experimental Farm, 'C' Unit, BCKV, Kalyani, Nadia, West Bengal.

Sl. No.	Treatments	Dose (g a.i ./ha)	Percent fruiting bodies damaged per plant after days of spray (mean of three replications).																
			Pre - treatment	Fourth Spray					Fifth Spray					Sixth Spray					Harvest
				1	3	5	7	10	1	3	5	7	10	1	3	5	7	10	
T_1	**Acephate 75% SP (KREPL)**	350	5.70 (14.42)	6.40 (15.23)	6.60 (15.45)	7.40 (16.32)	8.00 (16.95)	8.20 (17.15)	9.00 (17.95)	9.60 (18.53)	11.00 (19.82)	11.80 (20.53)	12.00 (20.53)	12.40 (20.70)	13.10 (21.05)	13.80 (21.64)	14.00 (22.22)	14.50 (22.38)	15.50 (23.58)
T_2	**Acephate 75% SP (KREPL)**	450	5.60 (14.30)	6.00 (14.77)	6.40 (15.23)	7.00 (15.89)	7.80 (16.74)	7.90 (16.85)	8.00 (16.95)	8.20 (17.15)	8.80 (17.76)	9.00 (17.95)	9.30 (17.95)	9.40 (18.24)	9.70 (18.34)	9.90 (18.63)	10.10 (18.81)	10.30 (19.00)	10.50 (19.37)
T_3	**Acephate 75% SP (KREPL)**	584	5.20 (13.81)	5.80 (14.54)	5.80 (14.54)	6.00 (14.770	6.50 (15.34)	7.10 (16.00)	7.30 (16.22)	7.60 (16.54)	7.90 (16.85)	8.00 (16.95)	8.20 (16.95)	8.30 (17.15)	7.40 (17.26)	7.80 (16.32)	8.00 (16.74)	8.20 (16.95)	8.50 (17.46)
T_4	**Acephate 75% SP (Chemnova India Ltd.)**	584	5.00 (13.56)	5.80 (14.54)	6.40 (15.23)	7.00 (15.89)	7.80 (16.74)	8.00 (16.95)	8.40 (17.36)	8.80 (17.76)	10.20 (19.09)	11.00 (19.82)	11.20 (19.82)	11.60 (20.00)	12.00 (20.36)	12.60 (20.70)	13.40 (21.22)	13.80 (21.89)	14.20 (22.54)
T_5	**Fenvalerate 20% EC**	100	5.80 (14.54)	6.20 (15.00)	7.00 (15.89)	8.60 (17.56)	9.20 (18.15)	9.60 (18.53)	10.20 (19.09)	11.40 (20.18)	12.00 (20.70)	12.60 (21.22)	12.90 (21.22)	13.00 (21.47)	13.60 (21.56)	13.80 (22.06)	18.40 (22.22)	18.90 (25.77)	20.40 (27.20)
T_6	**Cypermethrin 25% EC**	70	5.00 13.56)	6.40 (15.23)	6.60 (15.45)	8.00 (16.95)	9.80 (18.72)	10.00 (18.91)	10.80 (19.64)	11.60 (20.36)	11.60 (20.36)	13.40 (21.89)	13.80 (21.89)	14.00 (22.22)	14.80 (22.38)	15.00 (23.03)	16.8 (23.18)	17.00 (24.58)	18.30 (25.70)
T_7	**Control (Untreated)**	-	5.00 (13.56)	7.20 (16.11)	8.60 (17.56)	10.80 (19.64)	12.00 (20.70)	12.50 (21.13)	13.40 (21.89)	14.80 (23.03)	18.60 (25.91)	20.60 (27.35)	21.00 (27.35)	21.80 (27.62)	23.20 (28.18)	24.40 (29.13)	26.00 (29.93)	27.60 (30.98)	29.50 (33.21)
	C D (5%)				0.16	0.13	0.17	0.19	0.09	0.16	0.13	0.15	0.14	0.12	0.14	0.14	0.15	0.28	0.14

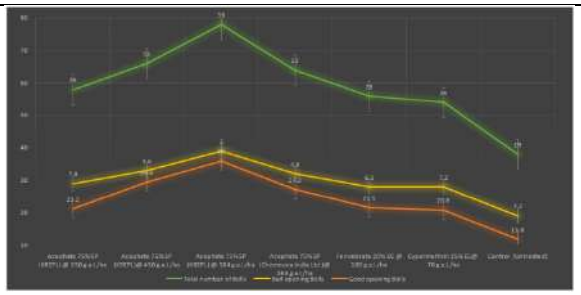

Fig 1: Effect of different treatments against Bollworm complex in cotton crop during 2017-18 at University Experimental Farm, 'C' Unit, BCKV, Kalyani, Nadia, West Bengal.

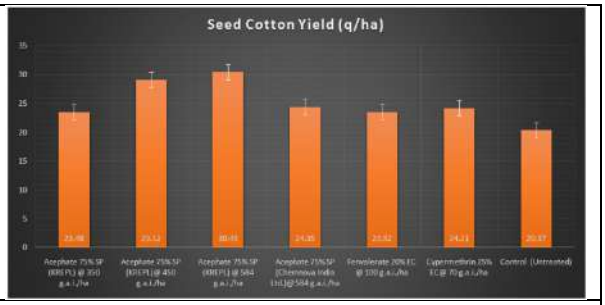

Fig 2: Effect of different treatments on cotton yield (q/ha).

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RESEARCH ARTICLE

Performance Analysis of Reversible Gates

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ABSTRACT

Reversible logic has developed as of late as a promising innovation with applications in low-power CMOS, quantum processing and computing advances. Reversible logic circuits have less power dissipation for each separate input as well as separate output distribution. The classic NAND, AND, NOR, OR, XOR and XNOR gate sets are not reversible. Attempts were made to minimize the circuit of all the gates of logic incorporating CMOS while making them reversible. Furthermore, the reversible logic was used to build the full and half adder reversible. This paper thus provides technical understanding for the design of reversible simple logic gates and combinational circuits

Keywords: Reversible Gates, Delay, Area, VLSI, Digital Circuits, Xilinx.

INTRODUCTION

In the present world dissipation of power is the main problem of the digital system. The use of reversible logical gates can reduce this problem. Low power and less area are the main criteria for high-performance systems. The dissipation of power from these gates is very low also they do not lose information. The quantum cost of such gates can be minimized by decreasing the number of reversible logic gates. VLSI circuit design aims mainly at very low dissipation and system performance [10].If the input can be restored from the output, any gate is said to be reversible. In future emerging technologies, reversible logic has demonstrated potential in broad applications to produce zero power dissipation under ideal conditions [1-2].

Types of Reversible Logic Gates

Fredkin Gate(3x3), Feynman Gate, Doppel Feynman Gate, Peres Gate(3x3), Toffoli Gate(3x3), Sayem Gate(4x4), New Gate(3x3), Peres Gate, TR Gate(3x3), BVF Gate(4x4) and TSG Gat(4x4) e are all major reversible gateway applications in reversible logic synthesis. The number of inputs must be equal to the amount of outputs in the reversible logic

circuit. There must be a unique output pattern for each input pattern. Each output will only be used once, that is, no fan-out is allowed. And the resulting circuit has to be acyclic. These are different types of reversible gates listed below:

Design of Full Adder Using Reversible Gates

In the conventional (irreversible) circuit synthesis, a universal gate library and a Boolean function specification usually begins. This program aims to find a logic system that implements the Boolean function and reduces a cost metric, for example the number of gates or the circuit [3].Reversible circuit synthesis on a high level is just a special case where there is no fanout and every gate needs to be reversible. The Full Adder modeling and simulation in four different ways will be presented here, combining both similar types of the Reversible Gate and different types of the Reversible Gate[4]. These combinations of New Gate Feynman, Peres Gate, Toffoli Gate and Toffoli are used.

Design Using Feynman + New Gate

The Full adder has 3 inputs and (A, B, C_{in}) and two outputs (Sum and Carry). By taking combination Feynman and New gate Full adder circuit can be implemented. It uses two New Gate in the first level and one Feynman Gate at second level. as shown in figure 9.

Design Using Toffoli Gate and Feynman Gate

In this design to obtain the outputs i.e. Sum and Carry combination of Toffoli Gate and Feynman Gate is used. From Figure 10 it can be seen that Toffoli Gate is used in level1 and Level3 where as Feynman Gates are used in level 2 and level 4 to obtain the sum and Carry outputs.

Design Using Peres Gate

The Full Adder outputs can be obtained by taking two levels of Peres Gate as shown in the figure11.

Design Using Toffoli Gate

Here by taking 3 level of Toffoli Gates the Full Adder outputs are obtained. In level one it uses one Toffoli Gate where as in level2 and level3 2 Toffoli Gates are used

RESULTS AND DISCUSSION

All the designs has been designed and synthesized in Xilinx tool using Veriolg programming. The RTL of all the designs are obtained and to validate the design the test bench codes has been implemented for each of the design. The synthesis reports are obtained to obtain comparative analysis between different designs.

Feynman + New Gate Implementation

Figure 13.a and 13.b shows the RTL diagram and Testbench output respectively. The RTL diagram consists of two number of New Gate and one Feynman which is as per our design. The Testbench waveform validates the correctness of design.

Toffoli Gate and Feynman Gate Implementation

Figure 14.a and 14.b shows the RTL diagram and Test bench output respectively. The RTL diagram consists of two number of Toffoli gate and Feynman which is as per our design. The Test bench waveform validates the correctness of design. From the Simulated result it can be checked when A=1,B=0 and C_{in}=1the outputs Sum=0 and Carry=1.

Peres Gate Implementation of Full Adder

Figure 14.a and 14.b shows the RTL diagram and Test bench output respectively.

The RTL diagram consists of two number of Peres Gate which is as per our design. The Testbench waveform validates the correctness of design. From the Simulated result it can be checked when A=1,B=0 and C_{in}=1the outputs Sum=0 and Carry=1.

Toffoli Gate Implementation of Full Adder

Figure 16.a and 16.b shows the RTL diagram and Test bench output respectively. The RTL diagram consists of 5 number of Toffoli gate which is as per our design. The Test bench waveform validates the correctness of design. From the Simulated result it can be checked when A=1,B=1 and C_{in}=1the outputs Sum=1 and Carry=1.

Comparative Performance Report of Reversible Gates

Table 1.Performance Report of Reversible Gates used for Full Adder

CONCLUSION

Reversible logic is becoming a modern method for the design of digital logic circuits. Full Adder's simulation and synthesis using a combination of different Reversible Logic Gates and similar types of reversible Gates are designed and simulated using Xilinx ISE tool. The synthesis report of individual design was obtained and comparison report is obtained in terms of number gates used, delay. From the analysis it can be concluded that design using Peres Gate has minimum delay and number of gates as compared to other reversible gates.

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Table 1.Performance Report of Reversible Gates used for Full Adder

Reversible Gate (Full Adder)	Delay	Number of Gates
Peres Gate	6.307ns	06
Toffoli Gate	8.40ns	10
Toffoli+ Feynman Gate	7.06ns	06
Feynman+ New Gate	12.09ns	15

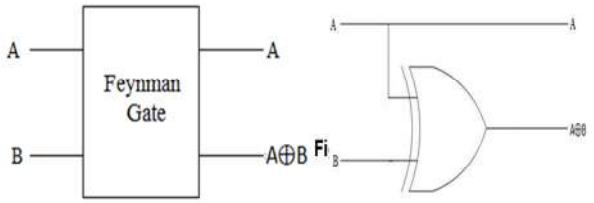

Figure 1. Feynman Gate and Its Internal Circuit [4]

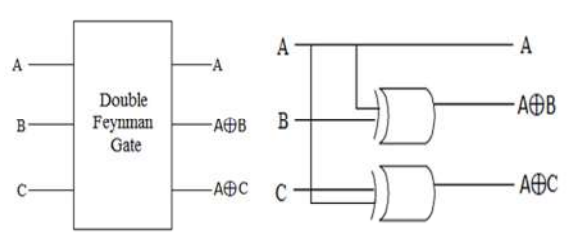

Figure 2. Double Feynman Gate and Its Internal Circuit

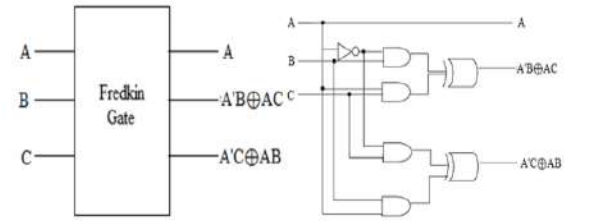

Figure 3. Fredkin Gate and Its Internal Circuit [3]

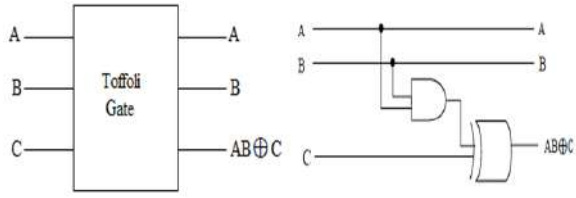

Figure 4. Toffoli Gate and Its Internal Circuit

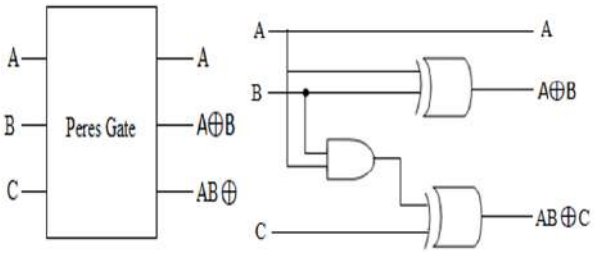

Figure 5. Peres Gate and Its Internal Circuit [5]

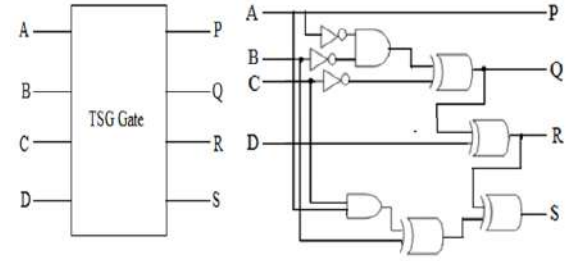

Figure 6. TSG Gate and Its Internal Circuit

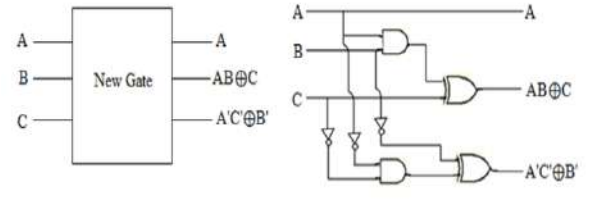

Figure 7. New Gate and Its Internal Circuit

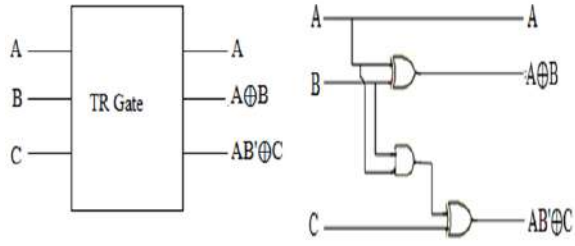

Figure 8. TR Gate and Its Internal Circuit

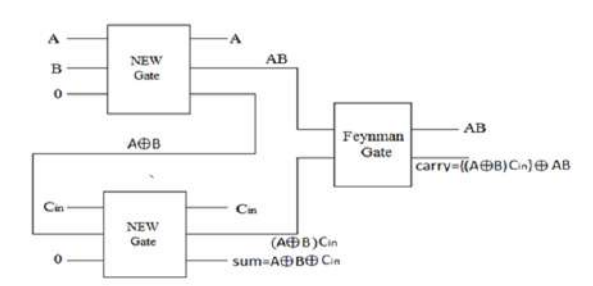

Figure 9. Design of Full Adder using New Gate and Feynman Gate

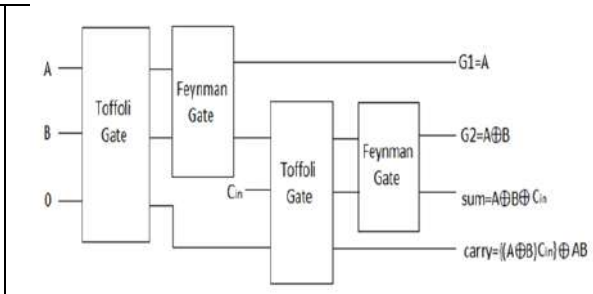

Figure 10. Design of Full Adder using New Gate and Feynman Gate

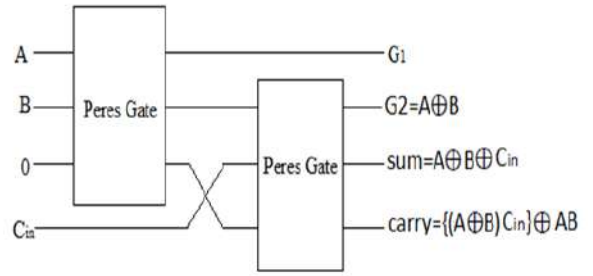

Figure 11. Design of Full Adder using Peres Gate

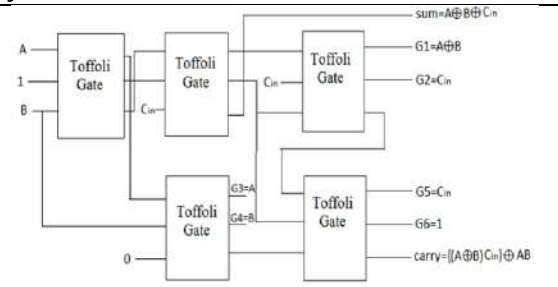

Figure 12. Design of Full Adder using Toffoli Gate

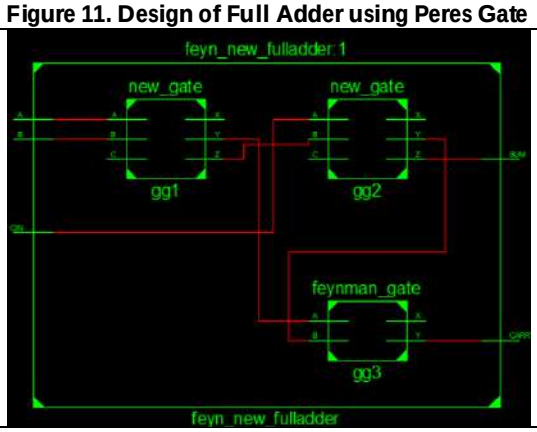

Figure 13.aRTL Diagram of Feynman and New Gate Implementing Full Adder

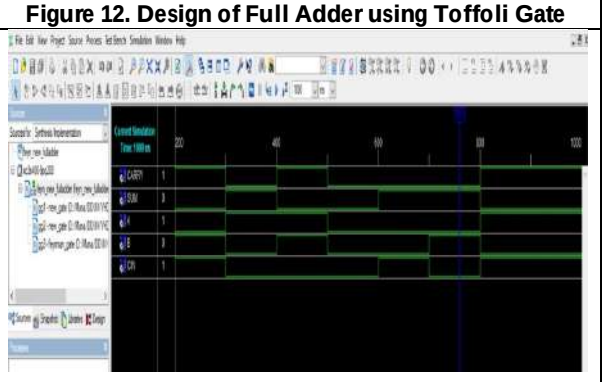

Figure 13.bTestbench Output of Feynman and New Gate Implementing Full Adder

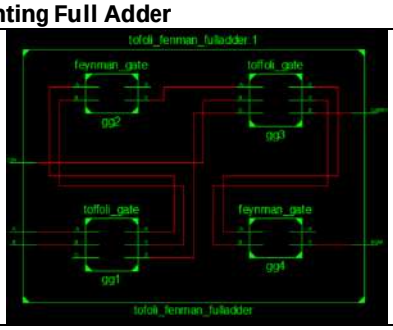

Figure 14.aRTL Diagram of Toffoli Gate and Feynman Gate Implementing Full Adder

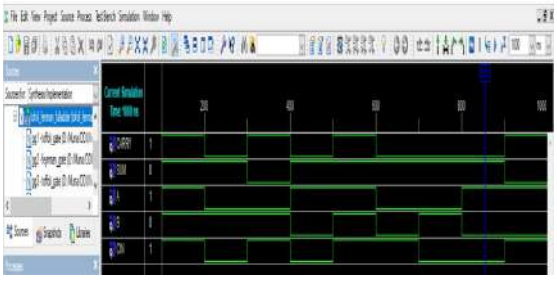

Figure 15.aRTL Diagram of Peres Gate Implementing Full Adder

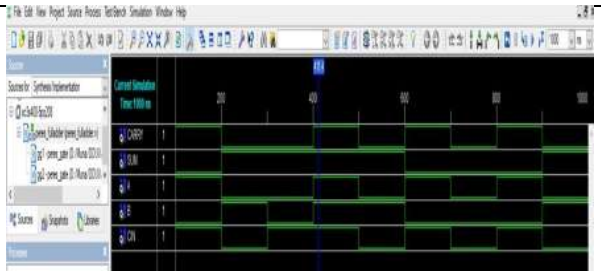

Figure 15.bTestbench Output of Peres Gate Implementing Full Adder

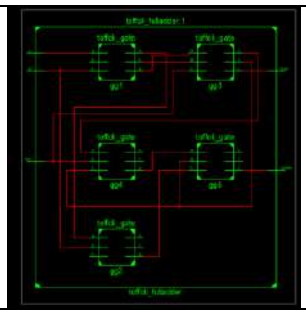

Figure 16.aRTL Diagram of Toffoli Gate Implementing Full Adder

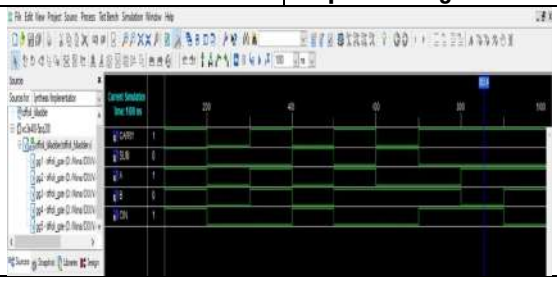

Figure 15.bTestbench Output of Toffoli Gate Implementing Full Adder

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RESEARCH ARTICLE

Perspectives on Conservation Agriculture for Sustainable Crop Production

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ABSTRACT

Agriculture is the backbone of any civilizations throughout the world. For past years many countries, including India, practised conventional farming. It is otherwise called industrial agriculture. In this farming technology usage of high fertilizers, pesticides, insecticides, and heavy tillage operations, injudicious water application are practiced. These practices create entirely ecosystem imbalance and less farm income by decreasing soil fertility and several other problems created by conventional agriculture. For the sustainability of future generations, practice of conservation agriculture is more reasonable compared to conventional agriculture. It maintains species diversification in an ecosystem. By conserving agriculture, we protect the soil, water, and weather etc. by boosting natural processes and biodiversity in above ground and below ground. Conservation agriculture (CA), opens food security windows for the under developed countries. It can balance every unjustified act done by conventional agricultural practices until now. Facilitation of integration of trees, animals and pastures into agricultural landscapes has opened sustainable intensification of agriculture. Adoption of conservation agriculture is on a rise throughout the globe bringing the hope of a better future.

Keywords: Farm Management, agronomy, species diversification, food security, sustainability.

INTRODUCTION

Sustainable farming systems and practices are indeed necessary in this present era as we have already approached ill effects of global warming throughout the world. Agriculture is responsible for 30% of the total greenhouse gas emissions of CO_2, N_2O and CH_4while being directly affected by the consequences of a changing climate (IPPC, 2014; Hossain *et al.,* 2021a; Maitra *et al.*, 2021). According to the Food and Agriculture Organization, Sustainable Agriculture is "a concept for resource-saving agricultural crop production that strives to achieve acceptable profits

together with high and sustained production levels while concurrently conserving the environment" (FAO, 2016). The term "Conservation Agriculture" was proposed by CIMMYT (International Maize and Wheat Improvement Centre) in the 1990s and the main theme of it lies in using the resources we have while without disturbing the nature. The extensive agricultural advancements have already made hazardous effects on the environment, resulting in reduced soil organic matter, enhanced soil erosion, chemical accumulation in the soil and atmosphere, etc. (Gathala *et al*., 2011, Jones *et al*., 2006; Singh *et al*., 2020). As a sustainable approach, conservation agriculture contributes towards healthy ecosystems by promoting a permanent soil cover, minimum soil disturbance and diversification of crop species (Montgomery, 2007; Kassam *et al.,* 2013; Dumansky *et al.,*2014; Hossain *et al*., 2021b). In conservation agricultural practices, soil interventions such as mechanical tillage are avoided or reduced to the minimum. Nutrient inputs which are applied externally are either of organic origin or if mineral origin applied optimally in least minimum quantity which do not harm the environment and its ecosystem services (FAO, 2014). In this review, we have discussed about the developmental phase of conservation agriculture and its current status in our country. Here we also have addressed various practices that can be considered for a sustainable agricultural process.

HISTORY AND DEVELOPMENT

The idea of no-till or minimum-till operation is the main ideal concept of conservation agriculture. In the 1930s in the Dust bowl in the USA no-till practice was started then later Edward Faulkner in his book "Ploughman's Folly" (Faulkner, 1945) and Masanobu Fukuoka in his book "One Straw Revolution" (Fukuoka, 1975) were explained about conservation agriculture. In the 1970's the demonstration about conservation agriculture was given by scientists, the farmers are ready to adopt this technology, in that time the fuel costs are raised and labor was not available for farm operations. So this technology was easily attracted to farmers and adopted by them at that time. Then later it slowly spread to remaining countries because of good results by this practice. The total area under CA in 2015 is estimated to be around 180 million hectares. During the 1960s no-tillage concept came into farming practice in the United States of America (Derpsch, 2004; Kassam *et al.,* 2004; Kassam *et al.,* 2010). Around the world CA crop production systems are experiencing increased interest. There are very few countries where CA is not practiced (Jat *et al.,* 2014). The total cropland area under CA during 2008-2009 was estimated to be 106 M ha (Kassam *et al.,* 2009; Derpsch and Friedrich, 2009). For the year 2010-2011 it was 125 M ha (FAO, 2011; Friedrich *et al*., 2012). But during the updating 2013, it was found that the total global cropland area under CA in 2010-2011 was some 145 M ha. For 2013, the global total CA cropland area was 155 M ha (Kassam *et al.,* 2014) but from then there is 157 million hectares increase in CA area in Argentina which had not been reported at the time of the 2013 (FAO, 2014). No-tillage CA is practiced on all farm sizes from less than half a hectare to few hectares (e.g., China, Zambia, and Paraguay) to thousands of hectares (e.g., Argentina, Brazil, and Kazakhstan). All crops can be grown adequately in CA systems (Derpsch and Friedrich, 2009). Major barriers to the adoption of CA practices in most countries are knowledge on how to do it, tradition, prejudice, inadequate policies, unavailability of appropriate tools and machines, facilitation of weed and vegetation management (Friedrich and Kassam, 2009; Jat *et al.,* 2020; Farooq and Siddique, 2014).

STATUS OF CONSERVATION AGRICULTURE IN INDIA

In India, conservation agriculture is still in the initial phases. It is an old technique, but it is not practiced highly in our country due to inadequate knowledge and awareness. Conservation agriculture firstly started in Western Indo-Gangetic plains with the rice-wheat cropping system of India in the 1900s. From the past few years, the adoption of zero tillage and CA has increase in size to cover about 1.5 million hectares (Jat*et al.,* 2020; www.fao.org/ag/ca/6c.html). Agricultural produces whole over the world has led to soil degradation, more pronounced in tropical countries and in moderate climatic zones. The world map of degraded soils points out that nearly all agricultural lands own certain levels of degradation in soil (FAO, 2006). In India, conservation agriculture started to adopt from a decade but in some areas and few farmers only adopt and accept this technology from last 8-10 years (Bhan and Behera, 2014). Major goal of conservation agriculture is better utilization of agricultural resources

through the integrated management of soil, water and biological resources such that external inputs can be reduced (FAO, 2001; Garcı´a-Torres *et al.*, 2003).

BASIC PRINCIPLES OF CONSERVATION AGRICULTURE

Conservation agriculture is the integration of ecological management with advanced, scientific, production of agricultural produce (Figure 1). Conservation agriculture deploys all modern techniques that improve the quality and ecological integrity of the soil, but the application of these is along with traditional knowledge of soil husbandry. This ensures the sustainability for those who practice CA. Main strength of conservation agriculture is the soil husbandry practices which is robust, cheaper, more productive and environmentally friendly farming system. These agriculture production systems are more sustainable than conventional agriculture systems because of the focus of producing with healthy soils in an environmentally friendly way. These practices create entirely ecosystem imbalance and less farm income by decreasing soil fertility and several other problems created by conventional agriculture (Owenya *et al.,* 2012; Palm *et al.,* 2014).

Conservation agriculture consists of the mainly three interlinked principles, those are;

- No or minimum mechanical soil disturbance,
- Biomass soil mulch cover
- Crop species diversification

Conservation agriculture aims at the perfect utilization of available resources without disturbing the environment, while giving good profits to farmers. It works on three main concepts and conjunction of working on eco-friendly, sustainable crop production, soil-water-weed-pest of integrated management.

PRACTICES OF CONSERVATION AGRICULTURE

The principles have become the practices in conservation agriculture

No/ minimum tillage and soil disturbance

Minimum or Zero tillage is practiced in CA. Minimum tillage means leave the crop residue more than 30% or at least 30% on the soil surface. Zero tillage means leave 100% of crop residue on the soil surface. It can be easily practiced in both small and large farms. Without soil disturbance operations we decrease the soil erosion and improves organic matter by previous crop residues (stubbles of an old crop). Improves soil fertility, structure and improves biological activities in soil. With less soil disturbance the soil microorganisms developed by this soil fertility will be more by their activity and improves soil pore space. When soil pore space increases it improves water holding capacity in soil. Table 1. indicates that the extent of no-tillage adopted is just over 95 million hectares (Derpsch, 2004; Derpsch and Friedrich, 2009). Not all of this land is permanently no-tilled or has permanent ground cover. Table shows details on the extent of no-tillage by country. Six countries have more than 1 million hectares. South America has the highest adoption rates and more permanent no-till soil cover. By adopting the no-till system, Brazil increased its production by 67.2 million tons in 15 years, with additional revenue of US$ 10 billion. Derpsch (2004) also calculated that at an average rate of 0.51 t/ha/yr, Brazil has sequestered 12 million tonnes of carbon on 23.6 million hectares of land where no tillage was practiced. Tractor use was also significantly reduced. This will again better the ecosystem.

Practices for minimum soil disturbance

In conservation agriculture, some of the instruments are used which makes very little disturbance during seed sowing time only (Chivenge *et al.,* 2007). The most popularized instruments in India and some other countries are mentioned below. Minimum tillage: This technique was first practiced in Punjab and some northern states of India for the wheat crop. By this method, the farmer cans easily some fuel and labor charges and their time. Happy seeder: By this technology, there is a possibility of direct drilling into heavy stubbles. It is used for sowing operation in combine-harvested and leaves the crop residue as surface mulch. Roto till drill: The machine which is used for

sowing seeds into the soil with minimum disturbance of soil with the help of equipment i.e. rotovator. Slit till drill: This equipment which is used for sowing seeds into the slits opening with the help of a rotary slit disc. It is easily used in stubble fields. Strip tillage: Under field conditions special strip till implements are used to perform tillage operations.

Biomass mulch cover

Mulch cover means close or cover the entire land surface with mulching material. Mulch covering should be done by covering crops of previous crop residues which are environmentally friendly (Derpsch, 2005). It reduces evaporation loss and gives a good result of minimum runoff rate. It manages soil moisture and soil temperature too. It reduces soil erosion to a great extent (upto80%).Mulching can be done with leguminous cover crops which improve nitrogen fixation and improves crop growth. the leguminous crops fix the atmospheric nitrogen and reduce the application of inorganic nitrogenous fertilizers. It balances water use efficiency.

Crop species diversification

Crop species diversification means maintaining more than one crop on a farm at a time. It balances the ecosystem and maintains different species of plants on a piece of land. Maintaining some fruit crops and timber crops around the field works as windbreakers to the main crop. By this practice, farmers get multiple benefits. Reduces economic risk. Reduces climatic vulnerability. Reduces insect, pest, and disease attacks. Helps in the better utilization of farm resources etc. (Dumansky *et al.,* 2014). The maintenance of different species of plants promotes biodiversity.

IMPACTS OF CONSERVATION AGRICULTURE

- Impacts on environment and ecosystem which includes soil system, water system, climate, biodiversity.
- Increases the quality of living of farmers and decreases labor costs.
- Decreases farm inputs and decreases the cost of cultivation of farmer.
- Balances and protects biodiversity along with the crop.
- Maximizing profits of farmers and insurance in tough times.
- Increases yields and profit.

ROLE OF CONSERVATION AGRICULTURE IN FOOD SECURITY

Now a days demanding for food is gradually increases along with increasing population, the main aim is not increasing quantity of food but also the food which is more nutritious (it means presence of vitamins and some essential amino acids). It is a big challenge for to scientists generate food and regenerate soil health, save the ecosystem (FAO,2018). The conservation agriculture technique is the best alternative method to conventional agriculture. For high yield, production cultivate developed varieties that are resistant and short duration (Nyanga, 2012). By the introduction of transgenic crops improves food security and reaches not only productivity and production of crops, increases the nutrient content value of food. Introduction of transgenic crops, we can improve biotechnological research, it creates more empowerment (Laborde *et al.,* 2019).

BENEFITS OF CONSERVATION AGRICULTURE

- This practice works efficiency on different soil borne pests and diseases.
- In CA soil have more biotic diversity because of less soil disturbance and maintenance of mulch on the ground surface.
- Increases the water use efficiency of crops.
- Decreases cost of cultivation and labor cost
- Maintains soil biological system.
- It reduces cost of cultivation, e.g. fuel, machinery operating costs, and maintenance, as well as reduced labor cost (Hobbs, 2007).
- Livestock maintenance is very beneficial in conservation agriculture, it gives another income to the farmer. The crop residue is used as fodder for animals.

- Global warming will decrease by avoiding crop residue burning, the high fuel consumption of machinery.

A study reported Yields in the rice-wheat (RW) systems of the Indo-Gangetic Plains in South Asia are higher with no-till practice because of timelier planting and better stand conditions. Yields of 200-500 kg/ha are obtained with no-till wheat in the same system (Hobbs and Gupta, 2004).

CONSERVATION AGRICULTURE AND SUSTAINABLE INTENSIFICATION

Sustainable intensification is a process to increase agriculture yields without negative impacts on the environment, considering the whole ecosystem. The goals of conservation agriculture and sustainable intensification are similar. Sustainable crop production intensification not only shows its impact on climate change like reducing greenhouse gases and it shows positive results of soil carbon sequestration (Rai *et al.,* 2021). It impacts on both below and above environment in ecosystem it gives better productivity and as well as great surroundings. Conservation agriculture is another way to increase sustainable intensification (FAO, 2018).

ROLE OF CONSERVATION AGRICULTURE ON SUSTAINABLE CROP PRODUCTION

Higher yields and potentially higher stable incomes can be obtained from conservation agriculture. The crop production, soil health and stable income of farmer can improves by conservation agriculture with proper nutrient management (Chauhan and Mahajan, 2012). Very suitable for Rice-Wheat cropping system, where wheat can be planted timely after harvesting of rice. This practice can reduce the issue of crop residue burning. Now a days demanding for food is gradually increases along with increasing population, the main aim is not increasing quantity of food but also the food which is more nutritious (it means presence of vitamins and some essential amino acids). By the introduction of transgenic crops improves food security and reaches not only productivity and production of crops, increases the nutrient content value of food (Andersson and D'Souza, 2014; Kienzler *et al.,* 2012).

REASONS FOR ADOPTION

- The cost of production is less compared to conventional agriculture.
- Improves soil productivity.
- Soil erosion is very less because maintains residual mulch or cover crops on the farm.
- It can be practiced in both clay and sandy soils (cultivate both tuber crops and root crops).
- Drought resistant; increases water use efficiency by crops.
- Environmental and social – protect the soil and make agriculture more sustainable.
- maintains carbon sequestration in soil.

CHALLENGES IN CONSERVATION AGRICULTURE

- Weed management is the main problem of CA in the early stages of practicing. However, the weeds can be managed using herbicides. Techniques like brown manuring, stale seedbed techniques may be used.
- Small-scale and medium-scale farmers machinery.
- Skilled and scientific manpower is required for types of equipment. Knowledge and information sharing mechanisms are needed.
- Farmers are not ready to accept new technology and search for instant profits.
- One of the important challenges in the adoption of CA is a lack of knowledge/awareness.

We can cross these challenges by creating awareness in farmers by extension workers, NGO's and need big support from government bodies. If local or state government bodies give more subsidies to farmers, they are interesting to adopt this technology (Carvalho and Lourenço, 2014). Compare to conventional agriculture conservation agriculture is less cost of cultivation but it is some expensive in starting years in that period government may give support to farmers it is easy to adopt. Farmers also give a positive response for CA then it is possible (Reicosky,2015; Sachan and Choudhary, 2019).

CONCLUSION

As the population is on the rise throughout the globe, agriculture production has to be increased to meet the needs of the humankind without compromising environmental sustainability in these prevailing climate change havoc period. Conservation Agricultural practices are very promising in this regard. Compared to conventional agriculture, Conservation agriculture changes soil properties and processes. The feasibility of conservation agriculture for rejuvenating degraded soils and increasing crop yields on low productivity is remarkable. Institutional developments need to me formulated by the governments to promote conservation agriculture with respect to the geographic and climatic conditions for the wellbeing of future generations.

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Table 1. Area under no-tillage adoption worldwide

Country	Area under No-tillage (million ha)
USA	25.30
Brazil	23.60
Argentina	18.27
Canada	12.52
Australia	9.00
Paraguay	1.70
Indo-Gangetic Plain	1.90
Bolivia	0.55
South Africa	0.30
Spain	0.30
Venezuela	0.30
Uruguay	0.26
France	0.15
Chile	0.12
Colombia	0.10
China	0.10
Others	1.00
Total	95.48

(Derpsch, 2004)

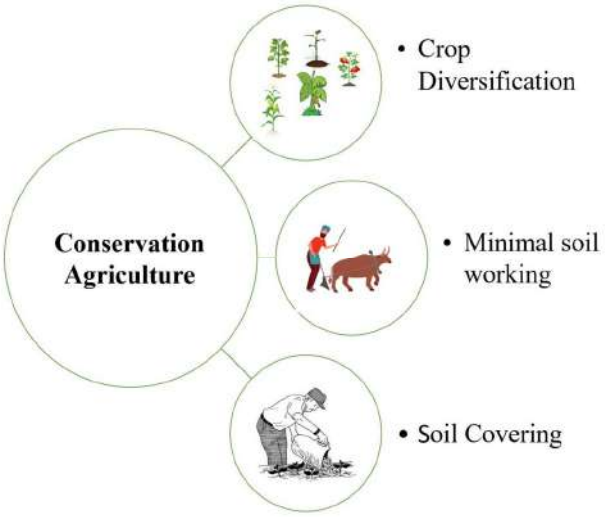

Figure 1. Principles of conservation agriculture

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RESEARCH ARTICLE

Liquid Biofertilizer-an Innovative Tool for Sustainable Agriculture

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ABSTRACT

Liquid biofertilizers (LFs) are containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity. To directly apply in soil, first biofertilizers are mixed with carriers like soil, compost, farmyard manure, rice husks or lignite and then directly put in the soil. Liquid biofertilizer formulation is one of the most promising and updated technology. In spite of many advantages over the agrochemicals left a considerable dispute among the farmer community in terms of several reasons major is the viability of the organisms. Now a days LFs are available in the market as one of the alternatives to chemical fertilizer and pesticide. The LFs are applied by spraying or by fertigation. Biofertilizers like rhizobium and phosphate solubilizing bacteria (PSB) plays an important role in increasing availability of phosphorus and nitrogen through increase in biological N-fixation and enhance the phosphorus availability to crop. Use of liquid biofertilizer significantly increases yield and quality of pulses.

Key words: Liquid biofertilizer, Shelf life, soil health, fertigation

INTRODUCTION

To feed the ever-growing populations, an agriculture-based developing country like India have to increase the per unit area productivity. As per United Nations Food and Agriculture Organization (FAO) estimations, in 2030 the average demand for the agricultural commodities will be 60% higher than present time and more than 85% of this additional demand will be from developing countries (Mia and Shamsuddin, 2010).To overcome nutrient deficiencies and to increase productivity chemical fertilizers are being used injudiciously and it has affected the environmental quality and soil ecosystem leads to several agricultural problems (Maitra *et al*., 2021; Praharaj*et al*., 2021; Shankar *et al*., 2021). Agrochemicals are extensively applied to obtain higher yield now a days and it resulted adverse effects on both beneficial micro flora and micro fauna as well as their activities (Asif *et al*.,2018). Several

research programs based on beneficial bacteria and fungi has resulted development of a wide range of bio-fertilizers which fulfilled the nutrient requirements of crops and increased the crop yield as well (Aggani, 2013; Palai *et al.*, 2021).Liquid formulations ensure more quality over the conventional carrier based biofertilizers. These liquid formulations facilitate long shelf life, minimum contamination, carrier free activity, handling comfort, storage and transport convenience, easy quality control, enhanced export potentials and are preferred by the farmer community as well as manufacturers (Pindi and Satyanarayana, 2012).Keeping in mind the disadvantages of the carrier based biofertilizers, LFs would be the only alternative for the cost-effective sustainable agriculture.

DEFINITION

Basically, LFs are the microbial preparations which contain specific beneficial microorganisms and are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity (Tamilkodiand Victoria, 2018). LFsare suspensions having agriculturally useful microorganisms, which fix atmospheric nitrogen and solubilize insoluble phosphates and make it available for the plant (Verma *et al.,* 2018).LFs are the microbial formulation containing beneficial microorganisms which are capable of fixing, mobilizing or solubilising the important plant nutrients by their biological activity (Chopra and Mali, 2020).LFs are generally produced from fermentation of effective microorganisms was recommended to be used within three months (Nagampimol and Kunathigan, 2008).Nowa days, ready to use LF from effective microorganism is becoming available in market (Maheswari and Kalaiyarasi, 2015).The potential microorganism composition in liquid biofertilizer is *Pseudomonas sp*., *Bacillus sp*., *Kebsiella sp*., *Aspergillus sp*., *Azotobacter sp* (Neneng, 2020).

ADVANTAGES

Liquid formulation technology has more advantages than the carrier-based inoculants using as an alternative to conventional carrier based biofertilizers. It has been developed from the Department of Agricultural Microbiology, TNAU, Coimbatore(Trimurtulu and Rao, 2014). The advantages of LFs over conventional carrier-based biofertilizers are: Specialized nutrients that ensure longer shelf life up to 12-24 months (Tamilkodiand Victoria, 2018), tolerant to ultraviolet radiations (Mahdi *et. al*., 2010)No contamination, no loss of properties due to storage up to 55º C, Greater potential to fight with native populations, high populations can be maintained up to 12 to 24 months. Easy identification of LF is possible by typical fermented smell. Quality control protocols for LF are easy and quick, better survival on seeds and soil and no need of running biofertilizer production units throughout the year. LFs are very much easy to handle and dosages are 10 times less than those of carrier-based powder biofertilizers, high export potential and they also have very high enzymatic activity since contamination is nil.

Chemical fertilization can be minimized by using eco-friendly LF to a great extent (Asif *et al.,* 2018). Now a days several LF products are available on the market, which contain potential microorganisms with various advantages, but in the field the success of the application of these microorganisms are highly dependent on their ability to adapt to local environmental conditions (Neneng, 2020).It is very cost-effective and easy to use for farmers (Chopra and Mali, 2020).

FACTORS AFFECTING LIQUID BIO-FERTILIZER

Temperature

Temperature is essential for growth and development of any micro-organism and the shelf life of microbial products depends on temperature. Optimum temperature and range of temperature varies with different microorganisms (Verma *et al*, 2018. In liquid formulation strains used normally grow at 37°C and also able to tolerate temperature up to 45°C for two year or more but in solid base biofertilizer shelf life of microbes is hardly up to 3 months as rise in temperature beyond 35°C and start rapid decline of organisms (Pindi and Satyanarayana, 2012).

Acclimatization

It is seen that the efficiency of the liquid is almost identical in all environments, but in the case of solid base, efficiency can reduce 20-25% in various climatic conditions. (Verma *et al,* 2018).Normally in liquid formulation an

organism remains active for a long time after application, ideally throughout the period of the crop (2 years to the maximum), or in soil throughout the crop cycle. But generally, temperature, humidity, leaf surface exudates and competitors, etc are the critical factors which affect the microbes and they may be physically lost from the target location by the action of wind, rain or leaching depending on the time and place where the product is used. (Pindi and Satyanarayana, 2012).

Sunlight intensity

It is most effective for microorganisms. UV-B (280-320 nm) and UV-A (320-400 nm) have direct effect on microbes which are the most harmful rays reaching the Earth's surface (Verma *et al*, 2018).However, microbes are sensitive to the wave-lengths outside this range. To counter the harmful effects of high temperature sunscreens are added to the formulation and they act by reflecting and scattering physically or by absorbing radiation selectively, converting short wavelengths to harmless longer ones butin solid base no such types of sunscreens are available to resist the effect of sunlight(Pindi and Satyanarayana, 2012).

pH

In the manufacture of liquid inoculums, the pH of a product is critical. The beneficial organisms are inactivated at extreme high and low temperatures; consequently, a buffer is maintained by adding various additives that provide the liquid a longer shelf life. The pH of a product plays an important part in liquid inoculum manufacturing, so it must be kept within certain ranges (Verma *et al*, 2018). Maintenance of optimal pH improves shelf-life of some of microorganisms like *Azospirillum, Azotobacter*, Phosphorus Solubilizing bacteria (PSM), Potash Mobilizing Bacteria (KMB), *Frateuriaaurantia* (Pindi and Satyanarayana, 2012).

Humectants

Moisture content affects storage, stability, and activity of microbes to a greater extent. Carrier-based inoculums become dry during transport and storage and the organism becomes stressed due to several reasons.Bacteria requires a wet plant surface to establish themselves and demonstrate their activity, which can only be met by liquid formulations that contain humectants(Pindi and Satyanarayana, 2012).

METHODS OF APPLICATIONS

There are mainly four different methods for LF applications in field and they are seed treatment, root dipping, soil application and with irrigation water(Trimurtuluand Rao, 2014).

Seed treatment

For all forms of inoculants, seed treatment is the most common and effective strategy. 5-6 ml LF should be mixed with an equal amount of 10% starch solution or 10% jaggery solution per kg of seed in a plastic bag and the mixture should be coated uniformly on the seed.Squeeze the bag for at least 2 minutes, or until all of the seeds are evenly moistened. The bag is then opened, inflated, and gently shaken shade-drying for 20-30 minutes after the bag is opened. Another option is coating huge quantities of seeds in a bucket and mixing the inoculant by hand.Seed treatment with Rhizobium, *Azotobacter, Azospirillum*, and PSM can be done and treatment with any two or more than two bacteria can be done without any side effects. The most important thing is that the seeds must be covered with *rhizobium*, *azotobacter*, or *azospirillum* and the PSM inoculant must be coated as an outer layer.

Root dipping

This approach is used to apply *Azospirillum*/PSM liquid biofertilizer to paddy and vegetable crops. 250-300 ml LF inoculants must be combined with 5-10 litres of water in one area of the field and seedling roots must be dipped for at least half an hour before transplantation(Trimurtuluand Rao, 2014).

Soil application

For one acre of main field, 250-300 ml liquid inoculants of each organism can be utilised. All inoculants should be diluted in 10-15 litres of water before being mixed with 200 kg of powdered farm yard manure, vermicompost, or any other compost and incubated overnight before being applied. This mixture should be administered at the time of seed sowing or transplantation, particularly in the furrows beneath the seed surface, and soon after the administration of microbial inoculants in tropical soils(Trimurtuluand Rao, 2014).In an experiment conducted by Maheswari and Elakkiya (2014), the combined inoculation of LF*Azospirillum* + *Rhizobium* + *Azotobacter* showed a better response in all parameters such as leaves number, height, shoot length, root length, and biochemical constituents such as chlorophyll, carbohydrate protein and carotenoids content.

Foliar spray and fertigation

LFscan be applied by spraying or by method of fertigation (Bhawsar, 2011).As LF are generally concentrated, they must be diluted with water before being applied to the field to avoid fertiliser burn. The biofertilizer is blended with water and other micronutrients in a tank during fertigation. Irrigation sprinklers/sprayers/piping provide it to specific plants. Fertigation is commonly used in greenhouses or shade netting.Farmers can use liquid inoculants with drip irrigation systems in their main field by putting 250-300 ml of each organism per acre in a drip tank and releasing it within 10-15 days of transplantation/sowing(Trimurtuluand Rao, 2014).

ADVANTAGES OF USING LIQUID BIOFERTILIZER ON AGRICULTURE

Impact on soil fertility and soil health

- Biofertilizer inoculation with and without different level of NPK fertilizer in low fertility inceptisolssoil order clearly increased $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ as well as available P (Setiawati, 2017).
- On average the LF treatment has increased soil nutrients in the form of N, P, K, in the treatment compared to controls. Increased total-N nutrients (%) by an average of 69.7%, an increase in phosphate nutrients an average of 4.7%, and an increase in potassium nutrients an average of 28%. The potential microorganism composition in LF is *Pseudomonas sp., Bacillus sp., Kebsiella sp., Aspergillus sp., Azotobacter sp*. (Neneng, 2020)
- The organic carbon was increased in soil samples collected after harvest of soybean crop as compared to initial soil samples (5.4 g kg^{-1}). The increase in organic carbon might be due to seed treatment with rhizobium and PSB increased the activity of microbes and due to better root penetration (Rajaa and Takankhar, 2017).

Impact on crop growth

- Highest survival of the seedlings due to the application of biofertilizers that helps in the well establishment of root system in soil which leads to better establishment of seedling than the plants which have not received any bio-fertilizers (control) (Vijendrakumar *et al*., 2014).
- From an experiment, Dunsin and Caleb (2016) reported that there are significant different in the number of lateral branches and plant height because of the essential elements contain in the liquid bio-fertilizer (alpha life) which are necessary for plant growth such as; nitrogen, phosphorus, calcium, potassium and magnesium.

Impact on crop yield

- Combined inoculation of LFs such as *Rhizobium Azospirillum, Azotobacter* enhance the morphological parameters of blackgram crop such as plant height, number of leaves, shoot length, root length, root number, root nodules, and biochemical constituents such as chlorophyll, carbohydrate, protein and carotenoids (Maheswari and Elakkiya, 2014).
- Application of 75% recommended dose of potassium along with soil application of KMB(*Frateuriaaurentia*) @ 1 litre in 80 kg FYM ha^{-1} resulted higher potato tuber yield (Chopra and Mali, 2020).
- LFs act as an important component of integrated nutrient management and showed significant effect on both pod and haulm yield of groundnut and the integrated application of 100% RDF with liquid NPK formulation + Zn solubilizing bacteria gave highest pod and haulm yield (Sing *et al.,* 2018).

- Application of 60 kg P_2O_5/ha + NPK and suitable LF resulted higher grain yield (5308 kg ha^{-1}), net return (Rs.44,834/ha) and B: C ratio (2.02) in Maize (Sivamurugan *et al*., 2018).

PRECAUTIONS IN THE USE OF LIQUID BIOFERTILIZERS

- Biofertilizers never mix with any chemical fertilizer.
- Biofertilizers are being never applied with the fungicides, plant ash at a same time.
- Not exposed to direct sunlight.
- Stored at room temperature not below 0^0C and above 35ºC.Store LF bottle in a cool and dry place.
- Keep agrochemicals away from biofertilizers bottle.
- After the expiry period use of biofertilizer is hazardous.

CONSTRAINTS OF LIQUID BIOFERTILIZER

- **Technical**: Unavailability of good quality carrier material, Use of improper, less efficient strains for production.
- **Infrastructural**: Non-availability of suitable facilities for production, Lack of essential equipment, power supply etc. for adequate incubation and storage of inoculants.
- **Human Resources**: Lack of technically expert staff in the production units. Lack of suitable training programme on the production techniques.
- **Environmental**: Soil characteristics like salinity, acidity, drought, water logging, toxicity etc.
- **Social:** Unawareness on the benefits of the technology, Lack of confidence towards different biofertilizer practices, Unawareness on the damages caused on the ecosystem by continuous application of inorganic fertilizers.
- **Marketing**: Lack of the market for the producers, Unavailability of proper transportation and storage facilities, Limited demand of LF (Singh and Kumar, 2015).

CONCLUSION

The rising need for raw materials in the agriculture sector is due to increased demand in food processing, packing industries, ready-to-eat meals, and so on. Because of the scarcity of agricultural land, vertical advances in agricultural input technology are causing changes in the farming community. Alternative agricultural technology likeLFis required to tackle the problem of global food crises. It has the potential to replace traditional chemical fertilizers and carrier-based biofertilizers and play a significant role in soil health restoration It enhances soil fertility as well as crop productivity. It is environment friendly because it reduces pollution which is caused by chemical fertilizers. Farmers will find it incredibly cost-effective and simple to utilise. LF have the capacity to replace the traditional chemical fertilizer and carrier based biofertilizer play a major role in restoring soil health. But a lot of measures in terms of technology, government support, and constructive awareness by well trained technicians one needed.

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RESEARCH ARTICLE

Analytical Method Development and Validation of Meropenem and Vaborbactam in Bulk Samples by RP-HPLC

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ABSTRACT

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of meropenem and vaborbactamin tablet dosage form. The estimation was carried out on PhenomenexLunaC18 (25 cm x 4.60 mm, particle size 5 µm) column with admixture of 10mM phosphate buffer (pH 6.8): Acetonitrile; 40: 60(v/v) as mobile phase. UV detection was performed at 260 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.95and 3.53 min for meropenem and vaborbactamrespectively and total run time was 10 min. at a flow rate of 1.0 mL/min. The calibration curve was linear over the concentration range of 40.00 - 240.00 µg/ mL for meropenem and 60.0 - 360.00 µg/mL for vaborbactam. The LOD and LOQ values were found to be 1.54 and 4.54 µg/ mL for meropenem and 4.60 and13.65 µg/ mL for vaborbactam respectively. The low percentage coefficient of variance confirms the suitability of the method for the simultaneous estimation of meropenem and vaborbactamin bulk samples.

Keywords: Meropenem, Vaborbactam, RP-HPLC, Validation.

INTRODUCTION

Due to counterfeiting, the drug quality has become a source of major concern worldwide, particularly in many developing countries. The most commonly counterfeited drugs are anti-infective or antibiotics. Use of poor quality antibiotics bears serious health implications such as treatment failure, adverse reactions, drug resistance, increased morbidity, and mortality. Among antibiotics, penems are much recently introduced, widely prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably [1-2]. Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. It is marketed in India by New Medicon Pharma with the brand name carbonem. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid [3]. Meropenem is bactericidal except against Listeria monocytogenes where it is bacteriostatic. It inhibits bacterial wall synthesis like other beta-lactam antibiotics. In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases. Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-betalactamases, or resistance to diffusion across the bacterial outer membrane [6-7].

Meropenem, present as a trihydrate (Fig.1) and it is official in Indian Pharmacopoeia (IP). It is a white to light yellow crystalline powder, with a molecular weight of 437.52. The chemical name for meropenemtrihydrate is (4R, 5S, 6S)-3-[[(3S, 5S)-5-(dimethylcarbamoyl)-3-pyrrolidinyl] thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid, trihydrate. The empirical formula of meropenem trihydrate is $C_{17}H_{25}N_3O_5S$ [1-4].

MATERIALS AND METHODS

Experimental Apparatus

RP-HPLC was performed with an Agilent chromatographic system equipped with 1200 series isocratic pump UV–visible and Rheodyne universal loop injector of injection capacity 50 µL. The monitoring software was Ezichrome Elite. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm x 4.6 mm i.d, 5-µm particle, Phenomenex–LunaC18column under reversed-phase partition chromatographic conditions. The flow rate was 1.0 mL/ min and injection volume was 20 µL, analyte were monitored at 260 nm and run time was 7 min.

Chemicals and reagents

Working Standards of pharmaceutical grade meropenem (MEP) and vaborbactam (VAB) were obtained as gift samples from Micro labs, Bangalore. All the chemicals and reagents used were of HPLC grade and purchased from Merck, Mumbai, India.

Preparation of standard stock solution

Standard stock solution of meropenem (MEP) and vaborbactam (VAB) pure drugs prepared by accurately weighing about 100 mg drugs and transferring in to 100 mL volumetric flask and dissolved in acetonitrile.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The parameters studied for validation were System Suitability, Specificity, Linearity, Precision, Ruggedness, Robustness, Limit of Detection and Limit of Quantification, Filter Validation and Solution Stability [22].

Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is

called selective. It has been observed that there were no peaks of diluents and placebo at main peaks. Hence, the chromatographic system used for the estimation of Meropenem (MEP) and Vaborbactam (VAB) was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 2.86 min for Meropenem (MEP) and 7.40 min for Vaborbactam (VAB). The results were depictinginTables and figures.

RESULT ANDDISCUSSION

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions weretried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with 10 mM Phosphate buffer (pH 6.8): Acetonitrile (40: 60) (v/v) at flow rate of1.0 mL/min was found to be robust method. The developed method was validated as per the ICH guidelines for the quantification of Meropenem (MEP) and Vaborbactam (VAB) in bulk samples. The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases% RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method is precise.

Robustness test was carried out by small variation in the chromatographic conditions and % change was calculated. The % change in the results was calculated and it was found robust as % change was below 2.0%. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 5.888 µg/mL for Meropenem and0.225µg/mL for Vaborbactam (VAB) and LOQis found to be 17.841 µg/mL for Meropenem (MEP) and 0.683 µg/mL for Vaborbactam (VAB).

METHODDEVELOPMENT

For developing the method, a systematic study of the effect of various factors were undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous analysis of Meropenem (MEP) and Vaborbactam (VAB) in a bulk samples. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short (7 min), which enables rapid determination of many samples in routine and quality control analysis.

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Table.1: Specificity of MEP and VAB

Name of the solution	Retention Time (min)
Blank	No peaks
Meropenem	2.95 min
Vaborbactam	3.53 min

Table. 2: Linearity and Range of MEP and VAB

S.No.	Concentration µg/mL	Area of Meropenem	Concentration µg/mL	Area of Vaborbactam
1	40	22988	60	16998
2	80	47605	120	31602
3	120	68028	180	50459
4	160	90704	240	67137
5	200	112120	300	84014
6	240	130654	360	101710
Concentration Range	40-240 µg/mL		60-360 µg/mL	
Slope (m)	547		282	
Correlation coefficient (r^2)	0.9987		0.9995	

Table.3: Intraday Precision data for MEP and VAB

Sample. No	Area of MEP	Area of VAB
1	110164	84015
2	110145	84037
3	111162	84052
4	111133	84062
5	110152	84034
6	111160	84014
Mean	110653	84036
SD	546.76	19.29
%RSD	0.49	0.02

Table.4: Interday Precision data for MEP and VAB

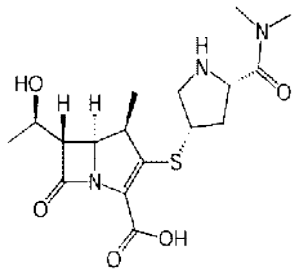
Sample. No	Area of MEP	Area of VAB
1	110165	85031
2	110138	85137
3	110222	85012
4	110142	85034
5	110163	84511
6	110122	85038
Mean	110159	84961
SD	34.98	224.60
% RSD	0.03	0.26

Table. 5. Robustness of MEP & VAB

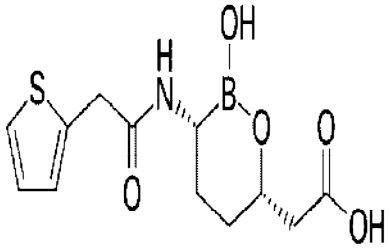
S.No	Parameter	Condition	MEP		VAB	
			Area (n=3)	% change	Area (n=3)	% change
1	Standard	Standard conditions	112120	0	84014	0
2	Mobile Phase composition (±2%)	10mM phosphate buffer (pH -6.8): Acetonitrile;(38: 62, v/v))	112232	-0.100	84054	-0.048
		10mM phosphate buffer (pH- 6.8): Acetonitrile;(42: 58, v/v)	112262	-0.027	84084	-0.036
3	Mobile phase pH (±0.2 units)	6.6	111232	0.917	84034	0.059
		7.0	112157	-0.832	84036	-0.002
4	Wavelength (nm)(±2nm)	258	111162	0.887	84042	-0.007
		262	112165	-0.902	84035	0.008
5	Flow rate (mL) ±0.2mL	1.2	111169	0.888	84042	-0.008
		0.8	111165	0.004	84040	0.002

Table. 6. LOD and LOQ of MEP & VAB

Parameter	MEP	VAB
LOD(µg/mL)	5.888	0.225
LOQ(µg/mL)	17.841	0.683



A



B

Fig.1: Chemical structures of A) Meropenem and B) Vaborbactam

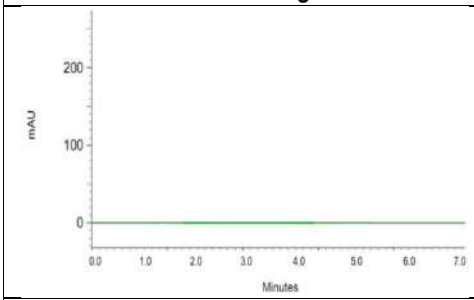


Fig.2: Blank chromatogram

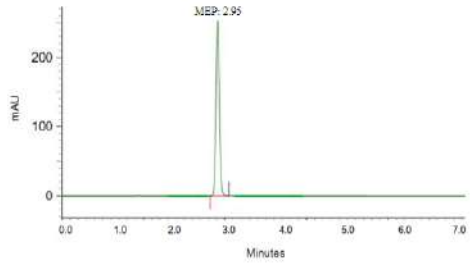


Fig.3: Standard chromatogram of Meropenem

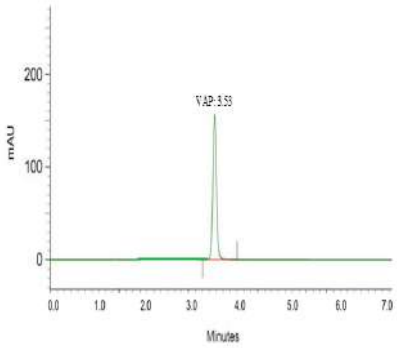


Fig.4: Standard chromatogram of Vaborbactam

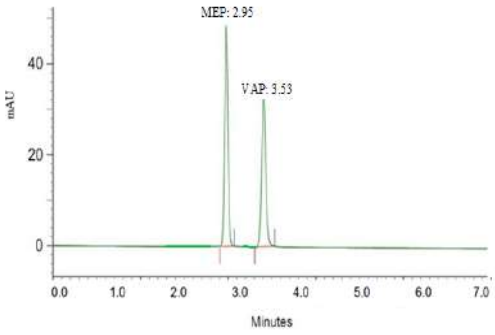


Fig. 5. Standard chromatogram of Meropenem (40 µg/mL) and Vaborbactam (60 µg/mL).

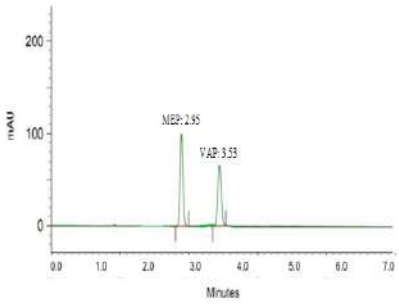


Fig. 6.Standard chromatogram of Meropenem (80 µg/mL) and Vaborbactam(180 µg/mL).

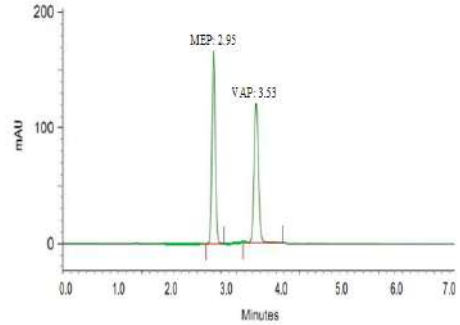


Fig. 7.Standard chromatogram of Meropenem (120 µg/mL) Vaborbactam(120 µg/mL).

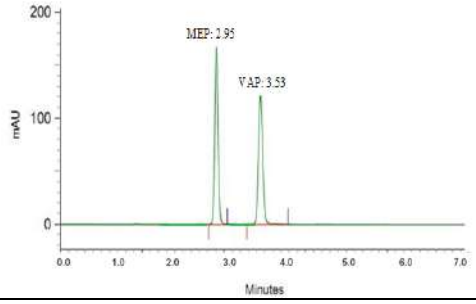


Fig. 8. Standard chromatogram of Meropenem (160 µg/mL)and Vaborbactam (300 µg/mL)

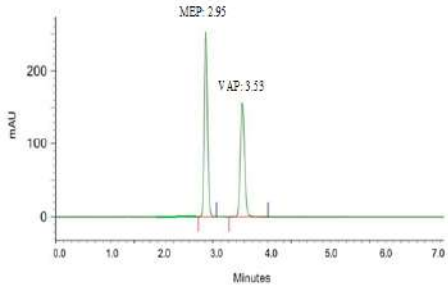


Fig. 9. Standard chromatogram of Meropenem (200 µg/mL) and Vaborbactam (240 µg/mL).

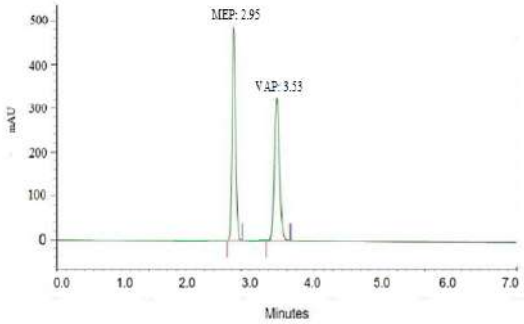


Fig. 10. Standard chromatogram of Meropenem (240 µg/mL) and Vaborbactam(360 µg/mL).

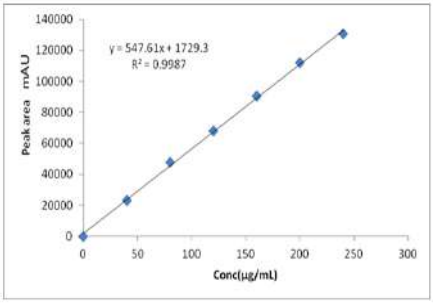


Fig. 11: Linearity of Meropenem

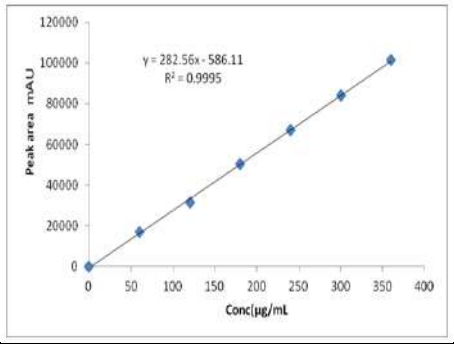


Fig. 12: Linearity of Vaborbactam

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RESEARCH ARTICLE

Design Solution to Minimize The Power Consumption of a BLDC Driver

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ABSTRACT

Low cost, reliable and durable solutions are required in all industries. In automotive industry, BLDC (Brushless Direct Current) motors are widely used due to its advantages over brushed DC motors, which also brings more complex commutation schemes and increased development cost. Despite the fact, BLDC motors require low maintenance and provide reliable results in compare with brushed DC motors, the cost of development of control stage and required hardware topology has been a competence area. The requirements of the application tailor the required hardware topology and control strategy. In this paper, BLDC motor commutation control hardware topologies to implement a BLDC motor based air throttle control circuit, power dissipation and EMC considerations are addressed.

Keywords: BLDC, Motor driver, Low power, Heat minimization, ASIC, Current Control, sustainability.

INTRODUCTION

In industries, it is important to lower the cost while reducing or keeping the maintenance requirement in acceptable levels and improving the performance where possible. The application type tailors the required performance matrix by giving its way to the specifications of the unit. The development cost extends the term performance matrix and includes the maintenance costs thus having durable solutions are as important as functionality. In heavy duty vehicle sector such as at Scania where this paper work was conducted, BLDC motors are widely used due to their advantages over Brushed DC motors such as better dynamic response, higher speed ranges, better torque characteristics, no sparking, less noise, less subject to electromagnetic interference, lower electrical resistance, less maintenance requirement [1][2]. BLDC motors are used in a wide range of applications such as user oriented applications, e.g. CD player, the mechanical setup to open and close the windows, seat position etc., to mechanical applications, e.g. air throttles, compressors, gear shafts etc. Despite the fact, it is advantageous to use BLDC motors, they require more complex commutation schemes and harder to control.

Historical Background

BLDC motor control has been a developing and a challenging field in automotive industry due to its harsh operating conditions. Technology evolution gives its way into smart solutions and enables sensor based and sensor less commutation control circuits, which improves the performance while adding additional development costs [3]. Hardware implementation of a circuit is critically important to minimize losses and increase durability. Software optimizations, whereas is equally important, require hardware to enable optimizations. Thus, hardware implementation and optimization for continuous development that will result in minimized losses, efficiency and durability are important facts [6]. The parameters and specifications to realize the optimal hardware differ depending on the application [7].

Aim

This paper aimed to implement a customized optimal hardware solution to realize minimizing the power consumption thus heat dissipation. The solution further targeted to enable a benchmark to deliver information in comparison with the sub-supplier.

METHODOLOGY

A development board was ordered from ASIC producer and an easy development platform Arduino for implementing the control logic was provided to the other. In the meanwhile, a literature study was conducted for deciding an optimal solution. By comparing the results of literature studies, input of the control student, results of the first experiments, a new hardware concept was developed and implemented for benchmarking. Benchmarking was done in order to enable comparison with respect to heat dissipation, current consumption at the time angle is settled and EMC considerations.

Commutation Strategy

There are several strategies and methods in BLDC motor commutation. This paper intends to focus on understanding the power loss at the commutation stage to benefit during the implementation.

Three Phase Bridge

Three phase inverter bridge or three phase full bridge is a common implementation for three phase BLDC motor commutation by using PWM. PWM can be read in [4]. The basic working principle of full bridge is to turn on one high side of a phase and one low side of another phase transistor simultaneously to let the current flow through the phases and alter the phase at a time that current flows by switching transistors in a sequence to create a certain magnetic field to rotate the rotor. During commutation, two transistors that are connected to the same phase cannot be active at the same time which would result in shoot through where basically the supply is short circuited with transistor connections, which would result in damaging the transistors. For this purpose dead time is inserted between the commutation points to ensure protection where high side or low side transistors are ensured to be off before a transistor connected to the same phase is turned on. By using 6 MOSFETs and switching them in a certain sequence, 120° phase differed three phase voltage is produced for commutation. When a transistor is switched on a magnetic field is created, during switching off due to windings inductive behavior, this created magnetic field has to be removed, thus a current has to flow. As also can be seen from the figure 1,

Four Transistor method

During the literature research, several methods and strategies are found which aims to optimize the cost, area and power consumption but were based on optimization of algorithm or software loaded hardware changes where load is referred to software is expected to fulfill fail safe hardware functionality. It is suggested using 4 transistors instead of using 6 and by using a current sense control 120° PWM signals are driven as shown in fig-2 [5]. The solution requires fast computational power and complicated driving scheme in compare with the classic three phase inverter

bridge. The advantages presented are less component usage, thus component and area reduction. Reliability was given as an open question since the operation is dependent on the software calculations instead of hardwired driving schemes.

Design Strategy

As can be seen from the figure 3, the commutation requires reliable energizing the windings at a certain time at a sequence such as when at least one of the Hall sensor output changes, the winding to be energized should also be changed. At this point, following three factors are considered in the solution.

• Control loop and hardware development must be in parallel, thus the chosen solution should give its way to a prototyping platform within the shortest time.

• The solution should enable diagnostics and fault detection.

• When the final product is ready, control loop and hardware integration shouldn't behave different than the results gathered by the development board based prototyping platform.

RESULTS

The BLDC motor without the flap connected as shown in fig-4, and microcontroller and CAN bus connections had been tested by running the motor as open loop in one direction every second, speed reference was sent via CAN bus feedback was visualized via CANalyzer and functionality was verified. CANalyzer is a software that enables user to monitor, send and receive signals on CAN bus with a user defined speed rate. CANalyzer provides comprehensive user friendly functions, such as signals can be named, grouped, timing of the signals can be seen, CAN messages can be defined as signals and visualised as it is in an oscilloscope, e.g. received CAN message's most significant bit can bet set as a digital signal X and value of it can be shown as 0 to 5V and visualised. One of the important functionality of the software is, databases with CAN messages (a .dbc file) can be created and used for visualizing the CAN messages. In this paper, a CAN database is written and sub supplier and our throttle unit are monitored by using this software. As a computer, a standard laptop equipment with an USB interface is used and any computer that supports the USB interface would have had been used. Details are explained in the following sections. While running the motor open loop, PWM frequency is set directly by modifying the PWM frequency parameter in the basic control algorithm. This test intended to check if everything in hardware was functional. Basic control algorithm here refers to where no control parameters are used except direction and PWM frequency. Transistors are simply driven with the given PWM frequency. By changing the direction and PWM frequency parameter of the algorithm, the hardware and its functionality is verified visually by means of motor to turn. To be able to check if there is any problem regarding soldering faults or not, several throttle units are run with the same PWM frequency and 0.6 A is measured as the drawn current from supply the with a standard multimeter. If the drawn current was not equal to 0.6 A, this was accepted as an indicator for a faulty soldering, and soldering faults are found and fixed.

CONCLUSION

Hardware implementation and practical observations showed that, Arduino platform is a time saving development platform. It is also important to state that, Arduino bootloader took care of low level implementation details such as registry settings of the microcontroller that resulted in fast and efficient development and enabled people to put the focus on functionality and optimizations. During control loop and CAN bus implementation, unstable behaviours and dead lock were observed caused by the start up algorithm. These problems resulted in time loss in the project. So, the suggestion would be to rewrite the algorithm into the actual microcontroller language and eliminate the Arduino environment to achieve a stable implementation.

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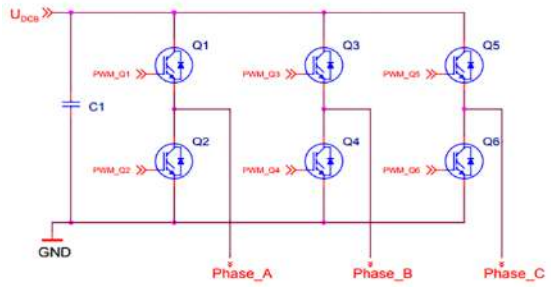


Fig-1:- Three phase bridge

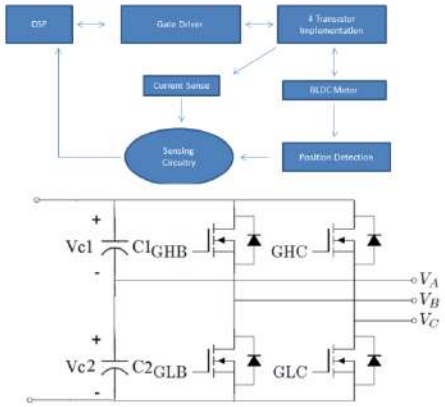
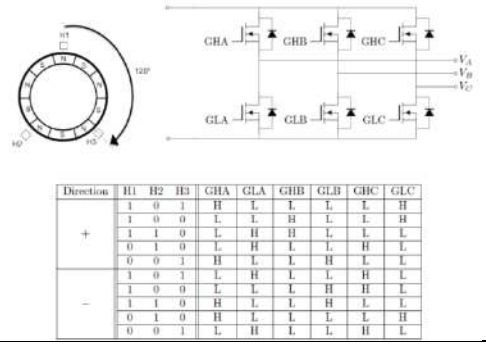


Fig-2:- Four transistor topology[7]



Direction	H1	H2	H3	GHA	GLA	GHB	GLB	GHC	GLC
+	1	0	1	H	L	L	L	L	H
	1	0	0	L	L	H	L	L	H
	1	1	0	L	H	H	L	L	L
	0	1	0	L	H	L	L	H	L
	0	0	1	H	L	L	H	L	L
−	1	0	1	L	H	L	L	H	L
	1	0	0	L	L	L	H	H	L
	1	1	0	H	L	L	H	L	L
	0	1	0	H	L	L	L	L	H
	0	0	1	L	H	L	L	H	L

Fig-3:- Sequence of Energizing commutators

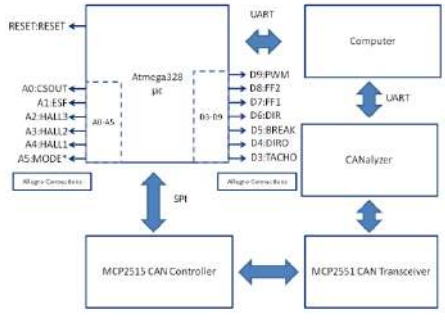


Fig-4:- Experimental set up

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RESEARCH ARTICLE

Application of Remote Sensing in Agriculture

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ABSTRACT

Remote sensing is an advanced technique for collecting data by the reflectance of the electro magnetic radiations involving satellites, aircraft and ground-based and its application in agriculture is increasing significantly. This article discusses the application of various remote sensing technologies and their optimal profitability on agriculture production, productivity and environmental protection. Remote sensing has wide range of applications namely, Crop acreage estimation, crop growth monitoring, soil moisture estimation, soil fertility evaluation, crop stress detection, pest incidence and other agricultural applications like drought and flood monitoring, crop yield for ensuring the sustainability of estimating, weather forecasting, and precision agriculture systems and boosting the country's economic growth. Remote sensing technology enables us to acquire the data, process and analyze the extracted data to synthesize and visualize the important geographic information by the implementation of remote sensing. In the present day agriculture remote sensing based precision agriculture technology, use of unmanned aerial vehicles for hyper spectral and multi spectral image processing and data collection has become a new revolution for smart agriculture techniques. The availability of large amount of satellite data, data analysis algorithms and data processing tools and machine learning programmes further advanced the application of remote sensing.

Keywords: Remote sensing, image processing, drones and hyperspectral camera.

INTRODUCTION

Remote sensing is the art and science of obtaining information about real-objects or areas from a distance without having direct or physical contact with the object. Remote sensing is a technique for monitoring the earth's resources that combines satellite technology with surface observations for higher precision and accuracy**.** Climate change, irrigation sources, crop monitoring, resource management are challenges to agriculture production system across the world. Over the last few years, there has been a significant decrease in the number of agricultural workers and shortage of human resource in agriculture sector. To overcome these challenges in agriculture sector the

advancement of remote sensing along with significant advancements in GPS receivers, microcomputers, and geographic information systems (GIS) can improve the crop monitoring and improve crop simulation models, as well as remote access (Bhattacharyay et al., 2020a, b).

Application of remote sensing in agriculture can have many advantages like precision nutrient management, drought stress management, efficient water use models, yield mapping, and yield estimation and crop phonological stages(Huang *et al*., 2018).The hyper spectral and multispectral images that are taken from earth orbited satellites or aerial vehicles can provide the variability of the fields through which the soil mapping and crop mapping can be done through image processing. The application of GIS can monitor the crops a locality and individual crop monitoring can be observed through GIS system. Remote sensing is also having its roots into precision farming, which is a management strategy that includes a combination of advanced information, communication, and data analysis techniques into the decision-making process to improve crop production while reducing water and nutrient losses as well as improving the nutrient use efficiency. The application of remote sensing for sustainable agriculture development and resource conservation measures are described briefly in this article.

REMOTE SENSING IN AGRICULTURE

The Indian remote sensing programme is governed by the philosophy articulated by Dr Vikaram Sarabhai, the father of India's space programme, which states that the nations should be "unrivalled in the world." Accordingly, remote sensing is one of the important application required for current generation agriculture in India. The payload specifications (spatial, spectral, temporal, and radiometric resolutions) for on board Indian remote sensing satellites were highly accurate in agriculture data collection. It helped in the development of digital image processing in India. Remote sensing technology has rapidly developed in recent years with some sensors are provided to higher temporal, spatial and spectral resolution images. The data acquires a data based remote sensor are mounting with multiple platforms, including satellites, drones and some ground-based vehicles. The satellites type can cover a large area which permits the collection of various types of datasets on global scale (Huang *et al*., 2018). He gave a summary on agricultural data sources. Huang *et al.,* 2018 presented 28 optical and synthetic aperture radar satellite for plant vegetation studies with the resolution of spatial which varies from 0.3km to 1 km. the satellite sensors mostly focus on research with the application in agriculture related to crop type classification (Sicre *et al.,* 2020), soil proper determination, crop mapping and spatial statistics and the forecasting of crop yield, estimation of canopy parameters and irrigation/drought evaluation.

AGRICULTURAL APPLICATIONS – BASIC ASPECTS

Remote sensing has the advantage of supplying information repeatedly without destroying a sample of the crop that can be used in delivering important data for precision applications in agriculture. Remote sensing is a technique for gathering information from a low-cost data acquisition across a vast geographic area (Dutta *et al*., 2015). In India, satellite remote sensing is mostly utilised to estimate agriculture acreage and production of crops for agriculture. The use of remote sensing technology has the potential to change the world agricultural resource detection and characterization based on the biophysical characteristics of soils and crops (Estel *et al*., 2018). Remote sensing satellite data can be utilized for a variety of purposes such as yield estimation (Doraiswamy *et al*., 2005; Bernerdes *et al*., 2012), detection of stress situations (Gu *et al*., 2007), disturbances and crop phenological information (Sakamoto *et al*., 2005).Remote sensing in conjunction with GIS is extremely useful for developing Spatio-temporal fundamental informative layers that can be successfully applied and used in a variety of fields, including flood plains surface mapping, hydrological modelling flow of energy, urbanisation, and land usage adjustments, crop growth tracking, and stress management detection (Kingra *et al*., 2016).

Monitoring of vegetation cover

In the fields of agricultural classification, crop acreage and yield estimation remote sensing plays a crucial role. Aerial pictures and digital image processing were used in numerous research investigation techniques. However, remote sensing is a technology field that reduces the human efforts and it enhances the precision of a set of estimates

(Kingra *et al*., 2016). Hyperspectral data has a great capacity to improve the characterization, classification, and modelling when it comes to crop and vegetation mapping as compared to multispectral broadband the term "remote sensing" is well-known.(Thenkabail *et al*., 2011). The Normalized Difference is the most often used index for assessing vegetation condition. The NDVI has become the most widely used metric. (Calvao and Palmeirim, 2004, Wallace *et al*., 2004) initiatives have been made to develop additional Indicators that can lessen the soil's influence on the impact of context and atmosphere on the outcomes of observations in the spectral range. Soil Adjusted Vegetation Index (SAVI) is an example of a vegetation index that reduces the impact of soil on remotely sensed vegetation data (Bernardes *et al*.,2012).

Crop condition assessment

Remote sensing provides timely spectral information that can be used to analyse crop health. The biophysical plant health indicators and physiological changes that take place in plants may modify their spectral characteristics as a result of stress.As a result of the reflectance/emission propertiesin the detection of stress that can be detected remotely for sensing (Prasad *et al*., 2006).Crop monitoring is required at regular times throughout the growing season to take suitable steps and also aware of the potential for production losses as a result of any stressor Crop development phases and their progressions are determined by several variables, such as soil availability moisture, planting date, and air temperature and soil conditions. These elements are in charge of the plant's environment and their efficiency. Corn crop yields, for example, can be harmed if temperatures are too high during pollination. As a result, understanding the temperature at the pollination period for corn could aid corn forecasters in making more accurate predictions (Nellis *et al*., 2009). Drought renders the land unusable for agriculture and makes it uninhabitable for human environment, livestock population, biomass potential, and plant diversity species (Sankaran *et al*., 2010). Drought monitoring using satellite data has gained popularity in recent years, particularly the usage Vegetation Condition Index (VCI) and Normalized Difference Vegetation Value Index (NDVI) has been widely acknowledged around the world. Highlighting agricultural drought in various parts of the world ecoregions with a wide range of environmental conditions (Nicholson and Farrar, 1994; Kogan, 1995; Wang *et al*., 2001; Anyamba *et al*., 2001; Ji and Peters, 2003).

Irrigation water management

By using variable rate irrigation technology like center pivot system, remote sensing data can help determine the variations within the field and apply water effectively. Water stress caused by excessive wet and dry conditions can be mitigated using variable rate application circumstances to obtain uniformly high yields over the field while lowering water and nutrient use losses. (Evans et. al, 2013; McDowell et. al, 2017). Multiple indices and approaches for precision water management have been developed and tested using remote sensing in the optical, thermal, and microwave bands (Amani *et al*. 2016).Das *et al*.,(2018) developed a soil moisture and temperature map using a high-resolution land data assimilation system as a computing tool which is aimed at providing soil moisture and soil temperature at 1 km spatial resolution in real-time at four soil depths and vegetation root zones. With the increase in the development of hyper spectral bands in the thermal region, remote sensing has been playing a major role in understanding crop soil characteristics. Such information when linked with GPS will provide promising results which are more helpful in precision farming.

Monitoring of pest management

The wavelength of electromagnetic radiation affects how it interacts with plants. Depending on the state of health and vigour of the plant, the reflectance of the leaves can vary significantly measured (Luo *et al*., 2010). Due to significant absorption by photoactive pigments, healthy and aggressively developing plant leaves can be measured and analysed to find the diseased leaf. Insect defoliation has been assessed and monitored using a remote sensing method. Comparing the different spectral responses to chlorosis (leaf and foliage yellowing)decreases over some time, assuming that these differences can be correlated, classified, and interpreted (Franklin, 2001).Detecting and mapping defoliation, characterization of pattern disruptions, and other remote sensing applications have been used as well as giving information to pest management mechanisms that aid in making decisions (Lee *et al*., 2010).

Limitations of remote sensing

Most of the image processing tools are based on the measurement of surface physical properties such as surface reflectance and temperature. But the important barrier for implement of image processing is to conversion of digital image in to surface properties (Huang et al., 2018). Due to uncorrected and uncalibrated images it is difficult to assess the accurate image output data which leads to improper imaging. Remote sensing requires a special kind of training to analyse the images. It is therefore expensive in the long run to use remote sensing technology since extra training must be accorded to the users of the technology (Luo et al., 2010).The technique is very expensive for small areas requiring for one time analysis. Specific trained human resource is needed in order to analyse the image data and calibration. Large scale maps are difficult to prepare from obtained satellite data. For dynamic image processing repetatiatve aerial photographs are required which involves high cost. The selection of sensors, mounting of sensors, collection of data and its timings are determined by human beings which may lead to errors if care is not taken. Remote sensing equipment require regular calibration, failing to do so will result into uncelebrated remote sensing data.

CONCLUSION

Remote sensing gives opportunity to get information about any object or phenomena without coming in direct contact with the object in question. As it can help in collecting the observations non-destructively., hence they can be used in different experimentation. Remote sensing has multiple uses such as resource inventory, yield estimation, input optimization and deficiency detection, identifying pest damage etc. Considering all these benefits, it is clear that remote sensing tools can be useful in improving resource use efficiency, reducing resource loss and ensuring long term sustainability.

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RESEARCH ARTICLE

Intensive Farming: It's Effect on the Environment

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ABSTRACT

Intensive farming system is commonly followed in modern day agriculture owing to rise in population to meet the high food demand. In intensive farming, the farmer need high economic input and knowledge for managing the land and crop considering the fast paced crop rotation in the field and high input use. However use of higher amount of chemical inputs, injudicious exploitation of natural resources, poor maintenance of soil health etc. make such intensive agricultural practice unsustainable. Hence, careful management of natural resources that takes care of agricultural sustainability and promoting alternate sustainable practices is the need of the hour.

Keywords: Intensive farming, green revolution, organic farming, agriculture, climate change

INTRODUCTION

Agriculture has undergone a technological revolution after the green revolution, which has drastically altered farming operations. The effects of such changes on the environment are becoming increasingly alarming (1-4).Injudicious use of chemical inputs, improper maintenance of soil health, excessive irrigation without considering drainage or soil irritability etc. has caused multiple second generation problems in agriculture. Industrial agriculture is known as intensive farming. This is a kind of farming that uses a lot of chemicals. It is a farming style that involves more input and output per unit of agricultural land area for the crop. It involves higher use of inputs such as capital and labor and higher crop yields per unit land area(5-6). Environmental degradation reduces ability of human beings to meet basic food demands., which will exacerbate the food security issue. To strengthen productive capacity and protect environmental domains from degradation, small-scale farmers in countries like India need financial and technical support, including rapid dissemination of sustainable agricultural technology and practices with the necessary supporting services to increase food production. This would make a significant involvement in improving food security and environmental protection. The only way to achieve the aims of sustainable development and food security at the very same time is to promote appropriate farming techniques that cause an unnecessary amount of environmental damage while producing the most amount of food grains (7-10).

To avoid future food security issues, farmers must first incorporate sustainable agriculture into their agricultural techniques. To achieve a "real green revolution" that protects the health of nature and humans while also producing adequate food for a growing population, policymakers and farmers must have strong willpower, as well as demand-side backing from the business sector.

Advantages of Intensive Farming

1.High crop yield
2.Intensive Farming is Simple
3.variety of food can be produced
4.Affordable food prices
5.More efficient
6.Sustainable supply of food

High crop yield

High crop yields are one of the primary benefits of intensive farming. Agricultural products like fish eggs, meat, milk, pulses, and cereals are in high demand. Fulfilling market demands has only been possible through industrial farming because large amount of food is produced on a small piece of farmland. To increase crop yield, intensive farming employs higher inputs and advanced agricultural techniques. Pesticide and fertilizer use is high.

Intensive Farming is Simple

Unlike traditional farming, where a farmer would need to consider all the different crop types to ensure diversity (11-13), in intensive agriculture especially those involving monoculture, understanding of a single crop or animal type is required. The amount of equipment and methods required to harvest crops and livestock is minimal since it only involves a single type of crop or animal. All the equipment is standardized and works best in mono-culture.

More variety of food can be produced

Intensive farming necessarily involves a large amount of human power, money, and resources, making it more practical to concentrate on a single production area. As a result, different farmers' involved in various areas of practice such as industrial fruit production, industrial vegetable production on any of the numerous options namely potatoes, rice, corn, wheat, and so on. Every farmer can only grow one or two crops in a single year. As a result, it is more efficient and productive.

More efficient

It is more efficient for commercial farmers as it increases productivity per ha. The farmer makes more profit by getting more yields on a small piece of land in contrast told-fashioned farming methods, which required large areas of land but produced lower gains yield (14). Due to mass production of single crop in large area management is easy.

Affordable food prices

The use of industrial farming to produce crops and meat products has reduced food prices. The reason is industrial farming takes up less space and gives more yields. Furthermore, it has made a significant contribution to resolve the world's food problem. As a result, disadvantaged and poor people can afford to eat nutritious and balanced food. Cereals and pulses are grown in large quantities to meet human demand. Its high production leads to a low price of food.

Sustainable supply of food

With global food demand increasing due to an ever-increasing human population, industrial farming has the advantage of high crop output as well as the ability to meet food market demand. As it produces more output per unit input; hence; it contributes in meeting the increasing need for food supplies.

Effect on Environment

Some estimations state that industrialized farming costs the environment approximately US$3 trillion per year because it emits greenhouse gases, contaminates air and water, and destroys flora and fauna. Externalized expenses, such as the funds needed to the treatment of contaminated drinking water or treat diseases caused by unhealthy nutrition, are also unaccounted for by the industry.

1. Animal agriculture
2. Degradation of Land
3. Excessive use of Agro-chemicals
4. Deforestation
5. Environmental Degradation
6. Soil Erosion
7. Depends on Fossil Fuels

Animal agriculture

Meat is regarded as one of the primary contributors to the current biodiversity loss crisis. The 2019 IPBES Global Assessment Report on Biodiversity and Ecosystem Services found that industrial agriculture and overfishing are the primary drivers of extinction, with the meat and dairy sectors playing a significant role. It is one of the major emitters of greenhouse gases in the world, as well as one of the leading causes of biodiversity loss, and it is arguably the main source of water pollution in both developed and developing countries(15).

Degradation of Land

Land degradation, in one form or another, is a serious concern that threatens agricultural sustainability. Overgrazing and poor agricultural methods in forest and other plain areas expose the land to water and wind erosion. Water and wind erosion damage 141.3 million hectares of total land area(16).

Excessive use of Agro-chemicals

As previously stated, intensive farming includes the use of a variety of agrochemicals such as chemical pesticides, fertilizers, herbicides, insecticides .These poisons not only kill their intended targets, such as weeds, pests, and parasites, but they also contaminate food with herbicide residues. Insecticides and pesticides also kill beneficial insects, contributing to the loss of biodiversity. Chemical sprays have negative impact on workers and people nearby, and people who eat the food inadvertently consume the chemicals. Predatory bird and bug populations have seen a decline. resulting in the eradication of natural pest control. The large-scale use of toxic pesticides has a direct influence on human and animal health. A wide range of cases involving pesticide residues and human and animal consumption has resulted in health risks.

Deforestation and alteration of the natural environment

According to environmental studies and assessments, industrial farming has a wide range of negative consequences and affects the environment. Massive deforestation and soil erosion have occurred as a result of tree removal, slash and burn tactics, and the clearing of forest areas to make room for agriculture. As a result of the damaging behaviors that have repeatedly led to habitat degradation, natural habitats and wild species have suffered considerably. Chemical fertilizers and herbicides pollute soils, wildlife habitats, and water bodies like seas, rivers, and lakes. Fertilizers, in particular, are the primary source of eutrophication in the vast majority of the world's water bodies, including oceans, lakes, and rivers. The earth is losing more than 18 million acres of forest per year. It is estimated that 70% of deforestation in the Amazon basin is linked to the beef industry(16).

Deforestation

Deforestation is destroying the world's forests on a massive scale, causing widespread land damage. Clearing land for pasture or crops is one of the causes of deforestation. According to British environmentalist Norman Myers, cattle

ranching accounts for 5% of deforestation, logging accounts for 19%, palm oil plantations account for 22%, and slash-and-burn farming accounts for 54%(17).

Environmental Degradation

Environmental degradation is defined as the earth's disintegration or deterioration caused by the consumption of assets such as air, water, and soil; the destruction of habitats; and the extinction of animals. Deforestation is defined as the activity of clearing forest cover or trees on a large scale without replacements. This exercise frequently results in the destruction of land quality, weather, and even climate. The primary perpetrators of deforestation are loggers acting on both legal and illegal orders. According to research, approximately half of the world's tropical forests have been cleared through logging where no new trees have been planted. India is a country with a diverse range of agro-climatic zones that support a diverse range of animals and plants. In terms of plant diversity, India is estimated to be tenth in the world and fourth in Asia. A number of plant and animal species are becoming extinct as agriculture becomes more commercialized.

Soil Erosion

Soil erosion is a natural phenomenon at its heart. Simply described, it is the movement of topsoil, the ground's topmost layer, from one spot to another. This is significant because topsoil contains the most organic content and is best suited for farming and other fruitful activities, which is why soil erosion has the biggest impact on farmers and agricultural land.

Depends on Fossil Fuels

The high amount of automation and machinery used in intensive farming has a high requirement on fossil fuels. Many farms are still using fossil fuels to provide energy to the equipment since it is cheaper and more efficient than renewable sources.

The solution to reducing Intensive cropping

- Create efficient, self-sufficient, and cost-effective production systems that generate adequate income.
- Preserve and safeguard biodiversity and territorial integrity.
- Increase the efficiency with which natural resources are used.
- Manage the quality of soil, air, and water.
- Adopting a different cropping system.

Integrated Farming System

Integrated farming is defined as a biologically integrated system that integrates natural resources into farming activities through a regulated mechanism to maximize off-farm input replacement while maintaining farm income(8).

Objectives of IFS

1. Efficient farm and animal waste recycling
2. Keeping nutrient losses to a minimum
3. Maximizing nutrient utilization efficiency
4. Adoption of crop rotations and efficient farming systems
5. Farm enterprises that work well together

Advantages of Integrated Farming System (IFS)

1. Productivity
2. Profitability
3. Sustainability
4. Balanced food

5. Environmental safety
6. Waste recycling
7. Saving energy
8. Adoption of New Technology
9. Money Round the year
10. Availability of fodder, fuel, and timber
11. Employment round the year
12. Agro-industries
13. Increases input efficiency
14. Standard of living
15. Avoid degradation of forest etc.

Productivity:-Crop and allied enterprise integration aids in increasing economic yield per unit area per unit time.
Profitability:-Produce/waste material of an enterprise can be used for other enterprises at least for a crop, thus reducing the cost of production and increasing profitability per rupee investment.
Sustainability:-Huge quantity of inorganic fertilizers, pesticides, herbicides are required to meet the food requirement of the ever-increasing global population. The productivity of soil gets drastically reduced in due course of time. IFS provides an opportunity to sustain production through organic supplementation and effective utilization of by-products of linked components.
Balanced food:-It provide balance nutrient from various crop and animal product.
Environmental Safety:-IFS waste material, by-products of one composite are effectively recycled using for other component and a by-product of that component as organic manure to enrich the soil. The use of bioagent or crop protection also minimizes pesticides.
Recycling of waste:- West of the crop husbandry can be effectively recycled for preparation of compost. Some of the by-products can be used as feed. This reduces the cost of production of one enterprise at the cost of another.
Saving energy:-Energy crises can be served to the same extent by utilizing organic waste to generate biogas which can be used for cooking.
Adoption of new technology:- In IFS linking of cropping with dairy, mushroom, sericulture, floriculture there is a flow of money throughout the year. Small can not afford technology quickly.
Money round the year:-IFS provides a continuous flow of cash throughout the year by disposing of eggs, milk, edible mushrooms, honey, silkworm cocoons, and so on.
Availability of fodder, fuel, and timber:-IFS utilizes every part of the land. Growing fodder trees on the border will not only provide fodder but also enrich the soil by fixing atmospheric nitrogen.
Employment round the year:-Crop-livestock integration increase labor requirement through the year, other activities like mushroom cultivation, sericulture, apiculture also need labor. Hence IFS provides employment to family members as well as outside labor throughout the year.
Agro-industries:-Linking of various components in IFS, the production increased to a commercial level.
Increase input efficiency:-IFS provides better scope to use available inputs more efficiently. This leads to an increased benefit: cost ratio.
Standard of living:- It increases farmer living standards.
Avoid degradation of the forest:- IFS linked with Afforestration and provide safety against degradation of lands, besides supplementation of fuel, timber, and fodder.

Organic farming

Organic farming is the cultivation of crops, livestock, and other goods without the use of chemical fertilizers and pesticides, antibiotics, growth hormones, and other chemicals(18-20).

Benefits of organic farming

1. Using organic material to protect soil quality and encourage biological activity.

2. Indirect crop nutrient provision via soil microorganisms
3. Soil nitrogen fixation with the help of legumes crop
4. To control weeds and pests, numerous strategies such as crop rotation, biological variety, natural predators, organic manures, and suitable chemical, thermal, and biological intervention are utilized.

Precision farming

Precision farming is a strategy of farming management that collects, observes, measures, and responds to variability in crops, whether it is in or outside the field(21-23). In a simpler definition, it is a farming technique that uses technology to produce greater results with fewer resources while keeping minimum potential harm.

The benefit of precision farming

1.Refined set of cultivation practices and choice of crops based on suitability of land
2.Elimination of volatility and risk of the crop.
3.Waste management is best in this farming
4.Reduced production costs due to precise amount is given to the plant
5.Minimum environmental impact due to west material
6.Optimized use of fertilizers by the different systems which increases efficiency
7.Water management is the best class due to drip irrigation.

Vertical farming

Vertical farming is growing crops in layers that are vertically stacked one on top of the other. Controlled-environment agriculture, which aims to improve plant development, and farming without soil techniques like hydroponics, aquaponics, and aeroponics are popular (24-25). Buildings, shipping containers, tunnels, and abandoned mine shafts are used to house vertical farming systems which are chip place that has more space area to grow the plant. In these structures are closed spaces that work as a controlled environment.

Advantages of Vertical Farming

1. It guaranty crop production. it ensures constant crop production.
2. Uses space optimally due to its vertical nature. It produces crops in 3 dimensions.
3. Reduces Usage of water due to hydroponic application, which recycles water. It is a close loop system.
4. Cuts down on transport cost as it can grow anywhere in world.
5. Less labour costs because it is a completely automated indoor growing system.
6.It doesn't involve chemicals or pesticides as the plants are grown in control environment that prevents entry of harmful pests.

CONCLUSION

Intensive farming affects the environment. There is a need to balance intensive farming with other sustainable farming to make sure our food securities are not affected. IFS, Organic farming, vertical farming etc. can be introduced. In intensive farming areas focus must be on efficiency centric management. Care must be taken to minimize ecosystem damage while maintaining optimal productivity.

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RESEARCH ARTICLE

Plant Nutrient Management by Organic Inputs

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ABSTRACT

Plant nutrient management by organic input includes application of different organic manures, namely, bulky organic manures and concentrates and biofertilizers. Undoubtedly, these inputs are having low analytical value, but they are ecofriendly and improve soil health. The non-judicious application of huge quantity of chemical fertilizers resulted in declining soil health as well as environmental pollution. Hence, there is an urgent need for integration of all available resources of plant nutrients for enhancement of soil fertility and productivity. In integrated nutrient management, organic sources of plant nutrients play a significant role. Further, organic farming is catching up in the country where organic inputs are the only sources of nutrients. An attempt has been taken here to highlight the importance of organic inputs in sustaining agricultural productivity.

Keywords: Farmyard manure, composts, crop residue, green manure, compost, crop rotation.

INTRODUCTION

Organic nutrient management is the practice of supplying plant nutrients by using organic sources of nutrients such as organic matter, farmyard manure (FYM), compost, vermicompost, oil cakes, green manures etc. In organic farming, suitable crop cycles and rotations also contribute a lot for enhancement of soil fertility. The advantages of using organic manures into soil include increased water storage capacity and improved infiltration rates (Nayak *et al*., 2012; Maitra and Palai, 2018).Bunemann *et al*. (2018) reported that the major concern of plant nutrient management, according to organic farming principles is the buildup and maintenance of soil fertility and the soil's ability to supply water and nutrients for plant growth and reproduction. Nutrient cycling in soil under various nutrient management practices and organic nutrient use, as well as developing effective nutrient management strategies for increased crop yield, soil health and long-term sustainability (Venkatesh *et al.,* 2017; Maitra *et al*., 2018a). Organic matter has been used to increased soil health and plant nutrient supply since long time. The use of organic manures improves the nutrient uptake and plant uptake and improving soil fertility and productivity for essential (Vidyavathi *et al.,* 2012). Bhattacharya and Dey (2014) reported that the application of organic manure was found to be more successful than inorganic fertilizer treatments. The long-term productivity of agro ecosystem

depends on the maintenance of soil organic matter. During recent time, organic agriculture is catching up in the country where organic inputs play significant role in plant nutrition. Soil is the most important factor in plant growth and development and soil physical, chemical and biological properties are better maintained by the application of organic manures. Further, green manures improve soil biomass and increases soil microbial activities and fertility. The present article has described the requirement of organic nutrient management and various sources of organic nutrients and their application for agricultural sustainability.

Nutrient management of plants through organic sources

- Application of organic nutrients has several benefits as they:
- Increase demand of organically produced farm-products due to food safety and absence of synthetic chemical residues in food.
- Enhance the physical, chemical, and biological qualities of soil.
- Conserve ecosystem and environment.
- Reduce environmental pollution by recycling of organic wastes.
- Maintain soil fertility for the long-term basis by slowly releasing plant nutrients into available form.

Different organic manures

Bulky organic manures

As the analytical values of nutrients are less in bulky organic manures, they are used in large quantity. The most essential and extensively utilized bulky organic manures are farmyard manures, composts, and green manure (inclusive of green leaf manure). They supply all essential plant nutrients including micronutrients and thus, increase the availability of nutrients to plants. By providing support to earthworms and beneficial micro-organisms bulky organic manures increase the biological activity in soil.

Farm yard manure

FYM is made up primarily of cow dung, urine, waste straw, feed left-over and other animal farm wastes. It is highly useful considering the aforesaid benefits. When cow dung and urine are combined together, a balanced nutrition is supplied to the plants. Application of FYM improves soil fertility and stimulate activity of microorganisms that made plant food elements in the soil readily available to crops. Soil organic matter, on the other hand, it is an important component of soil quality because it influences nutrient mineralization, aggregate stability, aeration and water uptake and retention.FYM enhances water holding capacity as well as the amount of water available for plant growth and improves the physical properties of the soil.In general, a well-rotten FYM contains 0.5% N, 0.2% P and 0.5% K. To improve and sustain crop yields and soil fertility, it plays as important role in the organic nutrient management (Kundu *et al.,* 2005).

Compost

Compost is a product of decomposition of plant and animal wastes which undergo with microbial activities and various additives. Composting is the process of recycling organic wastes with or without dung to quickly utilizable condition for improving and maintaining soil fertility and supplying nutrients to plants. Organic matter that can be utilized as a soil conditioner in organic as well as in traditional agriculture (Termorshuizen*et al.,* 2004). The application of compost manure to agricultural field directly impacts on soil health, buffering capacity, water holding capacity and nutritional status and indirectly influences on various microbial activities.

Advantages of using compost in plant nutrient management

There are several advantages of using composts in plant nutrient management and these are as follows.

i. Composts improve soil physico-chemical and biological properties as quality of soil as a whole.
ii. Composts are known to produce healthy plants and reduce pests and diseases of crop plants.
iii. As composts are cheap and hence, they save money in terms of plant nutrient supply.
iv. Composts reduce risk of pollution.

Vermi-compost

The method of producing compost involves the use of earthworms, which reside in the soil and eat partially decomposed biomass. Vermicompost provides a nutrient rich organic manure as the analytical value of essential nutrients is more than other composts. Moreover, vermicompost contains plant growth regulators and enzymes and hence, it is known as superior to other composts. Vermicompost has been shown to improve plant growth. In order to maximizecropyields, vermicompost should be applied in integrated plant nutrient management. In organic agriculture, vermicompost can play a pivotal role in plant nutrient management. As continuous application of chemical fertilizers has destroyed the soil quality (Gupta *et al.,* 2014), vermicompost can be a suitable option for plant nutrient management for agricultural sustainability.

Vermiculture

Vermiculture refers to the scientific process of worm cultivation for the benefit of humus. It's also called worm manure. Vermiculture research is unfolding a revolution for many applications in environmental preservation and sustainable agricultural development (Sinha *et al.,* 2010).

Green Manure

Green manure is organic manure, that refers to specific plant or crop varieties that are grown and then incorporated into the soil to improve the soil quality and supply nutrients to crops (Maitra *et al*., 2018b).Certain fast growing plant species or crop varieties are grown for the purpose and incorporated them at their vegetative stage into the soil health enhancement. The examples of green manure crops are *dhaincha*, clover, beans, peas among the legumes and other fast-growing crops such as annual rye-grass, oats, rapeseed, *Amaranthus* etc. The practice of green manuring increases the biological properties of soil and contributes as the source of nutrients in production of eco-friendly crops. Earlier researchers carried out experiments to evaluate the effect of green manures and found the beneficial impact on crop growth and productivity (Tejada *et al.,* 2008; Midya*et al*., 2021a, b). Legumes because of their multiple benefits on soil health are preferred for green manuring (Praharaj and Maitra, 2020).

Types of green manure

Green manuring *In situ*: When green manures crops are grown in the field and incorporated into the soil of the same field,it'sknown as *in-situ* green manuring. In India and othe tropical and sub-tropical regions of the world, *in-situ* green manuring of dhaincha, sunhemp, cowpea etc. is a common practice during summer season before onset of monsoon.

Green leaf manuring: In case of green leaf manuring, crops are cultivated *ex-situ* especially in waste lands, field bunds and forestsnearer to the crop fields and green leaves and twigs of these crops are incorporated into the field. In case of green leaf manuring, neem, mahua, subabul etc. are used commonly.

Panchagavya and Dasagavya

Panchagavya is an organic product that has the ability to promote plant development while also offering immunity of plant. Panchagavya is made up of five different cow products, namely, cow dung, cow urine, milk, curd, ghee. Dasagavya is another natural product in which ten different products, including panchagavya and plant extracts are included. The plant extracts are made separately by soaking the different foliages for 10 days in 1:1 ratio with cow urine.The filtered extracts of all the plants are then added to 5 liters of panchagavya solution. The mixture of is allowed to ferment for about 25 days which encourage the growth of microorganisms. Application of Dasagavya foliar spray @3% concentration is recommended and it is also known to manage the pests and diseases population dynamics such as aphids, thrips and mites (pests)and leaf spot, leaf blight (diseases).

Concentrated Organic Manures

Concentrated organic manures are superior to bulky organic manures and these are made up of plant-based sources and animal of raw materials. Following are the examples of concentrated organic manures.

Oil cake: After the extracting oil from oilseeds, the byproduct produced appears like cake and used as manure in the field. Oil cakes are rich in nutrients and they contain more nutrients than bulky organic manures. There are two types of oil cakes:

Edible oil cake: Suitable for cattle and poultry feeding as well as source of plant nutrients. Examples of edible oilcake are groundnut cake, mustard cake, sesame cake, coconut cake etc.

Non-edible oil cake: Suitable for crop fertilization and examples of non-edible oilcake are castor cake, neem cake etc.

Blood meal: Blood meal is prepared from the blood collected from slaughterhouse. The materials are treated, dried, powdered, bagged and sold as blood meal. It contains 10-12% N and 1-2% P.

Fish meal: Fish meal is a dry powder made up of whole fish and fish filleting wastes. It is used as a protein supplement in aquaculture to feed the fishes. Fish meal contains 4-10% N, 3-9% P and0.3-1.5% K.

Biofertilizers

Biofertilizers are latent or living cells of microorganisms that provide nutrients to crops (Hegde *et al*.,1999). The microbial inoculants as biofertilizers when applied these rapidly multiply and act as nutrient provider to crops. Some biofertilizers such as *Rhizobium*, *Azospirillum*, *Azotobactor* fix atmospheric nitrogen. Rhizobium remains in symbiotic association with legumes, forms nodule in crop roots, fixes atmospheric nitrogen biologically. In cereal crops, application of *Azospirillum* is beneficial (Harika *et al*., 2019; Ramya *et al*., 2020). Biofertilizers are applied by seeds inoculation, seedling root dipping and soil application. In different pulses cultivation Rhizobium seed inoculation is commonly practiced. Bhattacharjee and Dey (2014) reported that when biofertilizers are amended to seed, root and soil, they ensure benefits to crops. Application of biofertilizers is considered as a suitable option in the organic agriculture for nutrient management.

Crop Residues incorporation

The vegetative agricultural crop material that's left on the field after a crop has been harvested and burned. The straw burning is in alarming situation in India. The straw of most cereal crop contains all essential nutrients. If these are incorporated into the soil, it can act as organic manure after proper decomposition. The application of microbial inoculants can improve decomposition process and thus, soil health can be improved (Turmel *et al.,* 2013). There are two types of crop residues.

- Harvest refuse – straw, stubbles haulms etc.
- Processed waste – rice husk, cobs of maize etc.

As incorporation of crop residues improve soil quality, nutrient availability and reduce over dependance on chemicals, the residues incorporation has enough scope for creation of suitable conditions for achieving agricultural sustainability.

Crop rotation

Crop rotation is the repeated cultivation of an organized succession of crops on the same land (Panigrahy *et al.,* 1995). In general, it is advised to grow different crops in sequential cropping for maintenance of soil health and proper resource utilization. Legumes are highly suitable as crops to be considered in crop rotation as they offer multifaceted benefits. In a crop rotation, after cultivation of cereals, a legume crop should be taken in succession. Cereals are known as soil draining crops; however, legumes are soil restorative crops.

CONCLUSION

Organic sources of nutrients are cheap, easily available and environmentally friendly and hence, considering the ill effects on over-dependence on chemical fertilizers, organic sources of nutrients are to be incorporated in plants nutrient management. In Organic agriculture, there is no scope for application of synthetically produced chemical

fertilizers and organic nutrients play a pivotal role. In conventional agriculture, there should be reduction in use of chemical fertilizers and nutrients are supplemented by organic inputs. Actually, integrated nutrient management practice should be adopted in conventional agriculture. Inclusion of organic inputs in plant nutrient management may lead us to achieve agricultural sustainability.

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RESEARCH ARTICLE

In vitro Regeneration Through somatic Embryogenesis in Chrysanthemum Cultivars

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ABSTRACT

Petals of three chrysanthemum cultivars (Poornima Pink, Poornima Red and Poornima White) were cultured on Murashige and Skoog (MS) medium supplemented with 4 mg l^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D)alone or in combination with different concentrations (1,2 and 3mg l^{-1}) 6-benzylaminopurine (BAP) or kinetin (KIN). Indirect somatic embryogenesis (SE) was induced in all media with different growth regulators tested but number of somatic embryos varied with the cultivar and medium. MS medium with 2,4-D (4 mg l^{-1}) has induced more number of somatic embryos in Poornima Pink (12.80) and Poornima White (17.30). MS medium with 2,4-D 4 mg l^{-1} and BAP 2 mg l^{-1} has induced more number of somatic embryos (22.60) in chrysanthemum cultivar Poornima Red. Cytokinins significantly decreased the number of somatic embryos in chrysanthemum cultivars. Observed conversion rate of somatic embryos was 72.20 per cent in Poornima pink, 77.60 per cent in Poornima Red and 18.00 per cent in Poornima White. SE in chrysanthemums can be useful for both rapid production of plant in *in vitro* and in the regeneration step of breeding (through mutation breeding and genetic transformation techniques).

Keywords: Chrysanthemum cultivars Poornima Pink, Poornima Red and Poornima White somatic embryos, MS medium

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* Tzvelev.) is one of the important and most widely cultivated herbaceous perennial plant belongs to Asteraceae family and commonly known as “Autumn Queen” or “Queen of East” and reported to be native of northern hemisphere, mainly Europe and Asia (Anderson, 1987).Chrysanthemums are most important cut flower after rose, flowering potted plant and herbaceous perennial markets worldwide.

Traditionally chrysanthemum is propagated by root suckers or terminal cuttings. These methods are very slow and the risk of transmission of diseases is very high. *In vitro* culture is one of the input tools of plant biotechnology that exploits the totipotency of plant cells. Application of new methods in plant breeding programmes requires efficient *in vitro* regeneration procedures. Somatic embryogenesis is a desirable and fastest method of plant regeneration. Somatic embryogenesis is similar to zygotic embryogenesis, because of morphological changes in somatic embryos are similar to those in zygotic embryos. In addition, somatic embryos are generally thought to arise from a single cell (Nomura and Komamine, 1999), so this technique should be useful for the clonal mass propagation of plants (Lutz *et al.,* 1984; Ammirato and Styer, 1985) and the dissolution of chimeras in mutation breeding and gene recombination breeding. This study was mainly aimed to find an appropriate method to regenerate plants via somatic embryogenesis for three chrysanthemum cultivars of chrysanthemum: Poornima Pink, Poornima Red and Poornima White.

MATERIALS AND METHODS

Plant material

A continuous supply of plant material for conducting experiment was obtained from mother plants which were grown in well established poly house which was located at Department of Floriculture and Landscape Architecture, Kittur Rani Channamma College of Horticulture, University of Horticultural Sciences. In this experiment three cultivars (Poornima Pink, Poornima Red and Poornima White) of chrysanthemum were used. Flowers of selected chrysanthemum varieties were collected from mother plants and were thoroughly washed under running tap water for 15 minutes. Thereafter, they were sterilized with 75% (v/v) ethanol solution for 60 seconds, followed by rinsing with distilled water and 0.1% mercuric chloride for 4 minutes in laminar air flow chamber and finally by 3-4 rinses with sterile water (Misra and Datta, 2007; Barakat *et al.,* 2010).

Induction of somatic embryogenesis

After surface sterilization, ray florets were excised from flowers of selected chrysanthemum varieties *i.e*. Poornima Pink, Poornima Red and Poornima White. The ray florets used as explants for induction of somatic embryogenesis were cut horizontally and the basal portion (abaxially) cultured on MSmedia (Murashige and Skoog, 1962) supplemented with various combinations and concentrations of auxin and cytokinins (2,4-D, BAP and Kinetin) with 3% sucrose and 7g l^{-1} agar to know the capacity of somatic embryogenesis. All the cultures were maintained under well defined condition of the culture room maintained at a temperature of 25 ± 2°C. Uniform light intensity (ca 1000 lux) was provided by fluorescent tubes (7200°K) over a light and dark cycle of 16 and 8 hours respectively. After six weeks of culture initiation, number of somatic embryos per explants was observed under compound and stereo electron microscope. After induction of somatic embryos, number of plantlets from somatic embryos were counted and expressed in percentage.

Data analysis

Anovas and Duncan comparison tests for each evaluated factor were carried out using the Statistical Analysis Systempackage (SAS) 8.0 for each of the three cultivars studied.

RESULTS AND DISCUSSION

In this experiment, somatic embryogenesis was induced directly from the ray florets of selected chrysanthemum cultivars Poornima Pink, Poornima Red and Poornima White. When ray florets of selected chrysanthemum cultivars were cultured on the medium containing the plant growth regulators of different concentrations and combinations of auxin and cytokinin i.e 2,4-D, BAP and Kinetin, they become swollen and turned greenish yellow after 4-5 days of culture initiation. Somatic embryos were noticed after 20-25 days mostly in the cut margins of the explants, within 6 weeks of culture initiation different developmental stages of somatic embryos were detected on the explants.

Although somatic embryo development was unsynchronized in nature, at the same time globular stage, heart shaped and early cotyledonary stage were observed. In the present study, it was mainly focused on capacity of somatic embryogenesis, number of somatic embryos per explant in the media with different growth regulator concentration and conversion rate of somatic embryos.

The number of somatic embryos were calculated after 6 weeks of culture with different concentrations of auxin and cytokinins (2,4-D, BAP and Kinetin). The number of somatic embryos was clearly specific to cultivar and concentration of growth regulators in media. Production of number of somatic embryos (17.10 and 17.30 in Poornima Pink and White respectively) were more in the MS media containing 2,4-D alone (4 mg l^{-1}) followed by MS media with 2,4-D 4 mg l^{-1} and BAP 1mg l^{-1} (12.80 and 4.76 in Poornima Pink and White respectively). Chrysanthemum cultivar Poornima Red has more number of somatic embryos (22.60) in the MS media with 2,4-D 4 mg l^{-1} and BAP 2 mg l^{-1} which was on par with MS media with 2,4-D 4 mg l^{-1} and BAP 3 mg l^{-1} (20.40) followed by MS media with 2,4-D 4 mg l^{-1} and Kinetin 1mg/l (16.20) whereas, there was no production of somatic embryos in control (MS media without growth regulators) in all three cultivars of chrysanthemum (Table 1).Similar observations were found byNaing *et al.,* (2013) in chrysanthemum. Mandal and Datta (2005) studied the somatic embryogenesis from ray florets of chrysanthemum and reported that maximum embryogenic response with maximum number of somatic embryos per explant was observed in 4.0 mg dm^{-3} 2,4-D with 2.0 mg dm^{-3} BA. Increase or decrease of the growth regulator concentration reduced the embryogenic frequency as well as the number of somatic embryos.

After induction of somatic embryogenesis, somatic embryos were transferred to MS media without growth regulator and regeneration media which was good for regeneration from callus then, observed the conversion rate of somatic embryos. After transferring somatic embryos on MS media containing 5 mg l^{-1} BAP and 1 mg l^{-1} NAA further it was observed for the development *i.e* germination and conversion. Observed conversion rate of somatic embryos was 72.20 per cent in Poornima pink, 77.60 per cent in Poornima Red and 18.00 per cent in Poornima White (Table 2). There was no conversion of somatic embryos in control (MS media without growth regulators).These results were not similar to previous reports, Naing *et al.* (2013) in chrysanthemum reported that 30% of somatic embryos of chrysanthemum cv. 'Euro' germinated on PGR-free MS medium but in this study there was no conversion or germination of somatic embryos in PGR-free MS medium. The medium containing NAA, converted 40% of somatic embryos into normal shoots (Naing *et al.,* 2013) in chrysanthemum and cytokinins seem to be essential for germination and conversion of somatic embryos in chrysanthemum in the regeneration step (Lema-Ruminska and Niedojadlo, 2014) in chrysanthemum.

CONCLUSION

Efficient regeneration method through somatic embryogenesis for three chrysanthemum (*D. grandiflora*) cultivars: Poornima Pink, Poornima Red and Poornima White were obtained. Acclimatization and transfer to soil of regenerated plants from all the three cultivars were successfully achieved. In Poornima Pink and Poornima White number of somatic embryos were more in the MS medium containing 2,4-D alone (4 mg l^{-1}). In Poornima Red, production of somatic embryos were more in the MS medium with 2,4-D 4 mg l^{-1} and BAP 2 mg l^{-1}.Standardization of growth regulator concentration for induction of somatic embryogenesis with reliable regeneration capacity will be useful for mass propagation and genetic improvement.

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Table 1. Production of somatic embryos from ray florets as influenced by different growth regulators combinations in chrysanthemum varieties

S.No	Treatments	Number of somatic embryos		
		Poornima Pink	Poornima Red	Poornima White
1	Control	0.00	0.00	0.00
2	2,4-D 4 mg/l	17.10	5.33	17.30
3	2,4-D 4 mg/l+ BAP 1 mg/l	12.80	6.77	4.76
4	2,4-D 4 mg/l+ BAP 2 mg/l	11.60	22.60	3.66
5	2,4-D 4 mg/l+ BAP 3 mg/l	4.00	20.40	1.50
6	2,4-D 4 mg/l+ Kinetin 1 mg/l	7.60	16.20	3.33
7	2,4-D 4 mg/l+ Kinetin 2 mg/l	4.00	8.15	4.33
8	2,4-D 4 mg/l+ Kinetin 3 mg/l	12.00	4.33	4.26
S. Em±		**0.80**	**1.02**	**0.72**
C. D. at 1%		**3.28**	**4.23**	**2.97**

Table 2. Conversion rate of somatic embryos (%) in chrysanthemum varieties

S.No	Treatments	Conversion rate of somatic embryos (%)		
		Poornima Pink	Poornima Red	Poornima White
1	Control	0.00 (0.28)	0.00 (0.28)	0.00 (0.28)
2	BAP 5 mg/l + NAA 1 mg/l	72.20 (58.30)	77.60 (61.90)	18.00 (24.60)
S. Em±		**1.62**	**1.33**	**1.76**
C. D. at 1%		**6.58**	**5.39**	**7.17**

Figures in parenthesis indicates arcsin transformed values

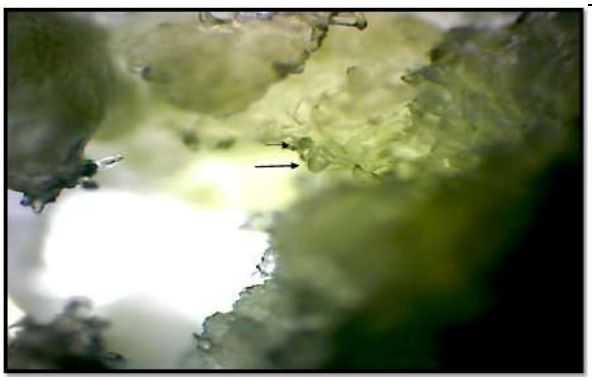

Figure 1. Early stages (globular and heart shape) of somatic embryos in chrysanthemum

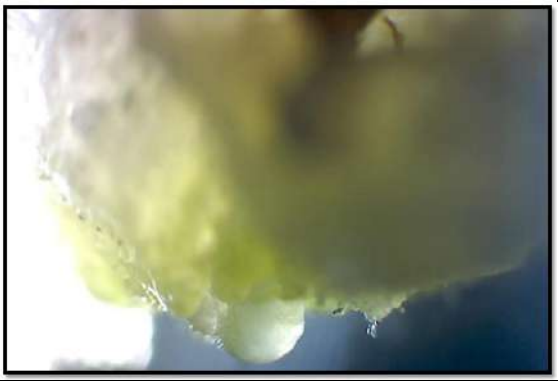

Figure 2. A somatic embryo at torpedo stage

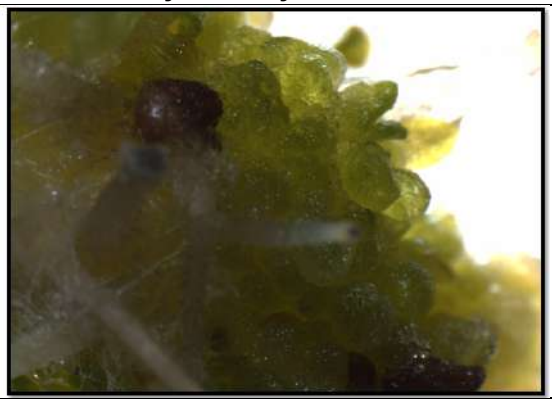

Figure 3. Mature somatic embryos

Figure 4. Initiation of shoot from somatic embryos

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RESEARCH ARTICLE

Analysis of Antioxidant Activity of White and Black Varieties of *Macrotyloma uniflorum* (LAM) Verdc under Nickel Stress.

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ABSTRACT

The present investigation was carried out to anti uretholethic activity of *macotyloma uniflorum*. under nickel stress. The different concertation of nickel was tested with two variety, Black and white with nickel for lethal dose The seeds were grown under nickel stess and harvested after 90 days .Seeds from the nickel stress plants were tested to evaluate the kidney stone dissolving potency. Different parameters such as chlorophyll content, protein and antioxidant activity were studied. It was evidenced black variety was quite significant for the marker character over the white variety

Keywords: *macotyloma uniflorum,* black, white, antiureothilic, chlorophyll content, protein and antioxidant activity.

INTRODUCTION

Macrotyloma uniflorum (Lam) Verdc. belongs to family Fabaceae and is commonly known as "Horsegram." Different parts of the plant (leaf, stem, root and seed) are used in the traditional system of medicine. Moreover the seeds of the plant are having antiurolithiatic activity. Metals in excess stimulate the $_2$production of free radicals and reactive oxygen species (ROS) such as superoxide radical ($O^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O^-$) and hydroxyl radicals ($OH^{\bullet}$) [1,2]. The ROS[2] has a role in lipid peroxidation, membrane damage and consequently in plant senescence. Antioxidant enzymes such as super oxide dismutase (SOD), Peroxidase (POX) and catalase (CAT) are involved in the scavenging of ROS. SOD is a metalloprotein that catalyses the

dismutation of superoxide to H_2O_2 and molecular oxygen.CAT found predominantly in peroxisomes, dismutates H_2O_2 into H_2O and O_2, whereas POX decomposes H_2O_2 by oxidation of co-substrates like phenolic compounds and /or antioxidants [2]. In this context the present investigation was based on the study of Nickel induced alteration in the protein profile and its correlation with the activities of antioxidant enzymes like catalase, superoxide dismutase and guaiacol peroxidase. The anti urolithiatic activity and the active principle of the white and black varieties will be studied under supplementationof various concentration of Nickel [3]

METHODOLOGY AND OBSERVATION

Seeds were collected and exposed to break the dormancy. The seedlings were allowed to grow in pots containing garden soil: cowdung : vermicompost in 1:1:1 ratio till they attained a height of 10 inch-1ft. Healthy plantlets were transferred to pots containing normal soil (control) and soil with different concentration of Nickel (50,100,150,200mg/kg of soil)[4,5].

Collection of seeds

- White and black varieties of seeds along with plant materials were collected from different localities of Odisha.
- Black variety seed particularly named as Gouri were medicinally potent than other other varieties.
- Identification of plant material was carried out in department of Botany, Utkal University

Determination of lethal dose

- Seeds of Macrotyloma were surface sterilised and exposed to different concentration of$NiCl_2.6H_2O$
- Lethal dose was determined in in vitro condition
- Germination was totally retarded at a conc. higher than 500 mg/kg Nickel.
- It was observed that seedlings grown with supplementation of 250 mg/kg Nickel showed the symptoms ofchlorosis and necrosis and completely perished within 10days.

Germination and seedling establishment under nickel stress

- Pots were filled with 10Kg of soil, compost and sand in ratio1:1:1
- Soil in the pot were treated with different conc. of Ni (50,100,150,200 and250mg/kg of soil)
- Pots were taken in triplicate with 20 seeds perpot.
- 20 seeds were sown in each pot
- Germinating seeds were allowed to grow for 21days.
- After 21 days the plant materials were harvested Chlorophyll a, Chlorophyll band Carotenoid content were estimated. Further to reiterate the anti oxidant activity, total protein, stress amino acid (proline) and antioxidativeenzymes like SOD, POD & CAT was estimated [7,8]

RESULTS AND DISCUSSION

Effect of $NiCl_2$ on chlorophyll a,b & carotenoid content

Biosynthesis of chlorophyll in *Macrotyloma uniflorum* increases at lower concentration of Nickel (50 ppm) but significant degradation in chlorophyll was observed with increasing concentration of Nickel. This might be attributed to ultra structure damage of chlorophyll due to excess lipid peroxidation. Replacement of Mg^{+2} of chlorophyll by Ni might be another reason for decrease in chlorophyll content. However enhanced biosynthesis of chlorophyll at lower concentration of $NiCl_2$ might be due to carotenoid which protects other photosynthetic pigments under stress condition.

- Analysis of Chlorophyll b showed a decreasing trend with an increase in concentration .of Nickel. It has been found that toxic effect of Ni was more on Chl. b as compared to Chl. a.

- Biosynthesis of Carotenoid was multiplicated with supplementation of higher concentration of $NiCl_2$(Fig.4).Increase in carotenoid content might be attributed due to increase in Reactive Oxygen Species and Carotenoid has the ability to quench the ROS effectively.

Effect of Nickel on protein & Proline

Protein content of *Macrotyloma* was more with supplementation of $NiCl_2$ at lower conc. and decreased thereafter with increasing conc. of Ni (Fig.5). Enhancement of protein content at lower concentration might be due to increase synthesis of de novo protein & also due to synthesis of antioxidative proteins / aminoacids and enzymes. Decline in protein content might be due to toxic effects of Nickel on protein. Proline accumulation is an important parameter to recognise the stress impact on plants. Correlation coefficient (R^2) value exhibited good linear correlation between different increasing conc. of Nickel & Proline accumulation in 21 days grown *Macrotyloma* seedling. Proline act as a osmoprotectant and maintains water level in the cell and stabilizes the membrane (yoshida et al1995).

Effect of Nickel on Antioxidative Enzymes

There was increased activity of antioxidative enzymes in response to Ni level indicating a strong induction of oxidative stress. Increased SOD activity was observed with lower concentration Ni (50,100mg/kg of soil).This increase in SOD activity is due to over production of superoxide radicals into hydrogen peroxide and oxygen. .Decline in SOD level at higher conc. might be due to over production of hydrogen peroxide that lowers the induction of SOD. A further indication of increased ROS generation due to excess Ni was provided by increase in POD & CAT activities that attacked hydrogen peroxide resulting from SOD activity. Decreased in POD & CAT at higher conc. of Ni might be due to change in assembly of Protein because of metal stress.

CONCLUSIONS

It is concluded that black variety has better efficacy for regulation of antioxidative enzymes as compared to white variety even in high concentration of nickel.

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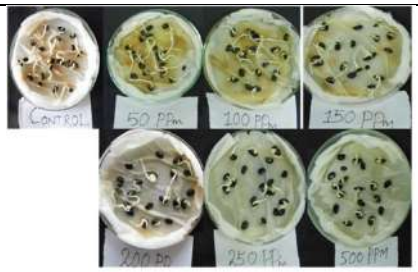
Figure – 1



Determination of lethal dose

Figure – 2

Seedling growth in different conc. Of Nickel after 7 days of treatment

Seedling growth in different conc. Of Nickel after 21 days of treatment

Figure – 3

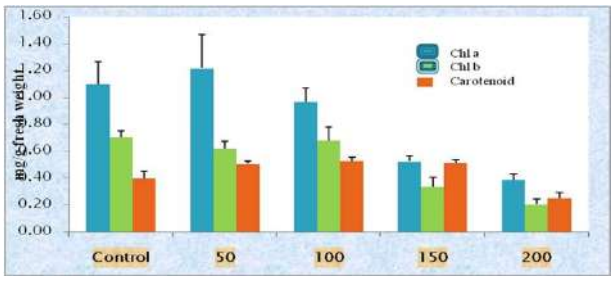


Figure – 4

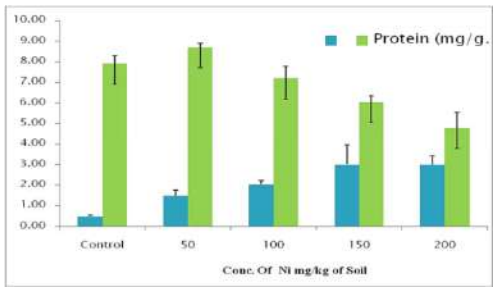


Figure – 5

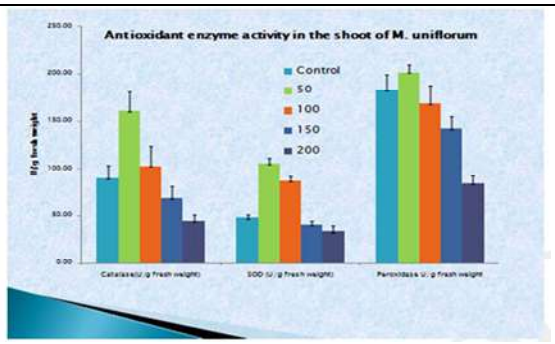

Antioxidant enzyme activity in the shoot of M. uniflorum
Control
50
100
150
200

Figure – 6

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REVIEW ARTICLE

A Review on Ethnomedicinal Plants used for Digestive Disorder

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ABSTRACT

Medicinal plants play a vital role in drug discovery and very useful to cure different ailments in human. Gastrointestinal tract infections are a very common and popular disease in all over the world which causes morbidity and can lead to mortality especially in the developing world where sanitation is deficient. A large part of the human population relies on medicinal plants for treating various disease, including gastrointestinal disorders. This review summarizes the traditional uses of medicinal plants to treat gastro-intestinal tract infections.

Keywords: Disease, Drug discovery, Gastrointestinal tract infections, Medicinal, Treating

INTRODUCTION

Ethnobotany is a rapidly expanding science. In the past nearly three decades it has considerably expanded both in its concept & scope. So, ethnobotany is the study of plants and their uses by the local people. These medicinal plants are very useful to both tribal as well as general people in daily life. Now includes studies likes conservational practices of tribals, ethno-pharmacology, ethnopharmacognosy, ethnomusicology, ethnogynaecology etc. [1]. Prior to the coining the term ethnobotany uses of plant by human beings found place in Sanskrit, Greek and Arabic literature, later systematic compilation works like indo-European Folk tales & Greek legends [2]. The about ethnobotany was proposed during the early 20th century by American botanist Johon William Harshburger. An introduction to ethnobotany (Faulks, 1958) is one of the important books in ethnobotay. It deals with the goods and services obtained from vegetation for food, physical and physiological troubles, influence of man on vegetation by way of destruction, conservation, relationship of vegetation with human civilization. Glimpses of Indian ethnobotany is the first book dealing purely with Indian ethnobotany [3]. It has a complication of articles on field studies in different phyto-geographical areas of India. The book contains tribal uses of more than1500 plants in different parts of our country. Jain [4] and others published a Bibliography of ethnobotany and complied a world dictionary of ethnobotanists [5].The world health organization (WHO) has listed 21,000 plants, which as used for medicinal purposes around the world. Among these, 2500 species are India, out of which 150 spp. are used commercially on a

very large scale. In India indigenous remedies of medicinal plants have been used for treatment of various diseases since the time of Charaka and Sushruta and Raman Sreedharan [6, 7] provided a comprehensive summary of ethnobotanical research carried out in different status and union territories of India until 1991.

GI tract infections

Digestive tract is a 30 feet long tube which starts from mouth and ends at the anus. In between the esophagus, stomach & bowels (intestine).The liver and pancreas aid digestion by producing bile and pancreatic juices which travel to the intestine. Gastrointestinal disorders are aliments affecting the functions of the digestive tract i.e. food and liquid absorption, digestion or excretion [8]. Such disorders are caused by infection by various kinds of bacteria, viruses and parasitic organisms [9]. Functional disorders are those in which the gastrointestinal (GI) tract looks normal but doesn't work properly. The common gastrointestinal disorders are stomach pain, diarrhea, dysentery, gastroenteritis, constipation, vomiting etc. In India, it is reported that traditional healers use 2,500 plants species and 100 plants species serve as regular sources of medicine. Local herbal practitioners in the study area are using a variety of plants species for the treatment of gastrointestinal disorders successfully [10].

Digestive Diseases

Twenty million Americans suffer from chronic digestive diseases. Digestive diseases are one of the most prevalent causes of disability in the work force. Digestive diseases rank third among illness in total economic cost in the United States. Digestive disease represents one of the nation's most serious health problems. In terms of discomfort for treatment, working hours lost and morality [11].

Treatment of GI Tract infections

The treatment of gastric diseases is very common and not affordable by majority of people. There are many common Phyto-medicinal remedies used by the tribes against gastrointestinal disorder. The remedies for stomach troubles have been found to be used against dysentery, blood-dysentery, diarrhea, stomach-ache, jaundice, worms (A.G. Devi Prasad et al., 2013). Proper treatment of digestive disease utilizes host to target the affected suhoshas, agni, amu. Healthy digestion leads to healthy life, when digestion is weak, the tissue of your body such as muscle, blood and nerve become weak and susceptible to disease. There were many herbs, nutrients and plants products that have been found to play a role in protective or helping to heal stomach and peptic ulcers. Except for a few phytogenic compounds limited clinical data are available to support the use of herbs as gastro-protective agents and thus, the data on efficacy and safety are limited. Finally, it should be noted that substances such as flavonoids, ascin, aloe gel and many others that possess anti gastric activity are of particular therapeutic importance as most of the anti-inflammatory drugs used in modern medicine [12]. The literature survey showed that concerted efforts made in documenting the traditional medicinal knowledge along with systematically explored flora of the world during the last two decades have paved the path to acquire more knowledge regarding the use and efficacy of medicinal plants. Available literatures about the concerned topic were through reviewed and following are notable plants showing for treatment of GI tract disease:

Quassia amara (Simaroubaceae)

Plants with a predominant content of bitter substances and a stimulating action of gastric secretion and gastrointestinal motility. Bitter substances are a heterogenous group of chemical compounds with the bitter test as a common feature. Their effect is not only me dieted via the mouth by bitter receptors but they also have a direct effect on stomach, leading to hyperemia of the mucosa, increased gastric secretion and even quicker gastric emptying. Amara may also have additional effects, such as an increase of bile secretion [13, 14].

Cnicusbenedictus (Asteraceae)

C. benedictus is used as an amarum due to its content of bitter sesquiterpene lactones (coincin), besides very small amounts of essential oil, supporting its use in stimulating gastric secretion [13].

***Matricaria chamomilla* (Asteraceae)**

The dried flower heads of this member of the Asteraceae family contain up to 1.5% of essential oil which is blue in colour. The lead constituents chamazulene and (-)-α-bisabilo are involved in the antiphlogistic activity of the oil. Chamomile has both carminative and spasmolytic effect, making it one of the most useful medicinal herbs in acute gasytric diseases. Besides essential oil, the flower heads also contain flavonoids, such as apigenin-7-glycoside,which contribute to the spasmolytic activity and may inhibit peristalsis. Chamomile is therefore used in painful gastrointestinal spasms as well as acute gastritis, ulcer and dyspepsia [15].

***Mentha piperita* (Peppermint)**

Leaves contain essential oil (menthol and menthone) and caffeic acid derivatives, such as rosmarinic acid. Other than chamomile, the spasmolytic activity is much more predominant, while an antiphlogistic effect is lacking. Therefore, the main indications are gastropathic states, including dyspeptic conditions [16].

***Aloe vera* (Lilliaceae)**

Aloe vera juice is used for consumption and relief of digestive issues such as heatburn and irritable bowel syndrome, although it bears significant potential to be toxic when taken orally, it is common practice for cosmetic companies to add sap or other derivatives. Other use for extracts of *Aloe vera* include the dilution of semen for the artificial fertilization of sheep, use as fresh food preservative, and use in water conservation in small farms. About 10 gm fresh leaf taken daily morning orally for 40 days, cures piles [17].

***Atropa belladonna* (Solanaceae)**

Among the alkaloids of this member of the Solanaceae family, hyoscyamine is the most important, next to scopolamine, apoatropine, and belladonine. These compounds are ester alkaloids and pharmacologically very active, for example, hyoscyamine leads to central stimulation. In the digestive organs, belladonna reduces the tone, lowers excitation, and diminishes the gastric and intestinal motilities. Indications in gastrointestinal diseases are spastic constipation, gastralgias, pylorospasm, intestinal spasms, in case of hyperacidic ulcers, the inhibition of gastric secretion is helpful [13].

***Chelidonium majus* (Papaveraceae)**

It contains several alkaloids, such as chelidonine, sanguinarine, and α- and β-homochelidonine, which have pharmacologically well characterized spasmolytic and analgesic actions. The extract has analgesic properties and a good spasmolytic action on smooth muscle, for ex-ample, bronchial smooth muscle and intestine, and stimulates bile secretion. It is used in spastic states of the gastrointestinal tract [18].

***Iberis amara* (Brassicaceae)**

It contains mustard oil glycosides. Mustard oil glycosides are known to be tonicizing and antiphlogistic. Their action is supported by the presence of small amounts of cucurbitacin's, bitter substances well known from the Cucurbitaceae (pumpkin) family. Flavonoids add to the anti-inflammatory action [13].

***Carica papaya* (Caricaceae)**

Papaya has been used for digestion problems. This product should not be used for intestinal parasitic infections because it may be infective. The effects of *Carica papaya* on exogenous ulcer and histamine-induced acid secretion were studied in rats. The latex of the unripen fruit were effective in protecting the exogenous ulcer [12].

***Embilica officinalis* (Euphorbiaceae)**

Indian gooseberry has used as a valuable ingredient of various medicines in India and Middle East from time immemorial. Aperient the green fruits are made into pickles and preserves to stimulate the appetite. Antibacterial, antifungal, antiviral medical studies conducted on Amla fruit suggest that it has antiviral properties and also functions as an antibacterial and antifungal agent. It is the primary ingredient used in one of the renowned Ayruvedic herbal formulae called Chayavanprasha which has great respect as a tonic [19, 20].

***Withinia somnifera* (Solanaceae)**

W. somnifera is considered to be one of the best leaves are used in Ayurvedic and Unani medicines. Ashwagandha root drug find an important place in treatment of rheumatic pain, inflammation of joints, nervous disorders and epilepsy. Dried roots are used as tonic for hiccup, cold, cough, female disorders, as a sedative, in care of senile debility, ulcers etc. Leaves are applied for carbuncles, inflammation and swellings. Bark decoction is taken for asthma and applied locally to bed sores. Ashwagandha and its extracts are used in preparation of herbal tea, powers, tablets and syrups [13, 21].

***Tinospora cordifolia* (Menispermaceae)**

T. codifolia is used in Ayurvedic and herbal medicine as a hepatoprotection, protecting the liver from damage that may occur following exposure to toxins, as well as in Thailand, Philippines [22, 23].

CONCLUSION

Many traditional medicines are now an accepted fact because of better cultural acceptability, better compatibility with the human body, lesser side effect and effectiveness. Some of the plants which have medicinal property are used as food by the tribal's and local community. The efficacy of the traditional medicine cannot be judged properly, although the ethnic tribal people used these plants for curing different types of diseases. Due importance should be provided for further research on these medicinal plants for their effectiveness, side effects(complication), mode of action etc. This study concluded that even though the accessibility of the modern system of medicine for simple and complicated diseases is available, many people in the studied area still continue to depend on medicinal plants, for the treatment of different types of diseases.

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Table 1. Plants used for diseases associated with digestive system [24-30]

Sl.no.	Botanical name	Local name	Family	Parts and mode of use
1	*Abrusprecatorius*	Kanicha	Fabaceae	A few leaves ground with little common dsalt to make a paste and is given with water twice a day for two days, cures stomach pain.
2	*Allium cepa*	Piyaja	Amaryllidaceae	The phytochemicals in onions that scavenge free radicals may also reduce your risk of developing gastric ulcers.
3	*Zingiber officinale*	Aada	Zingiberaceae	People who take ginger regularly in pitta condition or in pitta prakruti may develop pitta related problems. This may to release gastrointestinal diseases such as hyperacidity, hemorrhoids etc.
4	*Aegle marmelos*	Bela	Rutaceae	The dried bark along with curd drinking cures piles, The leaf juce drinking cures vomiting.

5	*Ageratum conyzoides*	Pokosunga	Asteraceae	The intake of leaf juice daily to reduce acidity.
6	*Aloe vera*	Lolesara	Liliaceae	About 10 gm fresh leaf gel taken daily morning orally for 40 days, cures piles.
7	*Calotopisprocera*	Arakha	Asclepiadaceae	Few tender vegetative buds ground with salt and pepper, made into small tablets. daily 2 tablets are given for a week, cures stomach pain.
8	*Piper longum*	Pippali	Piperaceae	The Indian long pepper is used to improve appetite and digestion, as well as treat stomachache.
9	*Terminalia chebula*	Harida	Combretaceae	About ten gm of fruit powder is taken with hot water daily 2 times for dysentery until cured.
10	*Centella asiatica*	Thalkudi	Apiaceae	The whole plant is grineded and mixed with the grinded tuber of Amorphophallus panifoliusthe leaves of Allophylus serratus the leaves of Allophylus serratus the leaves of *Clerodendrum serratulum* the heart wood of peenari and the fruits of Vallikarmoosa and applid to treat piles.
11	*Litseamonopetala*	Meda	Lauraceae	Bark decoction is taken orally 2-3 times daily in the treatment of diarrhea
12	*Heyneatrijkuga*	Komalisiuli	Meliaceae	Leaf decoration is taken orally 2-3 times daily in the treatment of cholera.
13	*Psidium guajava*	Dalimba	Myrtaceae	Few leaves ground to get extract and taken buttermilk twice a for a weak cure piles.
14	*Moringa oleifera*	Sajana	Moringaceae	One spoon leaf extract mixed with one spoon honey given with tender coconut water for dysentery until cured.
15	*Juniperus indica*	Dhupi	Cupressaceae	The leaf and bark of this tree very helpful for treating abdominal pain appetizer carminative diarrhea etc.
16	*Zizyphusmauritiana*	Bara koli	Rhamnaceae	Traditionally the plant parts are used as sedative abdominal pain constipation indigestion etc.
17	*Ocimum sanctum*	Tulsi	Lamiaceae	Improper and excessive use may aggravate pitta causing pitta and blood-related disorders and its leaf is very helpful for stomach disorders.
18	*Cuscutareflexa*	Kolanirmuli	Convolvulaceae	The juice kills intestinal worms. The leaf and root grind together and the paste is applied on the swelled
19	*Holarrhenaantidyse nterica*	Kurchi	Apocynaceae	Plant powder or decoration is taken 2-3 times daily for the treatment of acute and chronic diarrhea and dysentery.

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RESEARCH ARTICLE

Application of Data Processing and Decision Support System for Identifying Best Management Practices

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ABSTRACT

The aim of this study is to appraise the application of multiple criteria decision making (MCDM) methods in selection of tillage operations, doses of crop residues and inorganic fertilizer combinations suitable for conservation agriculture (CA). MCDM is one of the important tools for making precise decision out of many alternatives for the given criteria. Technique for order preference by similarity to ideal solution (TOPSIS) is an MCDM method based on choosing the best alternatives having the shortest distance to the ideal solution and furthest distance from the negative ideal solution. Therefore, the objective of this study is the application of TOPSIS method to identify the best management practice of wheat crop under conservation agriculture system. TOPSIS ranking showed that conventional tillage with certain organic residues and inorganic fertilizers dose performing better compare to zero and reduce tillage for those particular set of criteria.

Keywords: Conservation agriculture, Decision Support System, Multiple Criteria Decision Making, TOPSIS, Wheat

INTRODUCTION

Conservation agriculture (CA) necessitates minimum disturbance of soil, retention of crop residues and judicial crop rotations that help in conserving natural resources, increasing productivity and curving global warming even while in practice in small-holder farming systems. Conservation agriculture recovers soil health, system resilience and sustainability (Connor *et al*., 2003) and increases yield in the long run, (Gathala *et al*., 201; Hobbs,2007) although the yield potential is widely disputed (Pittelkow *et al*., 2014). Large scale adoption surveys in India reported lower production cost, higher net returns and benefit cost ratio (BCR), low water, labour and energy requirements for zero

tilled compared to conventionally tilled wheat as a result farmer are motivated by more than only yield (Saharawat *et al*., 2010). In the contrary some studies also mentioned lower wheat yield under zero tillage than conventional tillage (Tripathi *et al*., 2003). Hobbs and Gire (1997) reported that reduced and zero tillage may be a good option for establishing of wheat followed by rice in south Asia.

Under the above circumstances there are multiple management alternatives available for the betterment of soil health and ecofriendly environment. Based on the given soil and yield attributing criteria we judged the best alternative (s). Therefore, the concept of decision support system (DSS) tool like MCDM (multiple criteria for decision making) comes into play to choose the best alternative which is far from the worst but closest to the ideal one. MCDM is a part of operational research tool for supporting the subject evaluation of performance criteria by decision makers. Anton *et al*., (2012) used continuous multi-criteria methods for crop and soil conservation planning. There are many reported methods available to compute MCDM problems out of those technique for order performance by similarity to ideal solution (TOPSIS) is one of the major methods (Hwang and Yoon, 1981; Jiang *et al.,* 2010). TOPSIS considers both nearest distance from the positive ideal solutions (PIS) and the furthest distance from the negative ideal solution (NIS), and therefore preference is given to their relative closeness combining two distance measures (Hwang and Yoon, 1981).

Davarpanah *et al.*(2016), reported the application of TOPSIS methods for comparison of sustainable agriculture approach. Liu and Zhang (2013) evaluated the comprehensive assessment of economic, social and environmental factors in many agricultural regions of China through TOPSIS method. This method combines both quantitative attributes (like price, time, distance, and so on) and qualitative attributes such as reliability, quality of relationship etc. The ideal solution is originated from information contained in the criteria of the available alternatives. But there should have a certain assurance that the available alternatives are in suitable condition. That is why TOPSIS method should only use when the selection of one alternative out of many, regardless of suitability of desired alternatives (Toloei and Kalantari, 2011). In present study TOPSIS methods have been applied for selection of best alternative of tillage operation of wheat from the multiple choices. Three different tillage alternatives viz., conventional, zero and reduced are considered for decision making on the basis of the soil and yield parameters (criteria). Combined alternatives of tillage operations with each ratio of organic residues and inorganic fertilizers are also examined to find out the best combination by following the same TOPSIS process.

MATERIAL AND METHODS

Experiment Detailed

Crops Name: Wheat (DBW -38)
Wheat botanical name: *Triticum aestivam*
Season: 2018-2019
Experimental Site: Balindi Teaching and Research Complex, Bidhan Chandra Krishi Viswavidyalaya

Treatment's Detail

Tillage: Three kinds of tillage have been used as main plot (Table1.)
Organic residues and inorganic fertilizers: Each tillage has been divided into five different degree of nutrient fertilizer doses (Table 2.)

Database (alternatives and criteria)

Two different datasets were created from three different tillage (three alternatives; Table 1.) operations with each ratio of organic residues and inorganic fertilizers (fifteen alternatives). For each set of alternative eleven criteria have been taken for decision making. Nine soil parameters and two yield parameters have been taken purposefully for studying TOPSIS method (Table 3.). All criteria are belonging to benefit criteria. Any MCDM problem can be

considered as the rating of each alternative in respect of each criterion and the weights should be given to all criteria (Rahman *et al*., 2008). Apart from this, in MCDM structural process, an important element is to define all the criteria (Scheubreina and Ziontsb, 2006). Therefore, in this study all criteria and alternatives are discussed below-

TOPSIS procedure

If an experiment having K alternatives S_1, S_2… S_K. and there are N criteria to assess each alternativeS_k, which are denoted as C_1, C_2… C_N(Table 4.).The kth alternative's value on the n the criteria is obtained as X_{kn}.Which can be written as $S_k = (x_{k1}, x_{k2}, \ldots . x_{kN})andC_n = (x_{1n}, x_{2n}, \ldots . x_{nk} .. x_{Kn})$; k= 1, 2… K and n= 1, 2,… N. (Pavic and Novoselac, 2013)

Structure of initial data Matrix

In TOPSIS method the initial data structure and table consisting the alternatives which are placed as row wise and the criterion (parameters) arranged as column wise are shown in the below table 4.

The given number x_{kn} and their matrix

And their respective matrix

$$(\mathrm{X_{kn}})_{\mathrm{K\times N}} = \begin{pmatrix} \mathrm{X_{11}} & \cdots & \mathrm{X_{1N}} \\ \vdots & \mathrm{X_{kn}} & \vdots \\ \mathrm{X_{K1}} & \cdots & \mathrm{X_{KN}} \end{pmatrix}$$

Step-1 Calculate Normalized Matrix

The nth criteria vector C_n then normalized as TC_n, where

$$\mathbf{TC_n} = \frac{\boldsymbol{C_n}}{|\boldsymbol{C_n}|}$$

$$= (\mathbf{x_{1n}}/|\boldsymbol{C_n}|, \mathbf{x_{2n}}/|\boldsymbol{C_n}| \ldots \mathbf{x_{kn}}/|\boldsymbol{C_n}|) \quad (1)$$

n =1, 2, 3…N and k= 1, 2…. K. Where $|\boldsymbol{C_n}| = \sqrt{\sum_{k=1}^{K}(\boldsymbol{x_{kn}})^2}$is the Euclidian length or norm of $\boldsymbol{C_n}$ **,** so the new criteria vectors have the same length and thus unit free and directly comparable.

Step2: Calculating weight (By entropy theory)

Most of the time for TOPSIS problem weight is given but for this study we need to calculate weight. The concept of weight comes from the entropy theory. It is a criterion for the amount of information or uncertainty represented by a discrete probability distribution, $p_1, p_2, \ldots p_k$(Shanon and Weaver, 1947) as $E(p_1, p_2, \ldots p_k) = -\emptyset_k \sum_{k=1}^{K} p_k \ln(p_k)$, where $\emptyset_k = \frac{1}{\ln(p_k)}$ is a positive constant ranges from 0 to 1. It needs to assume that $p_{kn} = \frac{x_{kn}}{X_n}$, where $X_n = x_{k1} + x_{k2} + \cdots + x_{kN}$ as the probability distribution of C_n as

$$E(C_n) = -\emptyset_k \sum_{k=1}^{K} p_k \ln(p_k)$$

$= -\emptyset_k \sum_{k=1}^{K} \left(\frac{x_{kn}}{X_n}\right) \ln\left(\frac{x_{kn}}{X_n}\right)$, n=1, 2, 3…N

Define the weights as

$W_n = \frac{(1-E(C_n))}{\sum_{n=1}^{N}(1-E(C_n))}$n=1, 2, 3… N (2)

And $\sum_{n=1}^{N} W_n = 1$

LI *et al*. reported that the entropy weight represents useful information of the criteria. Thereby, the bigger the entropy weight of the criteria is, the more useful information of the index is and vice versa.

Step 3: Determination of Weighted decision matrix

Now the columns of the normalized decision matrix are multiplied by the associated weights,W_n, obtained in Eq. (2). And the weighted and normalized decision matrix is obtained by

V_{kn} = $TC_n W_n$ n=1, 2, 3…N; k= 1, 2... K (3)

Step 4. Determination of ideal solution

The positive ideal solution is composed of the optimal value of every attribute from the weighted decision matrix (4) and the negative ideal is composed of worst value of every ideal solution from the weighted decision matrix (LI *et al*. 2011), shown by (5).

$S^+ = (S_1^+, S_2^+, \ldots\ldots S_K^+)$ (4)

$S^- = (S_1^-, S_2^-, \ldots\ldots S_K^-)$ (5)

Now the ideal value and negative ideal value are determined by

$$S_k^+ = \{Max\, V_{kn} \;\; the\, benefit\, criteria\, or\, Min\, of \;\; V_{kn}\, the\, cost\, criteria\}$$
$$S_k^- = \{Max\, V_{kn} \;\; the\, cost\, criteria\, or\, Min\, of \;\; V_{kn}\, the\, benefit\, criteria\}$$

Step 4.Calculation of the distance every feasible solution from the ideal solution and negative ideal solution

$$S_k^+ = \sqrt{\sum_{n=1}^{N}(V_{kn} - S_k^+)^2} \quad (6)$$

$$S_k^- = \sqrt{\sum_{n=1}^{N}(V_{kn} - S_k^-)^2} \quad (7)$$

n=1, 2, 3…N; k= 1, 2... K

Step 5. Calculation of relative degree of approximation

Now the relative closeness to the ideal solution is determined by the following Eq.

$C_k = \frac{S_k^-}{(S_k^+ + S_k^-)}$, $(0 \le C_k \le 1; k = 1,2, \ldots . K)$ (8)

Therefore, higher the values of C_k , the better the rank is.

RESULT AND DISCUSSION

For the TOPSIS method application, two normalized decision matrices shown in Table no.5 and Table-8 using equation (1). And then these normalized decision matrixes were multiplied by the criteria weights using Eq. (2). The associated weights shown in Table 6 and Table 9. After that the distance from the ideal (S^+) and negative ideal solutions (S^-) are calculated by Eq. no (6) and (7), using equation (8) the score of relative closeness(C_k) to the ideal solution are measured from those distance and finally ranked them which are tabled in Table.7 and Table 10.

Decision making on tillage performance

Out of three alternatives reduced tillage (S3) is nearest to the negative ideal solution (0.090) and furthest from the positive ideal solution (0.166) and produced lowest score (0.350). In contrast, conventional tillage (S1) is nearest to the ideal solution (0.075) and furthest to the negative ideal solution (0.166). Therefore, it is high scoring (0.690) alternative and coming as the best alternative followed by zero tillage (Table.7). Bilalis *et al*., 2011 reported that wheat crop performs better under conservation tillage (Zero and reduced). In our study initial data was considered, therefore effect of previous tillage is still present. As a result, conventional tillage performed relatively better than conservation tillage.

Decision making on interaction of Tillage with combination crop residue and inorganic fertilizer

There were fifteen combinations of tillage (Table.10) with combination of crop residue and inorganic fertilizer out of these first alternative (Conventional tillage with 0% Residue +100% NPK) ranked first having scored .696 as it is very much close (0.07) to ideal solution and furthest (0.15) from the negative solution. Followed by alternative no. four (Conventional tillage with 50% Residues+75% NPK), seven (Zero tillage with 100% Residue+50% NPK), six (Zero

tillage with 0% Residue +100% NPK), four (Conventional with 50% Residues+100% NPK), ten (Zero tillage with 50% Residues+75% NPK) so on. Pittelkow *et al*., (2015a) investigated that zero tillage or no till produces low yield if residues are retained; for the current study TOPSIS method also created first rank to the conventional tillage with 0% Residue +100% NPK combination. Second rank alternative i.e., conventional tillage with 50% Residues+75% NPK also supporting the Pittelkow *et al*., (2015a) study. For the tillage zero and reduced with various combination of crop residue and inorganic fertilizer got similar rank, which may be due to the initial data.

CONCLUSION

In conservation agriculture, there are many parameters available for identifying best alternative. MCDM is such a decision support tool which makes best decision through multiple criteria extraction. This experiment identified conventional tillage with the combination of 0% organic residue and 100% NPK dose performed better but for having an impact of conservation tillage the experiment needs to go on for a longer year. This study applied TOPSIS method to solve this multicriteria problem on wheat crop under CA. The upfront advantage of this method is easy, normal and comprehensible. Result obtained from this study witnessed that TOPSIS could be used in decision making process for identifying the best tillage-based alternative for wheat crop under CA practices for screening the alternatives for the consecutive years.

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Table 1: Different tillage

Sl. No.	Tillage
1	Conventional tillage
2	Zero tillage
3	Reduced tillage

Table 2: List residue (rice crop residues) and Fertilizer dose

Sl. No.	Ratio of organic residue and inorganic fertilizer
1	0% Residue +100% NPK
2	100% Residue+50% NPK
3	100% Residue +75% NPK
4	50% Residues+100% NPK
5	50% Residues+75% NPK

Table 3: Criteria from soil and Yield parameters

Sl. No.	Criteria
Soil parameters	pH, Organic Carbon (OC%), Nitrogen (N), Phosphorus(P), Potassium (K), Zinc (Zn), Copper (Cu),Iron (Fe) and Manganese (Mn) Measuring unit: Kg per ha
Yield parameters	Plant height at harvesting (Cm), Yield (Kg per ha)

Table 4. Decision matrix

Criterion/Alternatives	C_1	C_2	…	C_n	…	C_N
S_1	x_{11}	x_{12}	…	x_{1n}	…	x_{1N}
S_2	x_{21}	x_{22}	…	x_{2n}	…	x_{2N}
…	…	…	…		…	…
S_k	x_{k1}	x_{k2}	…	x_{kn}	…	x_{kN}
…	…	…	…		…	…
S_K	x_{K1}	x_{K2}	…	x_{Kn}	…	x_{KN}

Table 5. Normalized decision matrix for three tillage alternatives

Normalized matrix	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
A1	0.58	0.56	0.60	0.57	0.59	0.57	0.56	0.77	0.65	0.56	0.44
A2	0.58	0.58	0.55	0.58	0.59	0.64	0.46	0.57	0.64	0.58	0.64
A3	0.57	0.59	0.58	0.58	0.55	0.52	0.69	0.29	0.40	0.58	0.63

Table 6. Weights of eleven criteria for tillage alternatives

Alternatives	Criteria										
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Weight(W1)	0.091	0.091	0.091	0.091	0.091	0.091	0.090	0.089	0.090	0.091	0.091

Tabled. 7S^+, S^- and C_k for tillage

Tillage	Alternatives (Si)	Distance from ideal solution (S^+)	Distance from negative ideal solution(S^-)	Score (C_k)	Rank
Conventional	S1	0.075	0.166	0.690	1
Zero	S2	0.091	0.133	0.594	2
Reduced	S3	0.166	0.090	0.350	3

Table 8. Normalized decision matrix for tillage and different dose of crop residue and fertilizers

Normalized matrix	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
A1	0.25	0.24	0.26	0.26	0.28	0.20	0.26	0.18	0.25	0.25	0.30
A2	0.25	0.23	0.22	0.25	0.25	0.23	0.23	0.31	0.30	0.23	0.20
A3	0.26	0.32	0.29	0.24	0.25	0.20	0.36	0.18	0.19	0.27	0.27
A4	0.26	0.24	0.23	0.23	0.27	0.25	0.20	0.35	0.32	0.26	0.21
A5	0.26	0.26	0.28	0.23	0.25	0.29	0.22	0.25	0.21	0.26	0.27
A6	0.26	0.23	0.27	0.25	0.23	0.18	0.39	0.10	0.15	0.27	0.28
A7	0.26	0.31	0.25	0.26	0.29	0.26	0.18	0.31	0.29	0.27	0.26
A8	0.26	0.27	0.36	0.24	0.28	0.31	0.39	0.40	0.34	0.24	0.13
A9	0.26	0.24	0.22	0.30	0.26	0.39	0.25	0.11	0.16	0.26	0.29
A10	0.26	0.23	0.24	0.26	0.29	0.27	0.23	0.27	0.33	0.26	0.31
A11	0.26	0.23	0.26	0.31	0.28	0.23	0.19	0.27	0.16	0.26	0.17
A12	0.26	0.22	0.22	0.31	0.25	0.30	0.17	0.24	0.24	0.26	0.28
A13	0.26	0.28	0.24	0.24	0.23	0.27	0.20	0.19	0.34	0.26	0.31
A14	0.26	0.28	0.25	0.24	0.23	0.23	0.22	0.35	0.32	0.26	0.27
A15	0.26	0.29	0.24	0.25	0.22	0.18	0.24	0.08	0.13	0.25	0.26

Table 9: Weights of eleven criteria for combination tillage and residues and inorganic doses

Alternatives	Criteria										
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Weights (W2)	0.091	0.091	0.091	0.091	0.091	0.091	0.091	0.090	0.090	0.091	0.091

Table. 10: S^+, S^- and C_k for tillage and residue and fertilizer combination

Tillage and fertilizer residue combination	Alternatives (Si)	Distance from ideal solution (S^+)	Distance from negative ideal solution(S^-)	Relative Score (C_k)	Rank
T1NR1	S1	0.07	0.15	0.696	1
T1NR2	S2	0.10	0.09	0.486	7
T1NR3	S3	0.12	0.07	0.376	14
T1NR4	S4	0.10	0.11	0.529	5
T1NR5	S5	0.09	0.11	0.566	2
T2NR1	S6	0.09	0.11	0.537	4
T2NR2	S7	0.09	0.11	0.556	3
T2NR3	S8	0.11	0.09	0.453	10
T2NR4	S9	0.10	0.08	0.469	8
T2NR5	S10	0.10	0.10	0.489	6
T3NR1	S11	0.11	0.09	0.457	9
T3NR2	S12	0.11	0.08	0.417	12
T3NR3	S13	0.12	0.09	0.419	11
T3NR4	S14	0.13	0.08	0.388	13
T3NR5	S15	0.15	0.05	0.260	15

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REVIEW ARTICLE

Oil Cakes and their Utilization in Aqua Feed

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ABSTRACT

Being rich in protein, oil cakes are utilized as protein ingredient in aqua feeds. However edible oil seeds have competition from the human food industry as it is considered ideal for food supplementation. With increasing emphasis on cost reduction of aqua feed, utilization of non-edible oil seeds cakes also finds its way forward. The nutritional composition and inclusion levels of oil cakes have been studied and compared by many researchers. The antinutrients such as phytate, tannin, lectin, saponin, oxalates etc. and their amelioration techniques like as soaking in water, steam cooking, roasting, autoclaving, solvent extraction were analyzed and documented. The information on nutrient profiling and inclusion level are very beneficial for feed formulators so that they can incorporate locally available low-cost oil cake in to the feed formulation. This mini review on oil cake nutritional quality will give a brief account on the availability and nutrient content of the oil cakes.

Keywords: Oil cake, Anti-nutritional factors, Amelioration techniques, Proximate composition

INTRODUCTION

There has been a gradual increase in the per capita consumption of fish from about 9kg per annum in 1960s to 20.5 kg per annum in 2018(FAO, 2018).The increasing global demand of fish has consequently hiked the culture of fishes in aquaculture which has led to heavy demand of fish feed for their culture. Known as a sub sector of animal husbandry, aquaculture represents itself as the largest utilizer of the fish meal for feed preparation (FAO, 2011). Feed is the major expenditure of aquaculture and protein content in it is a much-demanded nutrient for the culture of healthy fishes (Hossain *et.al*, 2018). Hence fish meal was introduced as an important feed ingredient for the

preparation of fish feed. It is produced by subjecting wet fish to various reduction processes where cooking, press-drying and milling of the fish is done. These reduction processes have led to its price inflation. Moreover, the dependency on fish meal has driven the world towards the concern of the sustainability of the fisheries which were utilized majorly for the fishmeal production and not directly for human consumptions (Naylor *et al,* 2000). Its richness in protein along with-it production processes raised its price which instead has hiked the price of the fish feed that is Rs30/Kg approx. in India and still varies regionally.

In order to sustain the fisheries utilized for the fish meal production alongside developing a protein rich fish feed at a minimal cost, the aquaculturist search out for certain alternatives that could be cheaper and could be plentifully available. One such alternative is plant sources. Plant ingredients such as wheat bran, wheat flour, rice bran, lentil bran, and molasses are good sources nutrients in feed. However, the major plant ingredients are obtained from the seeds of the plant and are placed under the category named as oil cakes. Oil cakes are the by-products of the edible oil industries which are available at a cheaper rate in the market. Oil cakes are the byproducts after removing the oil from the seeds. Some of the oil cakes available and utilized in the aquaculture are groundnut, mustard, sesame, coconut, flax seed, sunflower oil cakes. Oil cakes as compared to other plant ingredients are rich in protein content as well as essential amino acids such as arginine and methionine which are absent in case of soybean meal and other plant sources. To formulate diets including the oil cakes as a feed ingredient various researches and reviews were carried out. Proximate analysis of various oil cakes conducted was compared with the dietary requirements of the fishes to enquire if the oil cakes could be successfully replaced with the fish meal. A few conclusions of these researches carried out are summarized in this article.

Inclusion of oil cakes in feed

Anti-Nutritional Factors (ANFs) in Oil cakes

Most of the plant ingredients mostly oil cake consists of some Anti-Nutritional Factors (ANF). These ANFs are the anti-metabolites which are naturally present in the oil cakes and other plant ingredients and act as a survival factor in them but have a deteriorating health effect on aquatic and terrestrial organisms (Hajra*et al*, 2013). Oil cakes such as the sunflower oil cakes consists of protease inhibitors, saponins, arginase inhibitors, mustard oil cakes consists of tannins and glucosinolates, cotton seed oil cakes are rich in gossypol which are the anti-nutritional factors (Hajra *et al*,2013). Sesame oil cake also consists of phytic acid that is harmful for the aquatic organisms (Francis *et al,* 2001). Amongst the above factors protease inhibitors, tannins affect the protein utilization and digestion whereas the gossypol and glucosinolates affect the utilization of minerals (Francis *et al*, 2001). Moreover, tannin also diminishes the absorption of vitamin B12 and affects the kidney or liver (Hajra *et al,*2013). Hence, it is important to reduce the toxicity of the oil cakes before their inclusion in feed preparation. A few anti nutritional factors along with their harmful effects have been mentioned in the following Table 1.

Treatments before the inclusion of oil cakes

There are various procedures followed to reduce the ANF properties in oil cakes.

Soaking: It is considered as a traditional method as well as the easiest techniques for reduction of the anti-metabolites. The deoiled Sal (*Shorearobusta*) seed meals were soaked in water for about 16 hr in room temperature which reduced the toxicity of tannin from 34g/kg^{-1} to 7g/kg^{-1}present in it (Mukopadhyay& Ray, 1996). Also, the oil cakes such as groundnut, sesame, mustard, sunflower and canola before being incorporated in the feed of *Cirrhinus mrigala* were also soaked in water for about 24 hrs which resulted in the reduction of ANF properties in all except for the canola (Singh *et al,* 2002). The technique however is also said to eradicate many valuable nutrients in the soaking medium. This indicates that soaking the cakes in solvent is not enough to reduce the ANF properties.

Thermal treatments: The anti-metabolites are categorized as heat liable and heat stable factors. Some of them can be only destroyed when the ingredients (eg- canola, soybean etc.) are subjected to high temperature extrusion processing (Hajra *et al*,2013). Thermal treatments are a common alternative for the reduction processes. Reportedly,

the oil cakes such as the groundnut, sunflower, mustard, sesame and soybean which is rich in ANFs like protease inhibitor, tannin, phytates can be roasted, autoclaved or subjected to high temperatures certainly 80°-100°C to deteriorate the ANF properties or they can be steam cooked for a certain time period mostly 30mins to decrease the toxicity of the cakes (Hajra*et al*, 2013; Mohanta et al, 2007; Srivastava & Reddy, 2018).

Elemental supplementation: However, oil cakes such as the cottonseed oil cake is neither subjected to heat treatments nor soaked in water. It is subjected to iron supplementation which detoxifies the oil cake by destroying the gossypol content in it (Meric*et al*, 2010). The negative effects of anti-metabolites such as phytates or phytic acid can also be reduced or neutralized by incorporation of zinc and enzyme phytase in it.

Feed formulation and inclusion levels

The proximal analysis of all the oil cakes must be done before the feed formulation. The analysis protocol should be followed according to the AOAC (Association of Official Analytical Chemists) methods. The moisture is determined by subjecting the samples under high heat in the hot air oven. The crude protein estimation is conducted with the help of Kjeldahl's apparatus, the crude lipid with the help of soxhlet apparatus, the crude ash using the muffle furnace. The remaining are the carbohydrates. Despite being high in protein content some oil cakes are not included as a feed ingredient. These are non-edible oil cakes such as the neem oil cake, castor oil cake. Proximal compositions of a few edible and non-edible oil cakes have been presented in Table 2. The feed formulated must be an iso-nitrogenous, iso lipidic and iso-caloric diet. Feed with high protein content including essential amino acids must be prepared. Although being high in protein content the oil cakes are deficient with few essential amino acids, so they must not be utilized as a single ingredient in feed. When compared to the fish meal sunflower oil cake has a less amount of lysine and threonine in it (Hossain *et al,* 2018**)**. Even the groundnut oil cake being a good source of arginine is deficient in lysine, cystine and methionine (Srivastava & Reddy, 2018). Fish meal must be incorporated in feed due to its richness in essential amino acids and due to its functioning as a fish attractant. Plant sources such as rice bran, wheat must be included in feed to balance the carbohydrate levels. Moreover, wheat acts as a binder. At times multiple oil cakes are included in a single diet to balance the protein as well as the amino acid content in the feed.

The inclusion levels vary in different oil cake feeds. This variation may be present due to the deficiency of particular amino acids in them or may be due to the effect of the reduced ANFs on different fish species. The highest satisfactory inclusion level of groundnut oil cake is 40% (w/w) for the both *Labeorohita* fingerlings and also for the tilapia (Srivastava & Reddy, 2018; Jackson et al, 1982; Ghosh & Mandal,2015). However, the sesame oil cakes being rich in metheonine show an inclusion level of 50% in Rohu and an inclusion level of 25%-50% as an moderately accepted diet in common carp (Hasan *et al*,. 1997; Mohanta *et al*,. 2007). Non edible oil cakes such as the Neem oil cake once treated can be incorporated in aquatic feeds, up to a maximum inclusion level of 25% in Common carp without affecting its growth.(Daniel, 2016 ;Nandeesha, 1993). Research works showed variability in the proximate composition of oil cakes. High amounts of ANFs present in the oil cakes along with the deficiency of certain essential amino acids in it decrease the protein content in the feed, thus decreasing the inclusion levels. Deficiency of amino acid even affect the growth and survivability of the fish species. In such cases suitable methods must be followed to treat the anti metabolites or ANFs to reduce their toxicity level thus uplifting the protein content in them. In order to overcome the deficiency of amino acids in feed, partial incorporation of ingredients with high protein and amino acid content such as the fish meal, must be done. It is suggested that, oil cakes must not be used as a single ingredient in the preparation of fish feed.

CONCLUSION

Voluminous studies on the incorporation of animal ingredients as well as plant ingredients have been carried out in fish feed. Informations like proximate composition of oil cakes, presence of antinutritional factors, and their removal are very much crucial for fish feed industry. The use of oilcakes must also be tested not only for the reduction of

toxicity level and to test for the supplementation of particular amino acids that could lift the inclusion levels of the oilcakes in the fish feed, thus improving the feed uptake, growth and survivability of fishes. The oilcakes are locally and cheaply available ingredients which can be afforded by the farmers. If studied thoroughly this could help the farmers develop their own feed without the use of costly machinery that could improve their livelihood.

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Table 1: Anti-nutritional Factors Found in Oil cakes

ANTI-NUTRITIONAL FACTORS	HARMFUL EFFECTS
Protease inhibitors	• Inhibition of proteolytic activities of some enzymes (trypsin, chymotripsin).
Glucosinolates,	• Thyroid dysfunction. • Decreased feed intake, feed utilization, metamorphosis and maturation. • Decreased metabolism and growth.
Phytates	• Leads to insufficiency of phosphorus in body. • Diminishes the bioavailability of zinc leading to bilateral cataract, in feed efficiency, reduction of growth and proper thyroid function.
Saponins	• Damage of respiratory epithelium in gills. • Death of a fish.
Tannins	• Altered digestive processes. • Toxicity to kidney and liver • Reduction of absorption of vitamin B12.
Oxalates	• Deficiency of calcium
Gossypol	• Growth depression, internal organic abnormalities • Deficiency of amino acid methionine and lysine.

Table 2: Proximal composition of various oilcakes.

Oil Cakes		Moisture(%)	Lipid(%)	Protein(%)	Ash(%)	Carbohydrate(%)	References
Edible oil cakes	Sunflower oilcake	11.53	14.63	29.48	5.65	38.71	Hossain et.al, 2018.
	Groundnut oilcake	8.22	7.7	41.73	5.76	36.6	Ghosh et.al, 2015.
	Mustard oilcake	16.90	10.83	29.81	6.60	35.86	Hossain et.al, 2018.
	Copra oilcake	8.4	11.4	20.3	6.2	53.7	Nandeesha, 1993.
	Flax seed oilcake	8.30	43.90	21.34	2.66	23.8	Gutiérrez et.al, 2010.
	Sesame oil cake	6.9	9.2	35.5	9.6	38.8	Mohanta et.al (2007)
	Rapseed oilcake	11	0.9	35.9	6.9	45.3	Nandeesha, 1993.
	Soybean oil cake	15.58	8.40	43.24	5.35	27.43	Hossain et.al, 2018.
	Cottonseed oilcake	3.23±0.23	3.22±0.77	26.02±0.10	6.93±0.71	60.37	Jibrin et.al, 2020.
Non edible oil cakes	Jatropha oilcake	19.3	13.18	37.82	4.68	25.02	Belewu et.al 2010.
	Neem seed cake	10.12	5.11	31.81	8.26	44.7	Daniel, 2016.
	Castor bean cake	9.34	11.15	38.58	5.87	34.97	Odunsi et al, 2012.
	Karanj oil cake	9.42	18.43	28.99	4.38	38.78	Gopan et al.,2020

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REVIEW ARTICLE

Archetype for the Analysis of Brain Tumours, Present Techniques and Future Prospects: A Systematic Review

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ABSTRACT

Brain is known to be the most vital and critical organ of the human body, and to study and analyse the complex system of neuron network of the same is not only cumbersome but also a strenuous task. The detection and diagnosis of brain tumors is not at all easy and the manually ways of detection make the process even more complex. The identification, analysis and segmentation of the brain tumors requires high level of efficiency and preciseness as even a minor error would possess a threat to life. So, to overcome this lacking, several automated methods have been devised for afore said purpose. The automatic technologies like the Magnetic Resonance Imaging (MRI), Convolutional Neural Network (CNN), deep learning neural network are a few of the many machine learning methods which have been discussed in this paper of review. The methods along with their applications in the field of detection, 2D and 3D classification, analysis and treatment have been discussed briefly and also the benefits and shortcomings of the pre-existing methods and their proposed future plans have been mentioned.

Keywords: Deep learning, CNN, MRI, 2D, 3D segmentation, Brain tumor

INTRODUCTION

In today's world, people are detected with several hundreds of new diseases every day. One of these known diseases is brain tumor. The brain tumor is a very fatal disease which possesses serious threat to one's life. Cases of brain tumor are mostly higher in the developed countries as compared to the developing countries. Most patients of brain tumor are found in Australia. Asia records the highest number of deaths due to brain tumor. India, in 2018, ranked the same as the 10th most common kind of tumor in the country. Every year, in India, around 28000 patients are detected and around 24000 lose their lives to this deadly brain tumor. Brain is a small, yet, the most complex organ of

the human body, made up of billions of cells working together. Any kind of deviation in this can lead to some serious disorders. The tumors are caused due to the uncontrolled division of brain tissues, which forms a group of abnormal cells inside or around the brain, which destroys the healthy cells and affects the normal functioning of the human brain. These tumors are known to be life-threatening. Brain tumors are categorized into different grades depending upon their severity, like, benign or low grade (non-cancerous) [grade I and II] and malignant or high grade(cancerous) [grade III and IV]. The benign tumors are slow growing and do not spread into the other body parts, so are not considered too dangerous, while the malignant type of tumors are fast growing and have the ability to spread into various parts. Pre-malignant tumors are pre-cancerous stages of tumor that is not dangerous enough but in future can prove fatal. World Health Organization(WHO) classifies the brain tumor into 120 sub-types. Most common types of brain tumor in adults are meningiomas and astrocytomas (glioblastomas) while medullablastomas is common in children.

In the clinical practice, early detection of brain tumors is a very challenging task. Several factors contribute to make this challenging as the tumor associates itself with several psychiatric symptoms and neurological disorders, which may present the chances of many other diseases. The early detection of brain tumors is very crucial, as then only proper diagnosis can be performed and patient's life can be saved. The manual methods of detection of brain tumors is a time consuming and cumbersome task for the experts and hence may lead to error and even a minor mistake in the case of brain tissues will pave path to some serious threats, also death. To avoid all these threats and make the process easy and precise, in the modern world, techniques of artificial intelligence have been employed. Automated methods have been brought to use for the early detection of tumors and are devised in such a way, that an accurate and efficient differentiation can be done between the cancerous and non-cancerous cells. It also checks accurately for the particular symptoms of tumors separating it from all the other related diseases. Thus, the automated methods efficiently reduce the work load of doctors, enhance the working and process of treatment as a whole.

For the automatic observation and diagnosis, mainly the systems of machine learning, like, deep learning, Convolutional Neural Network (CNN) are utilized in order to incorporate the use of Magnetic Resonance Imaging (MRI), Computed Tomography (CT scan) and others for the scanning of the brain so as to locate the affected parts, know the history and thus perform the segmentation of the brain efficiently. Also, various evaluations and calculations are implemented to know the exact requirements of the treatment process. Upon performing the brain segmentation, the diagnosis is done through the images taken from different angles by the help of these automatic techniques (especially MRI) and hence the treatment becomes faster, more precise and a lot more effective and reliable. Even the progress and improvements of the patients can be better monitored by these new advanced methods. Thus, the progress in the field of medical science and especially in the branch of neuro-oncology has enabled for the utilization of these improved automated gadgets and technology as a better option for the diagnosis and alleviate the threat brain tumor possesses towards the patient's life.

EXPERIMENTATION AND ANALYSIS

Evaluation of Microscopic Diffusion Anisotropy

The disease and improvement of brain cells affects the in homogeneity of diffusion of water. These differences can be identified by utilizing the system of diffusion MRI and evaluated by use of Fractional Anisotropy (FA) inherited from DTI. FA is not only dedicated to these non-homogenous cells but also to the dispersion of their orientation. This serves as a great limit to using of FA as a standard for integration of tissues, majorly in the areas of difficult micro architecture. In this paper of research, it has been tried to work around this limitations by studying the ways in which this dispersed orientation affects the in homogeneity of microscopic diffusion. The ideas obtained by comparing the directional and homogenous diffusion coding were used to evaluate the order parameters (OP) and micro-FA. These values were then checked in the normal people, a patient with meningioma and other suffering from glioblastoma, which were further looked into to analyze the relationship between FA and micro-FA. It was

found that micro-FA was comparatively higher in the white matter than gray. Also, it was higher in case of patients of meningioma, leading to a conclusion that structural anisotropy was present in meningioma. Thus, it has been thus suggested that FA, micro-FA, Op if combined together can prove to be fruitful in clinical practices and research, as it provides for identification of in cell disintegration in the neurons, classification of fibrous tissues before surgery of the brain tumors and evaluation of in homogeneity at the microscopic level in the large homogenous tissues.

Identification using Automated White Matter Fiber Tract

The technique has been introduced to identify the key white matter fiber tracts automatically which is required for the neurosurgery. The technique is mapped in an attempt to face the hardships in neurosurgical tractography, including peritumoral edema, mass effect caused by mass lesions. Here, at first, the white matter fiber cluster atlas is learnt by utilizing group-wise registration and spectral clustering of multiple fiber tractography. Then, in the second step, the fiber tracts specific to patients are identified automatically by the help of tractography based registration and spectral embedding. When it applied on a group of 18 patients and observed that 94% of the 800 fiber cluster was found in around 16 patients. The outcomes indicate good colocalization. It thus shows capacity of automated methods for detection and its mapping is even helpful for patients with mass lesions.

Multimodal Functions and Data Clustering Problems

Actual requirements and cost for the designing applied issues help in tracking down the best outcomes that global advancement calculations can't understand. For precise and faster improvement, switching between the different nearby/worldwide arrangements and results is very vital. Here a social group optimization (SGO) method was introduced for tackling the several multimodal works and the information grouping issues as well. For taking care of worldwide improvement issues, the SGO inspired by the social conduct of humans towards tackling a compound issue was put in use. The SGO, a population based optimization calculation utilizes the arrangement populace to arrive at a worldwide solution. The simulation results looked at its execution with eight molecule swarm optimizer variations and shows evidence of the great performance of SGO. Application of new effective optimizing calculations and algorithms, mainly the SGO for evaluating the multimodal function results of data clustering. The performance is contrasted with the eight PSO variants on ten real data sets and two artificial data sets and the results hence obtained were reported. The values with an average of 40 simulations were used to get the algorithms convergence range. Mostly, SGO acquired lower errors but with a slow convergence in contrast to other PSOs. Usually, the SGO has demonstrated its effectiveness in data clustering and for multimodal function solving as well.

Kalman Filter Tractography- Two Tensor Model Evaluation

In order to view the important fiber tracts before operating, in strategy-making, MRI tractography has been widely used. But in the case of brain tumor patients, it becomes a difficult task, as water level rises in the tissues which in turn decrease the level of diffusion anisotropy, making it a challenge to detect the fiber tracts. In this experiment, it has been tried to employ a tractography technique to enhance the fiber tracking and make it more efficient than the conventional streamline tractography methods. The research conducted; keep its main aim to devote some ideas about the working strategy of UKF tractography with a two tensor model. Tests have been conducted to analyze the working of unscented Kalman Filter (UKF) method of tractography which is two-tensor. It attaches the diffusion design to the data at the time of tracing of fiber tracts. The presentation of many different techniques of tractography was contrasted. The accuracy and sensing speed of these were evaluated by checking the MRI activation achieved by tractography of each individual patient. In all the research performed, the best parameter to elevate the sensitivity of tracing was found to be generalized anisotropy limit, which helped in lengthening the fibers when they are decreased to 0.075.

Socio-Inspired Optimization Procedure

A technique of novel metaheuristic Socio Evolution and Learning Optimization Algorithm (SELO) has been discussed in this research enlivened by the social learning conduct of people coordinated as families in a cultural

arrangement. This populace based stochastic procedure can be ordered under the new and forthcoming class of streamlining calculations the socio-inspired algorithms. It is the social inclination of people to adjust to quirks and practices of others through perception. SELO copies the socio-development and learning of guardians and kids establishing a family. People coordinated as family gatherings (guardians and kids) cooperate with each other and other unmistakable families to achieve some individual objectives. Simultaneously, these family people gain from each other just as from people from different families in the general public. This assists them with advancing, improve their knowledge and all in all accomplish shared objectives. The proposed improvement calculation models this de-concentrated realizing which may bring about the general re cultural framework. SELO shows great execution on tracking down the worldwide ideal answer for the unconstrained advancement issues. The critical thinking achievement of SELO is assessed utilizing notable limit compelled standard test issues. The experiment contrasts the consequences of SELO and few other populaces based developmental calculations which are main stream across logical and genuine applications. Another ongoing socio-roused philosophy the Philosophy calculation results show that SELO exhibits tent amount execution to other examination calculations. This offers ground to the creators to additionally set up the adequacy of this metaheuristic by tackling intentional and genuine issues.

Analysis of Environmental Risk Factors For Tumors

The risk factors include various environmental factors but there is no scientific evidence supporting this fact. Ionizing radiations, some toxic agents, air pollution, radio-frequency electromagnetic waves have been posed as the environmental factors which associates with the carcinogenesis of brain tumors. In the children, exposure to brain-ionizing radiations constitute severe risk of these tumors, the brain ionizing radiation is known to possess serious threats in childhood, even at low doses. The N-nitroso compounds or pesticides as a risk for brain tumor (even for prenatal exposure) need proper scientific exploration. The studies considering relation of outdoor pollution with the brain tumor are all contradictory. Effect of mobile phones as a factor of developing brain tumors in children has not been explored yet.

Harmony Search Algorithm

Harmony Search, a population-based optimization algorithm developed on the basis of the idea that the musical instruments are played better when tuned to the best harmony state. Global Harmony Search (GSH), is the most popular of all the HS introduced till date. Although it has proved effective for solving these problems, a new updated mechanism being added to increase the efficiency and to help getting in a fix in local minima. The enhanced algorithm discussed in this research is called intersect mutation global harmony search algorithm (IMGHSA). The experimental results of comparison of IMGHSA with the several improved version of HS algorithm like basic HS, novel global harmony search and others, show that IMGHSA has achieved state of better performance than the other variations and it also provides better robust convergence in the terms of preciseness and effectiveness. Also, the proposed IMGHSA solves problems like parameter selection and processing of images and is easy to implement.

Detection of Brain Tumors using MRI

The detection of brain tumor in the early stage is a very arduous task for the radiologists. The size of brain tumor almost doubles in a span of twenty five days at an average, and the years of survival of the patients is reduced to a few months if not given proper attention and care. This is why the automatic system of tumor detection is employed for early observation. There is a difference between cancerous and non-cancerous MRI of brain found through automated methods. Various methods are employed for the segmentation of the patient's affected part and then the features chosen using the Support Vector Machine (SVM) classifier. The SVM puts along many cross validations on these chosen parameters and contrasts the accuracy of the proposed work. It shows the accuracy of 97.1%(avg.), 0.98 area under curve, 91.9% sensitivity and 98% specificity and hence is justified that it detects the tumor more efficiently than the other methods. . Most importantly, it can be used to accelerate the whole process of tumor detection and thus at an early stage it can be treated, before the onset of any complexity.

Meyer's Loop Tractography: Surgical resection has been used as an effective measure for treatment of temporal lobe epilepsy but at times it can create some visual field defects. These defects can only be alleviated if the surgeons could locate the ventral part of the optic radiation (OR), the Meyer's loop, accurately without any error. In the present day, there is an increase in dependence of the image guided surgery by utilizing the MRI process. Although a lot of endeavour has been put in improving the methods of analysis, a wide inconsistency has been observed in the reconstruction of Meyer's loop. Also, some differences are found in the accession of image on Meyer's loop which remains doubtful. Even, the limit of variance in data accession that leads to OR reconstruction variation is discussed in the paper. In the experiment conducted, 13 healthy people were taken and their Diffusion MRI data were obtained with different maximum grade amplitude (MGA). Meyer's loop was then redesigned on all of them and the stretch to the temporal pole was calculated and contrasted across the protocols and as a result a substantial response of data was observed.

Analysis through Magnetic Resonance Spectroscopy: The Magnetic Resonance Spectroscopy (MRS) is a device used for in-depth for calculation of bio-chemical basis of human diseases. The vivid demonstration of this biochemical basis enables us to depict the actual nature of the pathology and also to get the reaction to the treatment at the sub-cellular level. The brain tumors are the field that is constantly looked into and studied by the experts. Exploration of these factors by MRS plays a vital role in the management of the disease. In the paper, the scope provided by MRS into the biochemistry of human brain tumor is staged. Each metabolite depicted by MRS is visited and the importance of biochemical aspects of the pathologies in the management of the disease is explained. Also, the view of the disease according to the radiologist and biochemist is compared to prove that the preclinical work is of utmost importance. A proper mapping of the diagnosis has been presented to help in the classification of the lesions, after the different treatment options are available according to the images formed. The appearance of the images has been the chief tool to localize and characterize the lesion with MRS, which later helps in the sub-classification as stated above.

Tsallis Entropy and Region Growing Segmentation: The most common method used for the scanning and imaging in the field of medical science is Magnetic Resonance imaging (MRI) and thus the process for the disease to be identified becomes very easy. Again, to determine the severity and location of the abnormal detected, a suitable image processing is brought in use. In this work, the MRI is caught in contrast with improved TI modality (TIC) by a semi-automatic tool. The method combines Bat pattern and Tsallis along with domain growing segmentation. When experimented, the RGB images of brain MRI are recorded and then the features of the affected region are drawn with Haralick function. The clinical importance of the given method is checked by availing the brain images of BRATS obtained from TIC modality. The results of this whole research prove that the introduced process gives good enough values of dice (90.36%), sensitivity (98.27%), and accuracy (97.35%) for the BRATS brain MRI. The outputs prove that the above method (RG) is better than the other existing techniques. The clinical significance is also tested basing on the patients data set. The process discussed can be used in the future to estimate the contrast enhanced regions in MRI and magnetic angiograms (MRA) as well.

Brain Segmentation using MRI: For the researchers, medical image processing and its segmentation is the zone attracting attention. Through the discovery of CT scan and MRI, it has achieved heights in diagnosing the tumours. MRI is used in the identification of the tumor and segmentation is carried out on the part from the image. Various division techniques were used like watershed algorithm, morphological operations, K-means clustering and others using these MRI images. Different algorithms and division process has opened ways for the identification, observation and classification of the brain tumours. The various methods being used hive a different result on the basis of accuracy, efficiency, locating the brain tumours and its sensitivity. Again, the methods like Flower pollination algorithm (FPA) and Gravitation search algorithm (GSA) can also be used for the detection of tumours.

PET-MRI Imaging: New technologies have been introduced mainly considering the harmonizing nature of multi-parametric, multimodality imaging by use of MRI and PET. The latest MRI technologies like PWI, MRS, CEST and

others are considered to be far better than many pre-existing methods in examine the grade, condition and diagnosis response of the tumor. PET when done simultaneously with MRI also benefits us with some extra information like the metabolic properties of the tumor. Thus PET enhances the whole process of diagnosis and it proves the idea of post-treatment changes. These discoveries are capable to bring about a revolutionary change in the field of Neuro-oncology. The new technologies have enabled the experts to differentiate between the healthy and malignant brain tissue more efficiently. The use of Hybrid PET-MRI (FET PET) is looked upon as one of the most accurate and efficient techniques in planning before the operation and also in post-treatment care. Also the radio-genomics provide us with precise, non-invasive prognostic particulars by utilizing clino-pathologic, genetic and imaging data.

Advances in MRI Technology for Neuro-Imaging: The diagnosis of the primary brain tumor, over the lathe few years, has been highly dependent on the modern MRI technologies, in an attempt to predict the various patho-physiological characteristics of the tumor, like hemodynamics, metabolism and microstructure. The evaluation of the tumor grades, molecular subtypes from the MRI technique has opened an era of molecular imaging and radio genomics. This work of research has staged the basic developments in the field of quantitative neuroimaging by the application of radiomics analysis on the tumors, including the glioblastoma and low grade glioma. Results obtained from different sources like, diffusion MRI (dMRI), perfusion weighted imaging (PWI), and MR spectroscopy are sifted to ascertain the stand of quantifiable imaging in neuro-oncology. On one hand, it is proved that the use of MRI for examination of tumor aggressiveness, specific molar signature is important for the treatment, while, it has also been assumed from various studies that 2HG-MRS could occupy a principal role in the coming years for IDH status assessment during treatment and for treatment response assessment as well. Moreover, many improved technologies are waiting to be explored which will open opportunities for huge number of applications in this field of neuro-oncology. In future, the implication of these methods will enable to choose the techniques for better clinical practice.

Methodology for Detection, Segmentation and Classification of Brain Tumor

Detection of Abnormality of Lungs: It is found that lung abnormalities in humans are highly fatal. To reduce the risk, early diagnosis is required to enable fast and efficient treatment. The purpose of this research is to put forward a DL (deep learning) structure to test lung pneumonia and cancer. Two DL practises are proposed to evaluate the problem, first, a method called MAN (modified AlexNet) is executed to categorize chest x ray images into ordinary and pneumonia class. In MAN, the SVM (Support Vector Machine) is used to implement the classification and the performance is checked with that of Softmax. Secondly its performance is certified with the help of various pre trained DL methods like AlexNet, VGG16, VGG19 and others. Then in the second point, the other DL work involves an implementation of a combination of custom made and learned specifications in the MAN to achieve improvement of classification accuracy during detection of lung cancer and pneumonia. Serial fusion and Principal Component Analysis (PCA) based feature selection are employed for enhancing the feature vector. The performance of this DL is examined by the standard lung cancer CT images of LIDC-IDRI and a higher classification accuracy of 97.27% or more is obtained. It has thus been assured that the proposed MAN framework works more efficiently on the observed image datasets and when compared with the current state of the DL techniques, it enables to know that the proposed DL system grants better results having higher precision.

Detection of Epileptic Seizures: The working of the epileptic seizures when identified from the multiple path electroencephalogram (EEG) has always staged an important role for the in time diagnosis of the epilepsy patients. But in this, the detection if done manually by the doctors through EEG not only possess high chances of error but also is a tiring and difficult job. In order to reduce this chances of human error in clinical practice, there stands a need for a tool which can precisely act for detection of the same. In this work, a pattern has been presented by considering the use of multiple features and multilayer perception neural network (MLPNN). Many tests were conducted and the outcomes approved by the medical professionals. At first, to eradicate the power-line sounds, pre-processing was conducted on the EEG data obtained. Then, mainly few properties were abstracted like, entropy and spectral density. The extracted properties were then analysed and classified. The MLPNN then calculates the working of these features. . MATLAB was used as a tool to create a GUI based automatic application known as Aepitect, which

provides an automatic standard for these epileptic signals. The outcomes of the processing and evaluation were then checked which displayed different rates of sensitivity and specificity. It was even proved that this system can be applied for use in real life recording of multiple path EEG.

Fully Convolutional Network for Segmentation: Convolutional networks are regarded as the very efficient architectural designs that produce a variety of useful features. These models are trained on their own and have moved a step further in the art of semantic differentiation. The main goal is to produce a fully convolutional network set up that can accurately take in data of any size and give the respective output with effective learning. The design of this model network has been discussed in depth and its application in assuming the works thoroughly detailed and also their relation to the conventional modes of network has been mentioned. For the fully convolutional network, contemporary categorization networks have been brought in use and their learned portrayals are passed on to the differentiation processes. Again, a model has also been setup in order to attach the semantic data obtained from the rough, deeper coatings to the fine, upper coats to create a more precise segmentation.

Convolutional Network for Image Segmentation: A network and idea of training has been presented that is based on the study of augmentation of data in a view of utilizing the already prepared training samples more effectively. This model of U-NET has performed very brilliantly on the stages of various biomedical segmentation applications. It is convenient as it requires a limited number of annotated pictures and also has a training duration of approximately around ten hours due to the use of data augmentation methods. It is created in a way, that there is a path which contracts to catch the context and another path which expands symmetrically to allow accurate localization. The design can be made end to end from a small quantity of pictures and thus it is proved that this particular design has a better performance rate as compared to the previous models.

Classification Using CNN: The use of MRI is mostly in the case of brain tumors, but the data generated by MRI scans is huge and the classification of tumour versus non tumour manually becomes cumbersome. The precise measurements is provided for a specific number of images. Thus for to prevent the deaths and better diagnosis, automatic categorization is required. Due to the large variety and structural variability of the brain tumour, automatic classification is a difficult task. Use of CNN has been proposed to automatically detect the brain tumors. The main architecture models is designed by using the little kernels. Brain tumour classification is done by utilizing Fuzzy C Means(FCM) based division; texture and shape extraction and also SVM and DNN classification is implemented. It has low difficulty, low accuracy and more computation time. Thus to improve these factors, CNN method is used in the above scheme. CNN is one of the deep learning methods and also the OOP based programming language, python is used for its implementation. Image net database, a pre-trained model is used for classification. The raw pixel value, like length, breadth, height are drawn from CNN. To achieve high rate of preciseness, gradient decent based loss function is put to use. The training accuracy calculated as 97.5% and in similar way, the preciseness of validation is high and its loss is recorded to be low.

Classification using Deep Learning: Deep Neural Network categorizer is considered as one of the DL designs for grouping a dataset of sixty-six cerebral MRIs into 4 headings for example, glioblastoma, sarcoma and metastatic bronchogenic carcinoma tumors. The given technique integrates the discrete wavelet change (DWT) with the Deep Neural Network (DNN) to arrange the cerebral MRIs into typical and three varieties of brain tumors, as mentioned earlier. The latest procedural designs are similar to the convolutional neural networks (CNN) design and needs less device specifications and take suitable time for handling of huge size pictures (256×256). Furthermore utilizing the DNN classifier shows high precision in comparison to the conventional classifiers. The effective consequences accomplished utilizing the DWT could be utilized with the CNN later on and hence we can analyse the outcomes. The chief point is to highlight the state of art in brain Cancer area, like, the pathophysiology, imaging, classification of tumors, the methods of treatment, computer assisted algorithms for brain cancer categorization using methods of deep and machine learning. Also there has been a comparison between brain cancer and other related disorders. Further it has been concluded that the deep learning methods are getting more attention and have better capability in

contrast to the conventional methods. It is a fact that more lives can be saved if the treatment is done through fast and cost effective methods and also the detection is as early as possible. Hence, the need for such efficient yet cheap methods is of utmost priority, and thus the methods of Deep Learning if utilized to the best of knowledge with their full potential can prove useful for the same.

Classification of Epileptic EEG Signals: The patients diagnosed with epilepsy are checked for the detection of epileptic seizures basing on the EEG signals by the experts. It is a very crucial step and they employ visual inspection to detect the same, but it is recognized as a very tiring and difficult job. An automated design to perform the same operation by using the EEG signals can prove to be a great help. In this paper, a design based on determinant of matrix combined with Least Square Support Vector Machine (LS_SVM) has been proposed to identify the seizures. Here, each EEG channel has been segmented into various parts and these segments are again divided into number of sub-segments. The sub-segments are ordered in the form of matrix and the determinant is calculated. The results obtained are transferred to LS_SVM classifier. The above said design has been tested with the epileptic dataset and it has achieved a score of 100% in classification accuracy, sensitivity and specificity.

Techniques for Diagnosis of Brain Tumor

Blood Brain Barrier Posing a Challenge: Brain tumors have a high rate of deterioration and cannot be easily detected. The main reason behind the criticality and difficulty in the diagnosis of brain cancer is due to Blood Brain Barrier (BBB). This BBB has several molecular components and transport mechanism which creates an obstruction for the input of drugs into the brain. Hence, the need of the hour is to devise some suitable methods for the entry of drugs and plotting of various strategies in addition to the conventional methods to counter these hindrance that lie in path of successful treatment of brain tumor. Despite the treatment processes, the survival and curation rate of the patients is not good enough, the reason being, the heterogeneity of brain tumor, absence of proper system for input of drugs, all due to the presence of BBB. Effective and fast advancement is required in the field of brain tumor treatment and BBB research. The most efficient drugs found for the therapy are Temozolomide, Procarbazine, Carmustine (BCNU), Lomustine (CCNU) and Vincristine. The progress in the composition of the drugs or detection of some latest chemical bodies with increased level of effectiveness and less side-effects is the main aim of the experts today. Also, the development of drugs which can control the BBB or systems of transport can be the goal to work for in the future. The combinatorial therapy could also be an effective aim for the future, through which the BBB can be either be destroyed or modified and thus tumor cells be easily preyed, like, for example nanotherapy could be an effective tool for the aforesaid.

Immunity Developed by Radiotherapy and Vaccination: The reaction of the immune system to the antigens present in the central nervous system (CNS) is generally contracted as any kind of collateral harm can cause unwanted results. The importance of this is to make efficient tumor-targeted immunotherapy is not yet explored properly. In the experimental study, a B16 murine melanoma was taken up to contrast the reactions against the tumors found in the CNS and periphery. The cytokine analysis of the tissues from the tumor bearing mouse identified the increased secretion in the level of TGFb from microglia and it changed the liability of tumor antigen-directed CD8 T cells. Also, a diagnosis process utilizing focal radiotherapy and Listeria monocytogenes was tested to know about the immunologic activity and accuracy of this technique. The outcomes of this experimental setup showed that the CNS melanomas had more tolerance as compared to the similar tumors outside CNS due to the presence of CD8 T cells. The mouse tested had increased TGFb level, but it restricted TGFb signaling by a monoclonal antibody. Thus the tumor antigen-specific vaccine is the mixture of focal radiotherapy with increased survival. This process is generally linked to added poly-functionality of CD8 T cells, increased ratio of T effector to T regulatory cells and less secretion of TGFb from microglia.

Evaluation of Organotypic Brain Explant Culture: Organotypic brain segmentation culture has been brought under consideration for the studies of neurology, as a whole, which maintains the built modelling of the brain,

function of cells and the vascular network. Still, it is not yet acceptable to evaluate the anti-cancer medicines for brain explants. In this experiment, a replica of mouse for the severe glioma was considered basing on the representation of H-RasV12 in the stem cells of neuron to accept the brain explants that bears the tumor from the grown up mouse. The segmented slices were then tested with several compounds and evaluation of minimum assays was done to predict the consequences of the medications. Serial fluorescence-based imaging of tumor was regarded enough for the testing of cisplatin while the immunostaining of cleaved caspase-3 and Ki67 was sufficient for the analysis of working of temozolomide and immunostaining for histone H3 (phosphorylated) which allowed the displaying of specific outcomes. The cleaved caspase-3 was elaborated too in assessing the harmfulness of the drugs in the case of brain tissues of a normal person. The outcomes show that this organotypic brain segmentation culture is a helpful method for the evaulation of anti-glioma medicines.

Nanotherapeutic System for Treatment (Nanopore Sequencing): The most dangerous and critical type of brain tumors, including glioblastoma possess high rates of morality. Various treatment processes are used like, surgery, radiotherapy, chemotherapy and immune-therapy but the disease still continues to be fatal. Hence the experts have taken charge of developing some new methods of diagnosis and drug delivery systems (DDSs). By use of nanoparticle method, better drug release into deep tissues of brain is achieved. It is hoped that with time, the new projects of nano-scale treatment systems will come into being and will prove to be efficient enough. The optimization of the nano-particle based DDSs are required for specific localized treatment. With time and improvement of our knowledge in the field of brain cancer biology will pave the way for identification of more molecular targets along with nano-therapeutic system for the killing of specific brain cells.

Variations of a nucleotide in IDH1, IDH2, TP53, H3F3A, and the TERT advertiser area were recognized by utilizing the method profound amplicon sequencing. Nanopore sequencing gave a result of 0.1X inside a duration of 6 hours and coming about CN and epigenetic profiles in proper relation to coordinated microarray information. Analytically significant changes, for example, 1p/19q codeletion, and central intensifications could be reiterated. Utilizing impromptu arbitrary woodlands, we could perform directed pancancer arrangement to recognize gliomas and medulloblastomas, of various essential destinations. Identification of TP53 and TERT advertiser changes presents that grouping of whole qualities and GCrich locales is practical. Nanopore sequencing enables identification of underlying variations, point mutations, and methylation profiling utilizing a solitary gadget with trivial capital expenditure on that day itself.

Evaluation of High Affinity Fluoropyridine-Candesartan: Various cardiovascular diseases and renal sickness have been shown through the changes in the symbolization of the Angiotensin II type 1 receptors. The main motive behind this work is to highlight the use of Fluoropyridine-Candesartan in kidneys of rats as a PET imaging tracer. Assessments were done with the CHO-K1 cell membranes to show the AT1R in human beings. Evaluation of plasma proteins was done by ultra filtration. Analysis of the radiolabel metabolites in plasma of rats and kidneys of previously diagnosed animals were also carried out by the HPLC. The PET scans of Fluoropyridine-Candesartan were thus obtained for the male rats (Sprague-Dawley). In the outcomes, it was shown that this compound forms a strong bond with the proteins of plasma and metabolizes itself to give hydrophilic compounds that are radiolabel, that is, that the AT1R is more advantageous over AT2R in combination with the higher vivo stable modes as it enables higher proportion of kidney to blood.

Role of Long Non-Coding RNAs: Long non-coding RNAs, are capable of controlling the condition and expression of the genes at the transcriptions, post-transcriptional and epigenetic layers. It has also been discovered that these lnc RNAs with their abnormal characteristics in different cancers, stage a very pivotal character in the diagnosis of cancers and brain tumors. Many significant investigations detailed the connection of lncRNAs and EVs, which move the DNA, RNA, proteins, and lipids to different cells. This results in multiple phenotypic responses that assists in modulating the functionality of cell activities in receiver cells. There has been a progress in identification of

biomakers and drug development by elaborating the different functions of gene transcripts. Through the aid of genetic and biochemical studies, assessments for the present day study into biological functions of lncRNAs has been done. These lncRNAs have been explored to explain the genetic heterogeneity and their different roles from the biological point of view. The recent developments in the field of LncRNAs have enhanced the idea of cell free nucleic acids and are vital enough for the therapy of brain tumors.

Evaluation of Double Minutes in Diagnosis: Double minute chromosomes are extra chromosomal round-term DNA parts often located in primary cerebrum tumors. To comprehend their advancement, the double minute chromosomes are staged in prognosis and backside tumors from high-grade glioma of a child and four grown-up glioblastoma patients. The full designs of the significant double minute chromosomes utilizing a novel approach consolidating different kinds of genomic proof. From the different variations of double minutes determined in the paediatric patient, only one conveying EGFR was kept up at upper plenitude in the two examples, while two other variety were available in just following of the calculations at determination, however it was plentiful at relapse and the rest were discovered either in the relapse test or in the determination sample only. For the EGFR-conveying double minutes, tracked down a secondary somatic deletion in all duplicates at relapse, after treatment. Be that as it may, the somatic transition was available at exceptionally low frequency at diagnosis, suggesting expected protection from the EGFR inhibitor. This change caused an in-outline RNA record to skip exon 16, a novel record isoform missing in EST data set, just as around 700 RNA sequences of ordinary cerebrums that we surveyed. It was noticed that comparative examples including longitudinal duplicate number shift of double minutes in another four sets (analysis/relapse) of grown-up glioblastoma. By and large, in three of five matched tumor tests, it was found that albeit similar oncogenes were amplified at finding and relapse, they were amplified on different double minutes. The outcomes recommend that double minutes promptly develop, expanding tumor heterogeneity quickly. Understanding examples of double minute advancement can reveal insight into future remedial answers for mind tumors conveying such variations.

Toxin Resistant Therapeutics Stem Cells: The statistical analysis which makes it possible for the quantification of the diffusion metrics along the white fiber matters, was derived from Diffusion Tensor Imaging(DTI). Apart from this, many new and advanced techniques for better contribution in the field of brain tumor can be done by the use of modern MRI technologies, like NODDI, which enables for an in depth categorization at the micro structural level. In this paper, the experimental research was done on around fifteen healthy and twenty two affected subjects using both DTI and NODDI methods. A reference database was obtained from their micro structural analysis and then graphically plotted. This data was then compared and it was found NODDI derived data was more efficient. This research provides a base for the future studies dealing with comparison of healthy and affected people.

Adult and Childhood Brain Tumors

Management of Tumors in Children: Children are diagnosed with many kinds of tumors, brain tumor being the most critical one. In these recent years, the high-resolution genomic, epigenetic, and transcriptomic profiling of these tumors have put forward a molecular view and helps for a better classification of tumor into sub-types and proper therapy of these. Medullablastomas and many other types of tumors are classes under high risk categories while in recent years it is detected that they have four or more molecular subsets with unique clinical features. Similarly, glioma is also known to have molecular subsets which vary in terms of age, location and required diagnosis process. In ependymoma, as many as nine subsets have been found. Hence, in the present clinical practice steps are taken to move forward from the conventional methods and adapt to a better and more effective approach, like the molecular targeted therapy. . The molecularly targeted therapy has given treatment options for LGGs. The only difficult task is the molecular inhomogeneity of these high grade tumors and their habit to become resistant against these efficient treatments. So, for these high grade tumors various strategies are made to aim for the improvements in the control of these tumors.

Management of Adults with Metastatic Brain Tumors: In 2010, the Congress of Neurological Surgeons issued rules, standards and different modes for the treatment of adults diagnosed with metastatic brain tumors. As many years have passed by, an update to the existing data and treatment options is now needed. The main aim of this review is to develop the best ways for the efficient and effective management of the metastatic brain tumors based on proper clinical evidence, which can overcome the limitations present in the commonly used treatment and diagnostic methods. Several techniques and methods as per the literature searches like the radiotherapy, surgery, chemotherapy, use of steroids, prophylactic anticonvulsants, stereotactic radio surgery and others were discussed and considered as the options for the therapy of metastatic brain tumors. The quality and strength of these literature data were then tested for creating evidence based guideline. The limitations were further looked into and improvements made for better treatment.

Radiomic Analysis of Magnetic Resonance Fingerprinting: The capability of textural analysis of MRF diagrams by use of Radiomic to enhance the classification of intra-axial brain tumor was inspected in the adults and also the survival rate of glioblastoma was forecasted. In an experimental study, a group of 31 patients were selected and divided in 3 groups as 17 glioblastomas, 8 metastases and 6 low grade gliomas and then Magnetic Resonance Fingerprinting (MRF) was performed on them. The second order textural features for solid tumor and peritumoral white matter on T1 and T2 mappings were evaluated from the gray level co-occurrence matrices and gray level run length matrices. For survival analysis Kaplan-Meier method was used. The tumor types used the reverse of difference normalized values for peritumoral in T1 and T2 maps.

CONCLUSION

This review paper accomplished with genuine motive and understanding of the topic deals with the several methods and technologies of the past and present along with the plans for future projects for the error-free, efficient and precise evaluation and analysis of the malignant brain tumors in both children and adults. The paper has highlighted several key areas by drawing insights from a number of experiments and research papers, which in turn has enabled an in depth study of the causes, detection methods, automated imaging and segmentation techniques, treatment options, postoperative care and so on by bringing into use MRI, CNN, Deep Learning methods and even combining them with other machine learning techniques to get even better results. The automated methods for the examination of these cerebral tumors, their application, benefits, drawbacks, areas that need improvement, comparisons with other methods have been discussed elaborately. To conclude, the present methods of automatic segmentation give accurate and efficient outcomes and the succeeding experiments will add to the preciseness interface future lowering the risk that these brain tumor possess.

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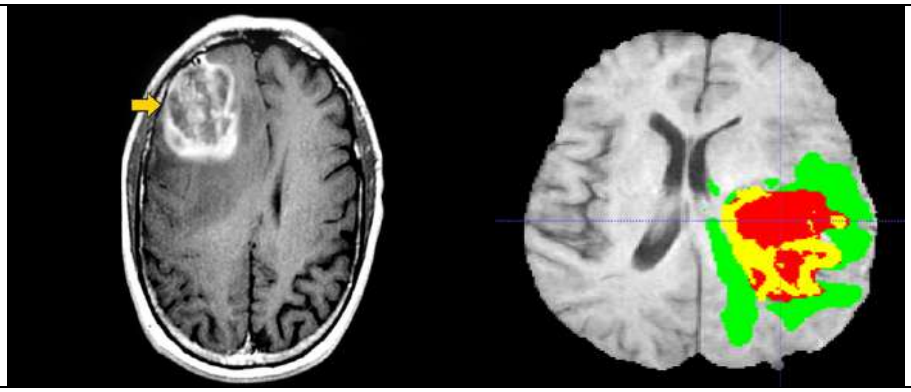

Figure 1: Images of Brain Tumors

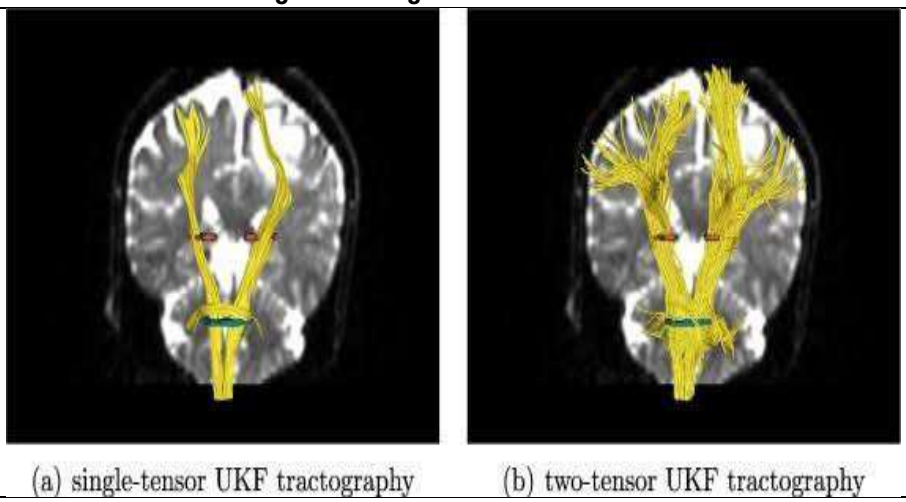

Figure 2: Single and double tensor UKF tractography

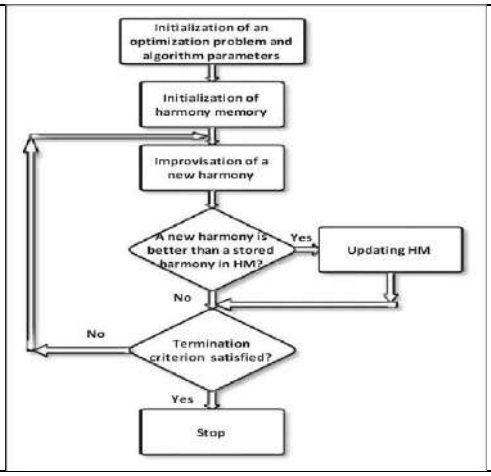

Figure 3: Algorithm for Harmony Search Algorithm

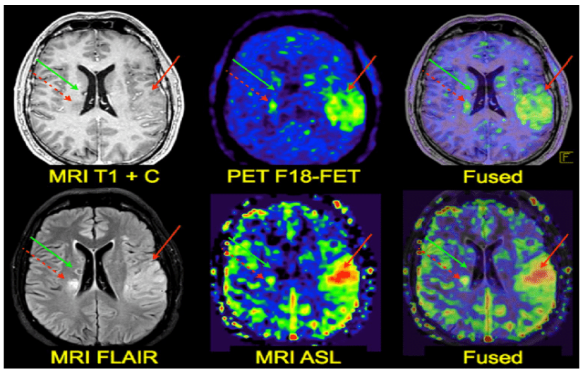

Figure 4: Different MRI Technology for Neuro-Imaging

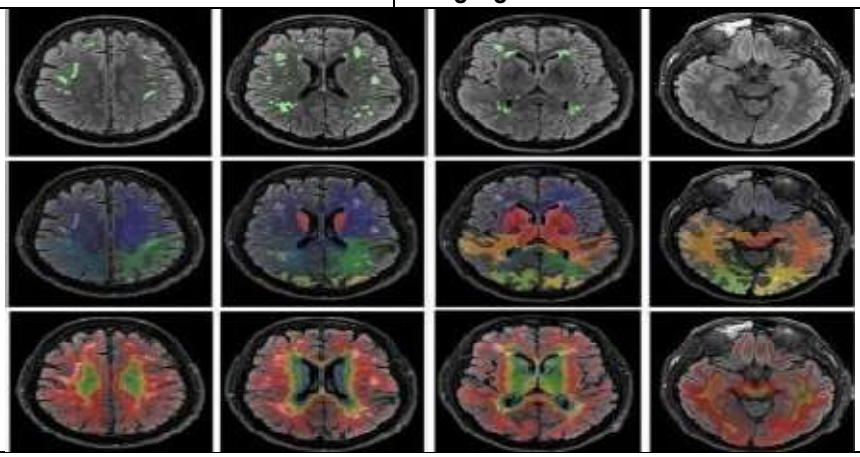

Figure 5: Segmentation by Fully Convolutional Network

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RESEARCH ARTICLE

Retardation of Candidiasis by Certain Antifungals

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ABSTRACT

Candida infections of virtually every tissue of the human body have been one of the current challenges which have directed several pharmacological studies. A number of significant works of paramount importance has been done on the extracellular protease enzyme that contributes its pathogenic behavior. To deal with these mycotic infections several synthetic drugs have been developed. In the present investigation, few polyene and azole drugs have been used such as Nystatin, Griseofulvin, Clotrimazole, Ketoconazole, Fluconazole, Itraconazole and Tinidazole. Furthermore, a relationship between the curtailments of growth of fungal isolates with the extracellular protease production has been established. A focus was made on current antifungal drugs and resistance mechanism. The main aim of the present work is to evaluate fungicidal activities of the tested antibiotics. It has been tested if any of the antifungal drugs is able to restrict the growth and enzyme production of the pathogen with selective toxicity for fungal cells. Also, an overview of new therapeutic alternatives for the treatment of *Candida* infections has been provided.

Keywords: *Candida*, Clotrimazole, Fluconazole, Griseofulvin, Itraconazole, Ketoconazole

INTRODUCTION

The infections caused by mycoses occur in every climatic zone, afflicts all strata of society and are of growing importance in the medical world today. Again, mycoses are the most ubiquitous [1] and also most difficult of all microbial infections [2, 3]which is largely due to limited therapeutic agents available and lack of reliable diagnostic methodologies[4].Clinically fungal infections are categorized according to the site and extent of the infection, route of

acquisition and the virulence of the causative organism[5].The high risk in fungal infection is related to prosthetics, cytotoxic chemotherapy, transplantation, increased use of powerful and broad spectrum of antibacterial, increased survival of premature infants in neonatal intensive care units and the Acquired Immuno Deficiency Syndrome (AIDS)[6, 7].

Candidiasis is a serious threat to public health and the main cause of morbidity and mortality all over the world [8-11]. The causal organism, *Candida* is a commensal of gastro intestinal and genitourinary tracts, the oral and conjuctival flora [12-14], can also affect skin and mucous membrane [15]or can invade the blood stream and spread the internal organs. Several contributory virulence factors other than biofilm formation to *C. albicans* pathogenicity in the host are secreted aspartyl proteinase and phospholipase activity [16]. Several reports suggested that the aspartyl proteinase secreted from *C. albicans* is directly related to virulence properties such as adhesion, tissue invasion and immune-invasion[17, 18].The problem of Candidal and other mycotic infections has not been solved truly by any of the antifungal drugs, which may be due to the interactive triangle between the host, fungus and the drug, so called devil's trident [19]. Again, the treatment procedure of fungal infection is more difficult than treating those caused by bacteria. This is because fungi like the host are eukaryotic. Therefore, it is intrinsically difficult to find agents with selective toxicity for fungal cells [9, 19]. Hence, several antibiotics are available for the treatment of bacterial infections in comparison to very few antimycotic drugs. Moreover, the weakness of antifungals and the lack of their fungicidal action against opportunistic mycoses with various side effects are associated with their use [20]and resistance have all served to dampen the enthusiasm for this class of compounds [21].

The opportunistic yeasts *C. albicans* and *C. tropicalis* accounts for about more than 80% of fungi isolated from patients with invasive Candidiasis and cause more fatalities than any other systemic mycoses[22, 23]. Inspite of their clinical importance, studies dealing with correlation between results of susceptibility test with antifungals *in vitro* and response to therapy *in vivo* is scarce [24]. There has been a great deal of interest in developing more sensitive forms of laboratory diagnosis including lysis centrifugation and serological tests [9, 23]. However, among the various measurements of susceptibility of bacteria and fungi, the *in vitro* determination of MIC (minimal inhibitory concentration) is assumed to be the simplest and most dependable comparative indicator. This method of determination originates from bacteriology and is suitable to prokaryotic cells but not to eukaryotic multicellular fungal isolates which show marked morphological and physiological differences between their parasitic and saprophytic life forms[25].It has been observed several times that the failure of drug treatment in fungal infection combined with improvements in performance and standardization of antifungal susceptibility testing have drawn attention to the problem of antifungal resistance. Hence, it is now evident that antifungal drugs can create clinical and epidemiological situations similar to those found with antibiotic resistant bacteria [21].

MATERIALS AND METHODS

To evaluate the effect of various antifungals, the fungal isolates were grown in modified[26] with the following constituents: Starch,1.0g; Casein powder, 10.0g; KH_2PO_4, 0.7g; $KHPO_4$, 0.3g; $MgSO_4$, $7H_2O$, 0.5g and yeast extract 1.0g in 1 litre of distilled water. pH of the medium was adjusted to 6.0. The medium was dispensed into culture tubes each containing 5ml and was then sterilized at 12 lb/inch^2 steam pressure. The antifungals tested were Nystatin, Griseofulvin, Clotrimazole, Ketoconazole, Tinidazole, Itraconazole and Fluconazole. Of these, Nystatin and Griseofulvin were made soluble in acetone water (1:1), while the azoles i.e., Clotrimazole, Ketoconazole, Tinidazole, Itraconazole and Fluconazole were dissolved in ethanol water (1:1). The different concentrations (i.e.1, 2, 3, 5, 7, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml) of the antifungals were added to the culture tubes containing 5ml aliquots. Finally, the volume was made to 10ml in each tube by the addition of sterilized distilled water. Apart from the culture tubes in triplicates, one control tube for each form of the fungus was run which was devoid of any antifungals. The culture tubes were inoculated and then incubated at 30±2^{0}C after about 11 days of incubation with undisturbed growth, the sets with antifungals at different concentrations were taken out, and the growth was

recorded turbidometrically. The effect of antifungals on the enzyme production was also determined following the Lowry's method [27].

RESULTS AND DISCUSSION

Nystatin, the polyene antibiotic showed greater fungistatic effect than Griseofulvin, extracted from the strains of *Penicillium*. Among the five azoles antimycotic antibiotics tested, Clotrimazole was observed to be most effective of all other azoles in its fungistatic property upon the isolates of *C. albicans*. The infection caused *by C.albicans* depends partly on the site of disorder and partly on the individual patients. However, in the pre-antibiotic era, relapse of the disease frequently occurred. It was difficult to eradicate the microorganism, particularly with the granulomatous form and in case of systemic involvement, the outcome was often fatal. However, the superficial and cutaneous infections were treated with several combinations of antimicrobial and keratinolytic agents like Castellani's paints, Whitefield ointment, 5% ammoniated mercury ointment, Pragmator ointment, Aquafor containing 3% salicylic acid etc [28]. The immune system works against microorganisms in three different ways- firstly, it prevents their entry into the body; secondly' it attempts to kill the pathogen if it gains access into the body; thirdly, it produces memory cells against the responsible pathogens so that it can mount effective immune responses against them if they enter the body in future. Mechanistically, antifungal agents are diverse, yet due to the alarming and rapid increase in drug resistant systemic fungal infections, new agents are necessary more than ever[29-31, 5]. The mechanism of drug resistance depends on the mode of action of antifungal compounds. The antifungal resistance is based on different mechanisms such as a) reduced drug intracellular accumulation b) decreased target affinity/processivity of the drug and c) counteraction of drug effect. The wide spread of antifungal drugs has increased the incidence of *Candida* resistance, ultimately leading to refractory fungal infections [32, 33].Although there are a number of diverse ranges of antifungal drugs used in clinical treatments, only few classes of antifungal agents are currently available in oral and intravenous forms.

A number of new synthetic antifungal agents have been developed for clinical use in both topical and systematic administration. Griseofulvin is one of the antifungal agents extracted from the mycelial homogenates of *Penicillium griseofulvum* by Oxford [34]. Then in 1946, the pure form was extracted from metabolic product of the culture filtrates of *Penicillium jancjewskii* [35].Gentles [36] first described the fungicidal action of this drug against experimental ringworm in guinea pigs and then it was used as an orally active antifungal drug against the ringworm in man by Williams *et* al.[37].It has been observed that griseofulvin acts as an inhibitor in the metaphase of mitotic cell division [38]. It is a tricyclic Spiro diketone that acts by disrupting spindle and cytoplasmic microtubule production, thereby inhibiting fungal mitosis [39, 31]. Again, it causes "Curling" of hyphae,a major growth abnormality in sensitive fungi which is associated with its fungistatic effect and its fungicidal effect results from the rupture of its cell wall [40]. However, the mode of action of this drug is manifold. The insensitiveness of *C.albicans* to griseofulvin has been reported by several workers [41, 40]. It has been also observed that there is no uptake of tubulin binding drug by *C.albicans*. It may depend on the variability of the fungal species, which, in turn, is determined by various internal and external factors.

The best-known polyene compounds applied in clinical purposes internationally, are Nyststin, Amphotericin B, Candicidin, Natamycin etc. of which Nystatin was first ever anti-fungal agent to be used therapeutically for Candidiasis [42]. It was discovered by Hazen and Brown[43] and was extracted from the filamentous bacteria belonging to *Staphylococcus* species or various substituted derivatives of the natural compounds[42]. Nystatin is an effective and broad-spectrum polyene fungicidal antibiotic which has been used for decades for treating superficial Candidiasis [44]. It is relatively effective and a safe drug for treating Candidiasis but associated with toxicity when given intravenously as it comes with side effects such as diarrhea, nausea, stomach pain, vomiting, allergic reactions etc. During any fungal infections, the cell membrane of the host maintains the integrity of the cell by preventing an excessive inflow of liquid which could lead to swelling and bursting and also leakage of intracellular contents such

as K^+ ions, an electrolyte required for cellular functions. Nystatin works by binding to the ergosterol present in the cell membrane of fungi leading to increased permeability of the cell membrane [45]. As a result, the essential contents inside the cell can leak away leading to the death of fungal organism. Thus, it is a preferred antibiotic as it only kills fungi and not the human cells because humans have cholesterol, not ergosterol. Here, in this study, Nystatin showed better funigistatic property than griseofulvin which stands contradictory to the previous observations made by several investigators, worked in this area [46].Although the MIC for the growth of the strains of *C.albicans* was more or less similar and in both the pattern and the percentage of growth was more or less similar, yet the production of enzyme percentage was much higher in griseofulvin than nystatin. This suggested that the growth of the isolates was not completely stopped but was merely suppressed by the application of these antibiotic drugs. However, the major role of antifungal is to damage the cell membrane as a result of which its permeability is altered. Therefore, the effect is fungistatic rather than fungicidal [42].

The other main family of antifungal agents is the azoles, whose efficacy against yeast isolates has been studied by several workers [47, 48]. It has been observed that azoles are activated at neutral pH or above neutrality or in other words, the inhibition of Candidiasis is marked when the azoles are unprotonated [49, 50].Again, add described that the physiological status of fungal cells also affect the inhibitory action of azoles on *Candida* species. It has been observed that the inhibitory effect of the drug not only depends on the mode of action of azole groups but also the kind of azole used. The primary target of the azoles is the haemoprotein that co-catalyses 14 L-demethylation of lanosterol-a cytochrome P^{450}. Differences in the extent of inhibition of fungal and mammalian cytochrome P^{450} accounts for the selective toxicity of azoles for fungi. A second aspect profoundly affecting the overall inhibitory action of azoles on *Candida* is the physiological status of the fungal cells [42].

Of the various azoles now being used internationally for the treatment of cutaneous, systemic and mucocutaneous infections caused by *C.albicans*, only five proven ones were tested such as Clotrimazole, Ketoconazole, Tinidazole, Itraconazole and Fluconazole. Moreover, these antifungals were tested for being used as orally, topically and topically-orally respectively by the patient. Very little is known about the mode of action of Clotrimazole. However, it is strikingly similar to those of polyenes. Odds [42], showed high efficacy of Clotrimazole against superficial *Candida* infections particularly those of skin, mouth and genitalia. The antifungal activity of clotrimazole against *C.albicans* depends on carbon sources, growth phase and morphology [51]. Clotrimazole in low concentration reduce the growth of yeast and filamentous forms by inhibition of ergosterole biosynthesis [52] in a fungistatic fashion. Clotrimazole concentration above 100μm are known to have fungicidal effects on yeasts, probably by a distinct mechanism independent of ergosterole biosynthesis via direct physico chemical membrane damage [51].It has been found that the drug is only used topically and is applicable to both dermatophytes and gram-positive bacteria apart from yeasts [53]. The antifungals are being taken by the patients orally, topically or topically-orally. However, the growth and enzymatic activity of the isolates was best checked by Clotrimazole whose MIC value was determined to be 2-3 ug/ml. This finding was supported by Odds [42] which showed similar results and also demonstrated the high efficacy of Clotrimazole against superficial *Candida* infections. Results of several studies showed that Clotrimazole is a more sensitive drug and has better effect than fluconazole in terms of growth minimum inhibitory concentration [54, 55]though it comes with few side effects like stomach pain, diarrhoea, fever, vomiting, nausea, itching, allergic reactions, difficulty in breathing etc. It has been observed that relatively high concentration potentiality damages the fungal cellwall and plasmalemma making them preferable to aminoacids, phosphates, intracellular macromolecular synthesis [56]. It also affected suppression of active concentration in lymphocytes [57].

Ketoconazole, another azole drug was first synthesized in 1976 and then introduced in India [58]. For the treatment of chronic mucocutaneous *Candida* infections, this antifungal drug has been used internationally [59, 60]. It has been studied that this drug helps in the accumulation of peroxidase by interfering in the synthesis of oxidase and peroxidase enzymes [58]. It also inhibits in the synthesis of sterol in the mycotic cell membrane by inhibiting the demethylation of lanosterol which is a precursor of ergosterol, required to maintain the integrity of fungal cell membrane [61, 62].Due to all the above reasons, the drug was recommended for being generally used orally,

topically and both orally and topically. Several studies reported no significant result in the reduction of *C. albicans* colonization by three azoles, ketoconazole, itraconazole and fluconazole at low MICs. As per the announcement of FDA, 2013, ketoconazole tablets should no longer be used as first line therapy for any fungal infections including *Candida albicans* because of the risk of severe liver injury [63],adrenal insufficiency and adverse drug interactions [64].

Fluconazole, another triazole derivative, is well established as a leading drug in treating Candidiasis and a variety of fungi and yeasts including *Cryptococcus neoformans* [65]but lacks activity against moulds [66]. Liss and Letourneau[67], made an *in vivo* study of fungal specificity of fluconazole which was proved to be meaningful because any drug-induced change in composition of the bacterial flora can favour opportunistic mycotic infection and disease [68].Thus, fungal specificity ensures targeted therapy against the causative pathogen and does not alter or diminish protective microbial species [67].Being the first member of a new subclass of synthetic triazole, it was selected here for determining its properties. It has been observed that the *in vitro* activity of fluconazole is very difficult to assess and its MICs are among the hardest to detect meaningfully. The fungal specificity of fluconazole depends solely on the culture medium in which it is supplemented to inhibit the growth and enzymatic activity of the fungus. However, in the present study, MIC value of fluconazole was determined to be 30ug/ml in which the growth of the isolates was completely checked, though complete inhibition of enzyme activity was found at 40-45ug/ml. These results proved that the strains of *C.albicans* are more resistant to fluconazole than ketoconazole, tinidazole, itraconazole and clotrimazole. Although several reports favour the present finding [42] while several others stand contradictory which suggest that among the azoles, fluconazole has been proved to be highly effective in treating mucocutaneous Candidiasis and is much better tolerated than amphotericin B [67, 9]. In the United States fluconazole resistance has caused significant additional hospitalization costs and deaths [69].The results of Liu *et* al. [18]showed reduced filamentous or morphogenesis of yeast to mycelial form in presence of licofelone combined with fluconazole as compared to those only treated with fluconazole. The combination of ibuprofen and fluconazole has been found to enhance fluconazole susceptibility to *C. albicans* by decreasing the MIC of fluconazole [70] several *in vivo* and *in vitro* studies demonstrated that CoA inhibitors such as ibuprofen, aspirin and indomethacin have certain antifungal activities by suppressing *C. albicans* PGE2 production and biofilm formation[71].Therefore, the use of novel compounds combined with azoles could be effective solution for *Candida* infections as it can expand antibiotic spectrum, improve antifungal efficacy and reduce the side effects [72].Besides the traditional azoles like clotrimazole, fluconazole, ketoconazole etc. two other azoles itraconazole and tinidazole were used here to know their antifungal effect. The retarding effects of itraconazole on the growth as well as extracellular extrusion of protease are probably new.

The antifungals can act variously like amphotericin B, impair membrane barrier function; Flucytosine, inhibit DNA and RNA synthesis; Itraconazole inhibit synthesis of ergosterol; Griseofulvin inhibit fungal mitosis and echinocandin derivatives, inhibit 1, 3, beta-D-glucan synthase etc.Azoles disrupt the cell membrane by inhibiting the activity of the lanosterole 14-α- demethylase [45], an enzyme involved in the biosynthesis of ergosterol. Many of the azoles are effective for both topicaluse and for the treatment and prophylaxis of invasive fungal infections [73, 69]. Unfortunately, long term applications of some azoles have been shown to produce carcinogenic effects [19].The long-term use of azoles may lead to nausea, vomiting, persistent diarrhea and elevated effect of liver functions, gynaecomastia in males and gastro-intestine reaction with some patients [74]. It has been also reported that certain strains are drug resistant [75]. The antifungal resistance based on different mechanisms continues to grow and evolve and exacerbate the need of new treatments against *Candida* infections.

CONCLUSION

Candidiasis is a serious threat to public health and the main cause of morbidity and mortality all over the world. A group of beneficial microorganisms are residing in our body that prevent the infection of harmful pathogens but they are disrupted by the use of antibiotics, stress, alcohol and poor diet as a result of which the body becomes vulnerable to infections by harmful pathogens. Studies have shown that even a single course of antibiotics can lead to change in

the microbiome that lasts for years and is accompanied by the uncontrolled growth of bacterial or fungal infections such as *Candida albicans*. The infection caused *by C. albicans* depends partly on the site of disorder and partly on the individual patients. However, in the pre-antibiotic era, relapse of the disease frequently occurred. It was found that all the antifungals tested were proficient growth inhibitors; but however, could not control the protease production by the isolates of the organism at lower concentrations. New formulations of antifungals, combination therapies and development of new bioactive compounds might be useful for a better therapeutic outcome. In spite of all the drawbacks, the contribution of antifungal chemotherapy to the medical science is tremendous. However, it is clear that, a broad spectrum of fungicidal drug is required which are safe enough to be used prophylactically over a long period of time to very ill patients. It should be observed when it induces resistance. The ongoing research on better understanding of these mechanisms may aid in detecting resistant isolates, identify novel drug targets, and inhibiting the rise of drug resistance.

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REVIEW ARTICLE

Mammography Breast Cancer medical imaging Segmentation and Classification using Machine Learning Model: A Systematic Review

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ABSTRACT

Breast cancer is the second leading cause of death in women, so accurate early detection can help reduce breast cancer deaths. Computer-based detection enables radiographers to efficiently detect anomalies. Several techniques allow radiologists to study the internal structure and they have generated a lot of interest in many different types of research. In several areas of medicine, each of these methods is of great importance. region. This review reflects the classification of breast cancer using multimodal medical imaging. It also provides detailed information on the methods developed to facilitate the classification of tumors, non-tumors and solid formations using different medical imaging techniques. Machine learning techniques followed by an overview of the various deep learning techniques and specific architectures for detecting and classifying breast cancer .We also provide an overview of the imaging techniques of different photographs to provide a complete overview of the field. In a similar context, this survey was conducted using various research databases as a source of information to access various field publications. Finally, this review summarizes future trends and challenges in the classification and detection of breast cancer.

Keywords: Breast Cancer Classification; Convolutional Neural Network; Mammogram Images Computer-aided Diagnosis System; Magnetic Resonance Imaging.

INTRODUCTION

Breast Cancer

Breast cancer is a group of conditions in which cells in breast tissue change and divide uncontrollably, often creating a mass or tumor. nipples (Society, 2019). Breast cancer is the most common cancer in women and the second leading

cause of death. Over the years, breast cancers have increased globally, and new breast cancers are reported annually (Hamidinekoo et al., 2018).This disease is fatal if not diagnosed in time (Arévalo et al., 2016).Breast cancer can be divided into two groups, normal and pathological, and it can be divided into two types: benign (not dangerous) and malignant (malignant). Benign tumors grow rather slowly, do not penetrate into nearby tissues and do not spread to different parts of the body (Mohammed et al., 2018). Breast cancer is often found before symptoms appear at the time of detection, or after a woman discovers a lump. (non-cancerous) and most breast tumors . When cancer is found, tissue is usually removed for microscopic examination with a needle (small or large needle) biopsy and, less commonly, with a surgical biopsy (Society, 2019).Early and reliable detection of this disease focuses on the analysis of data from previous diagnoses, and collects valuable information from previous data .Machine learning methods and medical images will help in this process.

Medical Imaging

Today, the rapid detection and diagnosis of tumors using imaging and machine learning techniques can be an important tool in improving the accuracy of breast cancer diagnostics. Medical imaging plays an important role in the diagnosis of breast cancer. defects in various organs such as eyes (Akbar et al., 2018), lungs (Akbar et al., 2018), brain (Rajinikanth et al., 2017), breast (Fonseca et al., 2017)., 2015) and (Khan et al., 2019). Medical imaging refers to certain techniques used to analyze the human body for the purpose of diagnosing, monitoring, or treating diseases. Each type of technology provides specific information about the area of the body being examined or treated, about a disease, injury or health effect that the human body has been examined to identify and track. Disease Monitoring (Ashour et al., 2016) Imaging medical research that seeks to classify the location, size, and characteristics of the organ in question is considered an effective method for extracting useful information that benefits from a wealth of information. Therefore, some researchers are intensively focusing on the creation and interpretation of medical images to identify most diseases, medical images, thereby contributing to the identification of diseases and helping to detect lesions, injuries, clinical treatment of patients and the identification of many diseases. ... Over the past decades, teaching and artificial intelligence have advanced significantly and play an important role in the field of medicine, such as medical image analysis. Medical imaging is the most effective way to detect breast cancer, with frequent use of a variety of techniques such as MRI, PET, mammography and CT, radiography, ultrasound and uplex (Dhawan, 2011) (Pluim et al., 2003) (Deserno, 2010) usually used for breast cancer.

Mammography Images

A mammograhy test, called a mammogram, helps detect and diagnose breast cancer early in women (Society, 2019). This is a human mammogram that uses small doses of X-rays to create an image of the breast (Dheeba et al., 2014). When assessing the risk of cancer in women without noticeable symptoms, while in patients with unusual symptoms or breast swelling, a diagnostic mammogram is performed to take a photograph that shows soft tissue, thick tissue, breast muscles, fibrous areas, etc. Professional radiologists can examine him for chest abnormalities. Some improvement in one to two years after two or more mammograms may indicate early cancer. A mammogram can show changes in the breast a year or two before the patient or doctor notices symptoms (Dhawan, 2011). In the early stages of cancer, more intensive therapy can be prevented and the breast's chance of survival is increased.

Ultrasound Images

Another method for detecting breast cancer is ultrasound, which typically uses low dose frequencies to create an image of the breast, but retains very little contrast. With large breasts Ultrasound can detect and identify nodules of breast mass and is mainly used for simplicity, volume, non-invasiveness and low cost (Qi et al., 2019).

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is another method for early detection of cancer cells, along with ultrasound and mammography. Instead of X-rays, MRI uses a magnetic field to produce highly accurate three-dimensional (3D) cross-sectional images. Magnetic resonance imaging of the human body requires a high dose of radiation to obtain an

accurate three-dimensional image of the breast. Thus, the difference in the infected area was very evident when we used the MRI scan, and therefore it did not reveal any cancer that could not have been seen otherwise. screening because it is expensive compared to mammography. As shown by mammography and calcification, MRI also does not take into account the first type of breast cancer. Magnetic resonance imaging has advantages over angiography and, in particular, provides clearer information about intra-abdominal masses than computed tomography in some situations. High spatial accuracy, specific differences between soft tissues can be seen by varying the acquisition parameters (Wilkinson and Graves).

Basics and Background

Machine Learning Overview

Machine learning is considered a branch of artificial intelligence that links the problem of learning data patterns to general inference principles (Tapak et al., 2019) and uses mathematical, statistical and logical methods to enable data-driven machine learning without programming (Montazeri et al., 2013) .By introducing artificial intelligence into games and pattern recognition algorithms, Arthur Samuel coined the term machine learning in 1959 to force computers to learn from their own experience. The key to machine learning is making predictions or decisions. Machine learning has become a powerful tool for modeling precise communication difficulties (Rahman, 2019). Through the use of multiple machine learning algorithms, powerful machine learning techniques have replaced many different parts of human participation (Goodfellow et al., 2016).Machine learning has become very successful over the years as the amount of data and computing power available increases. Several studies have implemented various machine learning methods (Das and Sengur, 2010) (Alkım et al., 2012) (Zheng et al., 2014).

Artificial Neural Network: Artificial neural networks (ANNs) are similar to biological networks of interconnected neurons in the human brain (Ripley et al., 1998). The answer is the most commonly used ANN to classify ANN problems using backpropagation learning algorithms. 2 shows the basic structure of a single neuron in the directly active ANN (Murtaza et al., 2019). A single neuron in the ANN takes input Xi from other neurons and multiplies it every day by the corresponding weight Wij, while using the activation function to obtain a weighted output signal f (Xj). This weighted output is passed back to the next layer as input for another neuron, and the same process is repeated until the output layer is reached.

Deep Learning Overview

Deep learning (DL) is a subcategory of machine learning and artificial intelligence that centralizes a complex hierarchy of image functions through its self-learning capabilities, unlike traditional machine learning extraction algorithms. progress in how computers extract information from images. medical imaging data cannot be identified by analysis in humans and to provide information on molecular status, prognosis, or response to treatment (Akkus et al., 2017). DL consists of layered neural networks that generate a hierarchy of functions through raw input images. Rapid improvement in GPU processing power has allowed the development of advanced DL algorithms capable of rendering millions of images and not sensitive to image displacement. DL has become more popular with recent success, especially in image segmentation and classification applications. Methods have been developed for various purposes, such as object recognition and segmentation in images, speech recognition, genotypic and phenotypic recognition and classification of diseases. (CNN) (Yap et al., 2017).

Convolutional Neural Network: Convolutional neural networks (CNNs) have become an important method for image analysis, especially when faces, text, human bodies, and biological images are detected or recognized (Yapet al., 2017). CNN is the most commonly used image algorithm. First introduced in 1989 (LeCun et al., 1989), CNN has been used with great success for image classification and segmentation (Krizhevsky et al., 2012), (Rusakovsky et al., 2015), (Deng et al. 2009) An accumulative neural network in deep learning is a type of deep neural network most commonly used to classify visual images. CNN is a feedback network capable of extracting topological properties of an image. CNNs are models driven by multilayer perceptrons. In general, layered perceptrons refer to fully

connected networks in which every neuron in one layer is connected to every neuron in the next layer. CNN is like a neural network with three different types of layers, namely stacking, grouping, and full connection. Each class has its own mission. The convolutional layer acts as a feature extractor. The fully linked layer uses the extracted function to determine which category the current record belongs to. The group layer is responsible for reducing the size of the feature map and network parameters.

Performance Evaluation

This section explains the performance metrics used to test CAD systems. Breast cancer can be classified as true positive (TP) or true negative (TN) if the diagnosis is correct and can be classified as false positive (FP) or false negative (FN) if the diagnosis is incorrect. Accuracy, sensitivity, precision, F measurement, AUC (area under the curve) and volume below the surface ROC are the most commonly used assessment criteria for the classification of breast cancer (Murtaza et al., 2019). They are briefly described as:

Accuracy (Acc): This section explains the performance metrics used to test CAD systems.

ACC = $\frac{(TP+TN)}{(TP+TN+FP+FN)}$

Sensitivity (Sn): This test only shows how many overall positives have been correctly assessed. In short, it reflects the exact estimated total number of patients with pathological breast cancer. $S_n = \frac{TP}{(TP+FN)}$

Specificity Index (Sp): Indicates how accurate the overall negative predictions are, and describes how much of the predictions are generally correct. $S_p = \frac{TN}{(TN+FP)}$

Precision Score (Pr): This score reflects only how much of the prognosis of abnormal breast cancer is correct. For medical imaging, the Sn and Pr content should be high to avoid misdiagnosis of cancer patients.

$P_r = \frac{TP}{(TP+FP)}$

AUC: The area under the curve is a numerical value that tells us how the model will perform in various situations.

AUC = $\frac{\sum R_i(I_p) - I_p(I_p+1)/2}{I_p+I_n}$

Where I_p and I_n denotes the number of positively and negatively images of breast cancer, and Ri is the rating of the ith positive image.

Machine Learning Techniques for Different Image Modalities

Various machine learning methods are used to detect, classify and diagnose breast cancer based on characteristics extracted from medical images. This section discusses and discusses breast cancer detection methods for various types of medical imaging; Mammography, ultrasound, MRI.

Machine Learning Techniques for Mammogram Images

This method has been tested on 95 mammogram images. In (Quellec et al., 2016), the authors proposed a methodology for classifying breast cancer as normal or abnormal using SVM in the DDSM dataset. The AUC measurement index is 94.4%. (Zemmal et al., 2015), semi-guided SVM was used in the DDSM dataset to classify tumors as benign and malignant, with an accuracy index of 93.1%. In (Liu et al., 2010), another method used SVM for volume classification. Experiments show that the area under the ROC is 0.7. The study used mammography images from the DDSM dataset. The authors in (Anitha and Peter, 2012) used the same concept as in the previous study to conduct a simulation experiment with 44 mammogram images in the MIAS database, and the authors indicate that the mass classification accuracy reaches 95%.

In (Mabrouk et al., 2019), researchers have proposed a CAD system that diagnoses and detects changes in breast cancer earlier, more reliably, and more quickly than control systems. Traditional CAD inspections based on image processing techniques. They start with preprocessing, segmentation, extraction. characters and finally the classification stage. The analysis described in this paper focuses on the integration of various characteristics such as shape, texture, and time-invariant characteristics. The data set is used by MMAS, the accuracy of the proposed system reaches 96% in the automatic ANN mode. While (Şahan et al., 2007), the researchers proposed a combined approach for detecting breast cancer using machine learning methods, integrating the fuzzy artificial immunity system and the neighbor of the species itself on the model, and tested the model in WBCD, 99.14% accuracy ... In addition, the authors introduced the neuro-fuzzy technique in (Nauck and Kruse, 1999) and go through this process in the WBCD. His system has an accuracy of 95.06%.

Machine Learning Techniques for Ultrasound Images

Researchers from (Mohammed et al., 2018) proposed a classification method using neural networks of different sizes and replication. For this study, 184 breast ultrasound images (72 irregular cases (tumors) and 112 normal cases) were analyzed. The results obtained show that the accuracy value is 82.04%, the sensitivity value is 79.39%, and the specificity value is 84.75%. In addition, (Chen and Huang, 2016) is another approach that proposes a backpropagation neural network for classifying breast cancer in clinical data.

Machine Learning Techniques for Magnetic Resonance Imaging

Many studies have used assistive vector machines to diagnose and classify breast cancer using MRI. A study (Hassanien and Kim, 2012) proposes a method for classifying cancer as normal or abnormal using SVM on a private dataset. The accuracy rate of this technique is 98%. (Hoffmann et al., 2013) also use SVM to detect minor lesions. A study (Soares et al., 2013) used SVM to detect breast cancer in a private dataset, and the inference accuracy of this method was 94%. (Agner et al., 2014) and (Yang et al., 2015) used SVM on a private dataset in which breast cancer was classified as malignant and benign. While the authors in (Waugh et al., 2016) propose a subtype classification using cross-validated kNNs on 200 private images, the authors propose a computer system for generating data on internal breast mass (Huang et al., 2014) and system performance. was tested on 61 images using Fuzzy CMeans. In addition, in (Weiss et al., 2014), exclusive diffusion imaging media were used to differentiate benign and malignant lesions.

Deep Learning Techniques for Different Image Modalities

Deep learning techniques have recently been developed to extract signs of displacement and improve the efficiency of medical image analysis. This section provides an overview of DL methods for detecting and classifying breast cancer using various types of medical imaging; Mammography, ultrasound, MRI.

Deep Learning Techniques for Mammogram Images

This subsection briefly describes recent advances in mammographic diagnosis and classification of breast cancer. The most impressive CNN models that are VGG16, ResNet50 and Inception v3. Instead of random initialization, the transformation from training using pretrained weights yields better results. The proposed system achieved an accuracy of 97.4% and 0.99 AUC in the DDSM database, an accuracy of 95.5% and 0.970 AUC in the DDSM database. INbreast database and accuracy 96.60 and 0.96 AUC in BCDR database. In the end, the extracted region of interest (ROI) was preprocessed and normalized from complete mammograms, and the authors pooled all the datasets to create a single vast collection of images and use them to fine-tune CNN. The accuracy index is 98.94%. In (Jiao et al., 2018), researchers classified various tumors in breast cancer tissue using a deep complex network to distinguish between malignant and mild cases, and proposed a deep metric neural network specifically to classify the system in (Wichakam and Vateekul, 2016). Which combines deep CNN and SVM for volume detection. The CNN model is based on mammography points. The image and data from the fully connected layer were finally used as a high-level representation of the image characteristics to form the SVM classification.

A quantitative analysis of various traditional mass inferences is presented (Oliver et al., 2010), and the advantages and disadvantages of the approaches used are qualitatively assessed, and deep learning methods are used to detect, segment and classify breast neoplasms for Let's start with mammography. In studies (Arevalo et al., 2016), (Kooi et al., 2017) and (Dhungel et al., 2017), the authors have proposed optimization methods for large classification, using deep learning techniques in feature extraction to automatically extract individual functions. classification, all characteristics are combined into classifiers to make a final decision. Deep learning systems are suitable for the simultaneous identification, positioning and classification of large objects in high-contrast, high-resolution natural images. This model was evaluated on the INbreast dataset. In addition, two publications (AlMasni et al., 2018) and (Alantari et al., 2018) propose a batch detection CAD system based on You Look Only Once (YOLO). 2020), the authors propose a system for identifying and classifying breast lesions. The two different datasets that evaluate this method are called DDSM and INbreast. The authors used the YOLO detector to detect breast lesions with an F1 rate of 99.2% for DDSM and 98.02% for INbreast. The classification is then done using three deep learning classifiers, namely CNN Regular Advance, ResNet50, and InceptionResNetV2. CNN classification models ResNet50 and Inception ResNetV2 achieved average accuracy of 94.5%, 95.8% and 97.5% for the DDSM dataset and 88.7%, 92.5%, respectively. AND 95.3% for the dataset INbreast, respectively.

Deep Learning Techniques for Ultrasound Images

There are several studies that have used DL to detect and classify breast cancer using ultrasound. The authors (Qi et al., 2019) developed a model using a deep complex neural network with multiscale cores and hopping connections. The model depends on two factors: the first determines whether the image contains melanoma, and the second detects large nodules the image. The authors in (Byra et al., 2017) presented a neural network for breast classification with three convolutional layers and two fully interconnected layers. The dataset used included 166 malignant tumors and 292 benign lesions. The average AUC was 0.912 with an accuracy of 83.0% and a sensitivity of 82.4%. The authors proposed using CNN in (Xu et al., 2019) to segment ultrasound images of the breast into four main tissues: skin, fibrous, three-dimensional (3D) and adipose tissue. Quantitative indicators for assessing the effects of segmentation, including accuracy, recall and F1 calculation, have reached more than 80%, showing that the proposed method is able to differentiate functional tissues in breast ultrasound imaging. In (Wang et al., 2020b), the authors propose a CNN-based CAD system for classifying breast lesions as benign and malignant. The proposed CNN uses a modified Inceptionv3 architecture to provide efficient feature extraction to extract features from multiple views from both views. The proposed CNN conducted training on 316 chest lesions. The AUC value was 0.9468, and the sensitivity and specificity were 0.886 and 0.876, respectively.

Deep Learning Techniques for Magnetic Resonance Imaging

In (Fang et al., 2019), the authors propose a classification method based on CNN and the Image Quality Assessment Algorithm (IQA). First, they used the CNN architecture to calculate the number of pixels in the lesion areas where the maximum clustering layers were used. The high-density pixel area is then assigned high-quality values that represent more of the texture and grayscale features. Finally, they generated a multiSVM image kernel using the quality values obtained to classify breast cancer. Although (Gibson et al., 2018) the authors present the open source NiftyNet platform for deep learning in medical imaging. The NiftyNet platform provides a scalable deep learning pipeline for a wide range of medical imaging applications, including segmentation, regression, and imaging. NiftyNet is based on the Tensor Flow system and supports standard features such as 2D Tensor Board and 3D rendering, as well as computational graphics.

FUTURE TRENDS AND CHALLENGES

This section presents directions for future research that can be used in screening for breast cancer, and significant efforts are needed to improve the effectiveness of breast cancer screening. and LD methods for detecting and classifying breast cancer .The review identified several key issues as well as inherent trends, directions for future research and issues discussed. comment below:

1. The first problem we saw in the reviewed articles was that the lack of complete training datasets was a major problem when training deep learning models for medical imaging. DL requires large training data because the performance of the DL classifier is highly dependent on the size and quality of the dataset. However, lack of data is one of the major obstacles to DL's success in medical imaging. In addition, generating big data from medical images is difficult because annotating data requires a lot of time and effort, not only from one person, but many experts to rule out human error. It is also difficult to construct big data from medical images, because annotating the data takes a lot of time and effort, not only for one person, but also for many experts to eliminate human error.
2. Most of the studies analyzed used different datasets from private cancer clinics or research agencies to evaluate and analyze them. The main limitation of this argument is that the performance of these models across studies is difficult to compare.
3. Unsupervised clustering techniques for the classification of breast cancer. Most of the selected primary studies used a breast cancer classification based on a supervised learning approach. Using tagged images for training has led to better results with these approaches. In life, it is difficult to collect correct images of breast cancer that have been noted by specialist doctors. In most cases, a significant number of vague medical images are available. Large numbers of blank labels are important sources of knowledge and cannot be used for supervised learning. Hence, a breast cancer classification model is essential and can be trained using various unsupervised clustering techniques.
4. A reinforcement approach to the classification of breast cancer. Providing machine learning models with the ability to simultaneously learn in their environment is a big challenge. It is important to provide enough sample breast cancer images to be representative of all cancers. The introduction of a learning-based reinforcement model can dramatically improve the efficiency and productivity of medical imaging methods for classifying breast cancer.
5. In future work, in addition to the previous points:

- Another interesting development that has emerged in recent years is the deep learning classifier. In recent years, interest in computer diagnostic systems has increased. DL evolution with hybrid architecture. Computer diagnostic systems include various imaging methods.
- Another highlight is the development of CAD systems based on 3D mammography, an emerging trend that can help improve CAD performance. These aspects must be taken into account in the future CAD production.
- Rather than just using these imaging techniques (mammography, ultrasound, MRI, and histology), certain types of breast cancer imaging, such as computed tomography (CT) or thermal imaging, can also be used to potentially improve the efficiency of breast cancer classification models. The same patient should undergo magnetic resonance imaging or computed tomography. Photos of all types of breast cancer cases will also be collected. Classes of models for the classification of breast cancer.
- Cross-validation is a model validation technique to test the versatility of a model, resulting in a invisible dataset. The goal is to classify the dataset in order to test the model during training and resolve issues such as inconsistencies, redundant pages, and to show how the trained model is generalized to an independent dataset.

CONCLUSION

Accurate recognition of breast lumps is essential for adequate treatment. This study proposes the development of an accurate system for detecting breast cancer tumors as a standard procedure for diagnosing breast cancer. Digital mammography is now the standard procedure for diagnosing breast cancer, several methods used to classify problems in medical diagnostics. Visualizing signs is an important step in mammogram classification. These features are extracted using image processing techniques. The review also focuses on the complex neural network and its deep learning architectures used to detect and classify breast cancer through a variety of imaging techniques. This review also provides an overview of medical imaging; Mammography, ultrasound, MRI.

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Table 1: Summary of the papers for mammogram images using SVM classifiers from 2015- 2017; AUC: area under the curve; Sn: sensitivity

Reference	Dataset used	Contribution	Performance Evaluation
(Abdel-Nasser et al., 2015)	- Mini-MIAS - INBreast	- Classification of breast density and mass or normal and classification	- Mini-MIAS: - Accuracy: 99%, AUC value: 0.9325
(Liu & Zeng, 2015)	- DDSM	- Mass detecting for diagnosis of disappointing areas	- Sn value: 82.4%
(de Oliveira et al., 2015)	- DDSM	- Classification of regions extracted as mass/non-mass	- Accuracy: 98.88%
(Wajid & Hussain, 2015)	- MIAS - INBreast	- Differenting between abnormality (mass or micro calcification) and (benign or malignant)	- Accuracy: 99% ± 0.50 - AUC value: 0.9900 ± 0.0050
(deNazare Silva et al., 2015)	- DDSM	- Masses detection	- Accuracy: 83.53%
(Liu et al., 2015)	- INBreast: 410 images	- Detection of micro calcification	- AUC value: 0.8676
(Sharma & Khanna, 2015)	- IRMA - DDSM	- Classify vector features as malignant or non-malignant	- IRMA: Sn value: 99%, Sp: value: 99% - DDSM: Sn value: 97%, Sp value: 96%
(Ponomaryov, 2015)	- MIAS	- Breast cancer classification	- Accuracy: 96.3%
(Khalaf & Yassine, 2015a)	- MIAS - DDSM	- Breast cancer classification	- MIAS: Accuracy : 95.80% - DDSM: Accuracy: 95.78%
(Khalaf & Yassine, 2015b)	- DDSM	- Breast cancer classification	- Accuracy: 94.44%
(Addioui et al., 2015)	- Private cases	- Breast cancer classification	- Accuracy: 98.33%
(Phadke&Rege, 2016)	- MIAS	- Classify abnormalities using fusion functions	- Accuracy: 93.17%
(Hiba et al., 2016)	- DDSM	- Classification of breast cancer	- Accuracy: 91.25%
(Khan et al., 2017)	- MIAS : 109 cases	- Normal and masses classification	- Accuracy value from 68% 100%

Table 2: Summary of the papers for mammogram images using ANN: Artificial Neural Network classifier.

Reference	Dataset used	Contribution	Performance Evaluation
(Pratiwi et al., 2015)	- MIAS	- Normal or abnormal classification then classify the abnormal into benign or malignant	- RBF(normal/abnormal): - Accuracy: 93.98, Sn value: 97.22% - RBF(benign/malignant): - Accuracy: 94.29%, Sn value: 100%
(Mina & Isa, 2015)	- MIAS	- Breast tissues classification into normal and abnormal grouping	- Classification rate: 91.64%
(Tan et al., 2015)	- Private: 1896 cases	- Breast cancer detection	- Sn value: 68.8% - Sp value: 95% - AUC value: 0.851± 0.046
(Rouhi& Jafari, 2016)	- MIAS: 57 images - 37 benign - 20 malignant	- Tumors classification: malignant and benign	- Accuracy: 90.94% - AUC value: 96.89%
(Peng et al., 2016)	- MIAS - BancoWeb:100 images	- Classification as a benign or malignant tumor	- Accuracy: 96%

Table 3: Summary of the papers for Ultrasound images using the SVM Classifier.

Reference	Dataset used	Contribution	Performance Evaluation
(Cai et al., 2015)	- 138 Privately owned cases	- Benign and malignant tumors discriminate	- Accuracy: 86.96% - Sn value: 86.96% - Sp value: 86.96% - AUC value: 0.894
(Prabusankarlal et al., 2015)	- 120 Privately owned images - Benign: 70 - Malignant: 50	- Breast masses detection and diagnosis	- Accuracy: 95.85% - Sn value: 96% - Sp value: 91.46% - AUC value: 0.9444
(Wu et al., 2015)	- 210 Privately owned images - Benign: 120 - Malignant: 90	- Evaluating malignant breast cancers	- Accuracy: 96.67% - Sn value: 96.67% - Sp value: 96.67% - AUC value: 0.9827
(Huang et al., 2015)	- 46 Privately owned images	- Detecting the tumor regions	- Accuracy:0.983 ± 0.013 - Sn value: 0.974 ± 0.035 - Sp value: 0.985 ± 0.019 - AUC value: 0.997± 0.003
(Chmielewski et al., 2015)	- 105 Privately owned images	- Classification of lymph node	- Sn value: 95% - Sp value: 90% - AUC value: 95%
(Moon et al., 2015)	- 169 Privately owned cases	- Differentiating normal from abnormal	- Accuracy: 94.81% - Sn value: 94.12% - Sp value: 96.72%

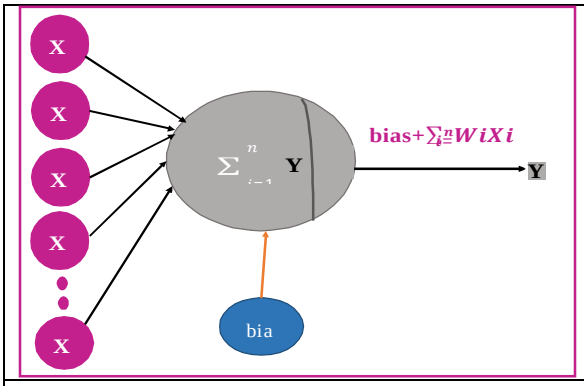

Figure 1: An artificial neuron

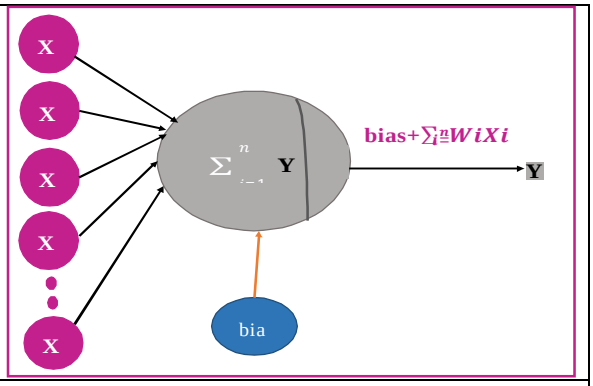

Figure 2: the basic ANN

Artificial Neural Network

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REVIEW ARTICLE

An Overview of *Boerhavia diffusa* Plant used for the Treatment of Various Diseases in Odisha, India

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ABSTRACT

From the early advancement, plants have been utilized as a medication for diseases. *Boerhavia diffusa* of the family Nyctaginaceae, a highly useful plant in the indigenous system of medicine (ISM), is a perennial creeping herb found throughout the waste land of India. It has been reported to possess the remarkable medicinal properties for various ailments like liver ailments, urinary disorders, chemotherapeutic ailments and antimicrobial agents etc. Its root is used in the treatment of cancer, jaundice, dyspepsia, inflammation, eye disorders, enlargement of spleen, abdominal pain and as an anti-stress agent. It possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. It is one of the oldest oriental medicines mentioned in Ayurveda as potential remedy for various ailments. The whole plant is rich in glycosides, steroids, flavonoids and also contains various polyphenolic compounds. Many pharmacological studies have demonstrated the ability of its antioxidant, ophthalmic, anti-inflammatory, spermatogenic, aphrodisiac, immunostimulant, hepatoprotective, antiasthamatic, supporting its traditional uses. This review focused on phytochemistry, enlistment of phytochemicals, responsible for therapeutic values, traditional uses and its reported pharmacological properties.

Keywords: Ayurveda, *Boerhavia diffusa*, Indigenous, Pharmacological, Phytochemicals

INTRODUCTION

From prehistoric time's man has always been dependent upon the plants for food, shelter and health. The relationship between man and plants is as old as history of mankind and likewise indigenous knowledge about plant is as old as human utilization [1]. Plants have been used in a number of systems of medicine in the world including India. Atharvveda suggests that man learnt the therapeutic value of plants by observing the behaviour of wild animals and birds in disease [2]. India is well known as important source of various medicinal herbs and shrubs. The use of plants to treat various diseases and disorders dates back to the times of 'Rigveda' (3500-1800 BC).

Later, most important and authentic works on Indian medicinal plants is found in the classics of Ayurveda that is Charak Samhita, Sushrut Samhita and Astanghridaya, which are believed to be written in the pre- Buddhist period, that is, before 500 B.C. These works incorporate 700-800 drugs of medicinal value used in several preparations for treatment of various diseases. They also serve as the basis for the medicinal plant research in India as well as in other countries [3]. In India, around 6000 plants are used in traditional, herbal medicines, etc. Due to the presence of 16 different agro-climatic zone, 25 biotic provenances, 10 vegetation zone, and 426 biomass India's diversity is unlatching. Since pre-history time plants have been a part of human culture. It is found that about 5000 species possess a distinct medical utility amid 250000 multi-story plant species on the globe [4].

In western countries, use of natural substances for medicinal purposes can be found since 78 A.D., when "De Materia Medica" written by Dioscorides, described thousands of herbal and medicinal plants [5]. According to WHO as much as 80% of population in developing countries is dependent on plants for primary health care, Statistical data reveal that as many as 3226 out of 4752 communities in India are dependent on the traditional medicine derived from plants [6]. A status report on ethnobiology in India, undertaken by Ministry of Environment and Forest has indicated that the tribal communities use over 7500 species of plants for medicinal purposes [7]. In the Indian system of medicine viz. Ayurved, Siddha, and Unani, it is the treasure house of healing herb which is being used [8].

About 37.64% of the total land area of the state i.e., one-third of Odisha is covered by forest. From most of the studies, it is found that most of the plant species help in curing stomach disorders. From the few audits examines it is accounted for that in a few pieces of India to shield the information on conventions from vanishing [9-11]. The use of any medicinal plants is based on its usefulness in treatment of various diseases by generations of physicians and traditional systems of medicine from different ethnic societies. Based on the experience of many generations, medicinal plants have been used to cure many ailments. Indian system of medicine (Ayurveda) is playing a vital role in control and management of various health disorders and it depends on plant resources and used as a whole drug[12, 13]. By analysing the phytochemicals which are responsible for therapeutic values found in medicinal plants and scientific validation of folk claims. Which may prove to be a new discovery of unique biomolecule to cure alarming health disorders in future.

Taxonomical Classification, Common Names of *Boerhavia diffusa* [14,15]

Taxonomic hierarchy
Kingdom: Plantae
Class: Angiosperms
Order: Caryophyllales
Family: Nyctaginaceae
Genus: Boerhavia
Species: B. diffusa

Scientific classification
Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Caryophyllales
Family: Nyctaginaceae
Genus: Boerhavia
Species: Boerhaviadiffusa

Common name
- **English:** Red hogweed, Tar Vine, Red Spiderling, Wine flower, Common Hogweed, Hogweed, Pigweed, Spreading hogweed, Spreading hog - weed
- **Odia:** Puruni Saga
- **Hindi:** Gadahpurna, Biskhapara, Shothagni
- **Sanskrit:** Punarnavaa, Shothagni, Varshabhu
- **Telugu:** Atakamamidi, Punar-nava
- **Tamil:** Mookkaratti, Sarandai, Sukuaetti
- **Kannada:** Adakaputta, Adakaputtanagida, Komme, Gonajaali, SanaadikaaBalavadike, Belavadaka, Shavaata, Shivaatike,
- **Nepali:** Punarvaa, Laal Punarnavaa, SaanoPaate, Laal GajPurnee, Aule Saag

Morphological and Anatomical Characteristic Features of the Plant

Boerhavia diffusa is a species of flowering plant in the four o'clock family which is commonly known as punarnava (meaning that which rejuvenates or renews the body in Ayurveda), red spiderling, spreading hogweed, or tarvine. *Boerhavia diffusa* is a perennial herbaceous plant, occurring as a wild weed in tropical region of the world including Peninsula, Afghanistan, Baluchistan, Syria, Tropical Africa, South America, Brazil, Britain and Nepal. In India, it is widely distributed throughout all the part of the country. It is variable, diffusely branched, pubescent or glabrous, prostate herb and distributed upto an altitude of 2000 meter in the Himalayas.

Habit:	A creeping, perennial, much-branched herb with stout fusi form roots.	
Root:	Elongate, stout, fusiform, woody.	
Stem:	Creeping, Branches divaricate, stem purplish, swollen at the nodes, normally 1-1.2 meter long and purplish.	
Leaves:	Long petialed, ovate or oblong-cordate, entire and sinuate, margins slightly pinkish, wavy, lower surface with small, white scales, base rounded.	
Flowers:	Flowers are small, around 5 mm in diameter. Bracteoles, acute. Perianth -tube constricted above the ovary, limb funnel-shaped, dark-pink, with 5 vertical bands outside. Stamens 2 or 3, slightly exserted, unequal. Ovary superior, oblique, ovule 1, erect, stigma.Red, pink or white, in small umbels and terminal panicles.	

Fruits:	Ovate, oblong, five ribbed viscid and glandular anthocarps.	
Seed:	Minute, albuminous with endosperm. Embryo curved	
Inflorescence:	Small umbels forming Corymbose, axillary and terminal panicle.	

Medico- Chemi- Botanical Survey of the Plant

Liver Aliments			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa	Not reported	Hepatoprotective	[16]
B. diffusa (Ethanol extract)	Not reported	Hepatoprotective	[17]
B. diffusa(roots) (Methanol extract)	6,11 dihydroxy, 9, 10, (dimethyl 4-methoxy rotenoid; 3 acetate 2,20-dihydroxy-5-ene 17 -propyl androstane	Anti hepatotoxic	[18]
B. diffusa	Alkaloids (Punarnavine)	Ascites	[19]
Urinary Disorders			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa (roots) (Methanol extract)	Lignans, Liriodendrin, Syringaresinal mono β.D. Glucoside	Diuretic	[20]
B. diffusa (Whole plant) (Ethanol extract)	Boerhavistrol, Boerhadiffusene, diffusarotenoid, Boerhavilanostenyl benzoate	Diuretic	[21]
B. diffusa (Methanol extract)	Not reported	Diuretic	[20]
B. diffusa (roots) (Methanol extract)	Not reported	Diuretic & Cholinergic	[22]
Chemotherapeutic Agents			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa(roots) (Aqueous extract)	Not reported	Antiviral	[23]
B. diffusa(roots) (Ethanol extract)	Glycoprotein	Antiviral	[24]

B. diffusa(roots)	Not reported	Anti amoebic	[25]
B. diffusa (roots) (Chloroform extract)	Rotenoids (Isoflavonoids)	Insecticidal & piscidal activity	[26]
B. diffusa (roots) (Ethanol extract)	Punarnavoside (2-gluco pyrano-4-hydroxy-5Jhydroxy phenyl) propionyl methane	Antifibrinolytic agent	[27]
Abdominal Ailments			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa (roots) (Ethanol extract)	C- Methylflavone	Laxative	[28]
B. diffusa (roots) (Ethanol extract)	Punamavoside	Contraceptive	[29]
Anti-stress Agents			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa (Whole plant) (Aqueous extract)	Punarvanine alkaloids, boerhavic acid, reducing sugars, KN03 tannins	Mitodepressive effects	[30]
B. diffusa(roots) (Aqueous extract)	Punamavine alkaloids, boerhavic acid	Mitodepressive effect	[31]
Miscellaneous			
Plant species	**Chemical constituents**	**Medicinal importance**	**References**
B. diffusa(roots) (Ether extract)	Boeravinone C (12 - hydroxyl rotenoid)	Not reported	[32]
B. diffusa (roots) (Ether extract)	Boeravinone A, B & C (rotenoids)	Not reported	[32, 33]
B. diffusa(roots) (Ethanol extract)	Not reported	Cures corneal ulcers & night blindness	[34]

CONCLUSION

The ethnomedicinal plant *Boerhavia diffusa* of the family Nyctaginaceae, a highly useful plant in the indigenous system of medicine in Odisha, India gives a description of the use of plants and plant parts as medicines. As the name affirmed Punarnava (Punar + Nava). Punar means - once again, nava means becoming new, really because of its multiple benefits and pharmacological actions, Punarnava proved itself as magical natural remedy by Ayurveda. This study will provide information regarding different parts of the plants used for the treatment of various diseases and curing many ailments by generations of physicians and traditional systems of medicine from different ethnic societies for management of various health disorders. The herbal traditional healers have been using several plants and plant parts for treatment of cancer, jaundice, dyspepsia, inflammation, eye disorders, enlargement of spleen, abdominal pain and as an anti-stress agent and has medicinal properties for various ailments (Liver ailments, Urinary disorders, chemotherapeutic ailments and antimicrobial agents etc.)

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RESEARCH ARTICLE

Investigation of Best Fitted Exchange and Correlation Potential for GaAs

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ABSTRACT

This article is on the popular quantum method for electronic structure calculation, Density Functional Theory. This theory can be used to, successfully predict the structural, electronic, magnetic and many other properties of various materials. In density functional theory method of calculation, the exchange and correlation functional has an important role to play. Selecting the correct exchange and correlation functional is a crucial parameter before starting serious verification of properties for different applications. In this work we apply different exchange and correlation functional to check the properties of GaAs. We have used LDA, GGA-PBE and GGA-PW91. The energy values are calculated, band energies are plotted. Total energy value and band gap are found. We also plotted the density of states. All these results found with different exchange functional are compared to check which one gives us the best results.

INTRODUCTION

Understanding the structure and properties of various materials is one of the basic steps for its application in any industry. This can be done with various methods. One of the most popular method is Density Functional Theory (DFT). Computer simulation based on DFT has gained popularity in predicting material properties and behavior under various conditions. This method uses several approximations during its calculations. One of the most important approximations is applied for exchange and correlation potential of the electrons. This value is precisely the difference between the exact and calculated band gap values. In order to find the exact value of exchange and correlation potential, several schemes are developed by different researchers. For example, Local Density Approximation (LDA) [1], Generalized Gradient Approximations (GGA) [2], Perdew–Burke–Ernzerhof (PBE) [3], PW91 [4] etc. These are applicable to different systems under different physical conditions. In this work the focus is on finding the best fitting exchange and correlation potential approximation for GaAs. This will help to get more exact values for all other related properties. Semiconductor materials have been playing an important role in the electronics industry. They have attracted attention since their conductivity can easily be increased and controlled by adding impurities and also, they can operate in various conditions of temperature and pressure without losing its

properties. Semiconductors are mainly classified according to their electrical conductivity and band gap. Group III-V compound semiconductor GaAs has a direct band gap. It attracts the attention because of its suitability in the field of optoelectronic devices. It has found its place in LEDs, lasers, photodetectors, various integrated circuits and filters etc. At ambient temperature and pressure GaAs exists in zinc-blende phase with experimental lattice constant 5.65 A^0 [5, 6]. Different demanding areas like LED lighting, printed electronics and oxide electronics, where GaAs can be used because of its suitable properties. High electron mobility and small dielectric constant are those characteristics of GaAs which makes it suitable for use in high temperature resistance, ultrahigh frequency, low-power devices and circuits [7]. This study started with calculating the lattice constant, band gap, plotting the band structure and plotting the density of states for cubic GaAs. The same calculations were repeated with different schemes like LDA, GGA and PBE. The results were compared and discussed in this work.

Computational Method

Electronic structure calculations can be used to understand the electrical, optical, vibrational and thermal properties of materials under different physical and thermal conditions. First principle methods of calculations using density functional theory (DFT) are popular and dependable methods for studying properties of materials theoretically. We are using a first principle total energy code available for electronic structure calculations i.e., Biovia. Here the calculations are done using DFT with any one of the functional LDA, GGA-PBE and GGA-PW91are done. Valence electrons are taken for calculations and the interaction between the valence electron and the cores is handled with the help of ab initio Vanderbilt pseudopotentials [8]. The calculations here are done using BIOVIA material studio. BIOVIA, a brand of Dassault Systems, is a scientific tool that can be used for research work. Biovia Materials Studio [9] is a tool for modelling and simulation in Materials Science, Physics and Chemistry to study the structure and properties of materials under different conditions. The cutoff values were taken as, kinetic energy cut off of 590.00 eV. A set of 2x2x2 K-points scheme is used for BZ sampling. The exchange and correlation energy per electron is described by Perdew and Zunger [10] parametrization of Monte Carlo calculations of Ceperley and Alder [11] method. The single particle Kohn-Sham [12] equations are solved and the eigen values are taken to interpret bulk band structure and density of states. The calculations for the structural properties of the cubic GaAs, was done taking a face centered cubic crystal. GaAs crystal has a tetragonal unit cell belonging to space group symmetry of T2d F-43m.This compound semiconductor is a combination of gallium of group 3 with orthorhombic trigonal crystal structure and arsenic of group 5 having a trigonal crystal structure. The unit cell is shown in figure 1. The primitive cell is shown in figure 2 with the BZ sampling.

RESULTS AND DISCUSSIONS

Density functional theory (DFT) [13] calculation for electronic properties of GaAs were done. The first step in calculations were carried out to find out the lattice constant of the primitive unit cell. After geometry optimization of the cell, lattice constant is 5.757 A^0 with cubic structure. Fixing of basic parameters like cutoff energy and K points grid were also done. K point set is 4*4*4 for band plot and 8*8*8 for density of states (DOS). These calculations require the determination of wave function and positions of all the valence electrons. The rest of the calculations were done with GaAs crystal with lattice constants as mentioned above.The knowledge of band structure energies is essential for the determination of electronic and optical properties of materials. The energy band calculations were done and plotted for several symmetry points. Non spin polarized calculations were done with energy cutoff value of 590 eV and ultrasoft pseudopotentials were used. The band structure energy of GaAs is found out along direction of high symmetry is shown in figure 3, 4 and figure 5. Figure 3 is for the LDA exchange and correlation potential. Figure 4 and 5 are for GGA-PBE and GGA-PW91 exchange and correlation potentials respectively. Energy is plotted in eV along the Y axis in both the figures. The highest value of valence band is taken as 'zero' along the energy axis. The electronic configuration of Ga is [Ar] $3d^{10}$ $4s^2$$4p^1$and electronic configuration of As is [Ar] $3d^{10}$ $4s^2$ $4p^3$. For this calculation, $3d^{10}$ $4s^2$4p1 electrons of Ga and $3d^{10}$ $4s^2$ $4p^3$electrons of As were taken as the valence electrons. Use of smooth norm-conserving pseudopotential for Ga and As helped in reducing the calculation effort.

The valence band maximum and conduction band minimum occur on the same symmetry line, Gamma, indicating a direct band gap. This is in accordance with published experimental results too [14,15]. The shapes of the energy bands are almost similar in both the cases. The calculated band gap is 0.496 eV for GGA-PBE and 0.478 eV for GGA-PW91 formalism. From the energy band structure, it is found that band gap is0.2eV for LDA formalism. The experimental band gap of cubic GaAs is 1.2 eV to 1.7 eV [14, 15] has been reported. The known drawback of LDA is, it reduces the band gap. This is also observed in this calculation. The smaller band gap compared to experimental value is due to the inherent drawbacks of theoretical calculations. So this can be said that GGA gives better results for energy value calculations and band gap. The total density of states (DOS) of GaAs using first principle DFT with LDA using pseudopotential are shown in figure 6. The same with GGA-PBE and GGA-PW91 are shown in figure 7 and 8 respectively. Here the calculated densities of states are plotted against energy. The dotted line represents the Fermi energy. Both figures are plotted taking Fermi energy as zero on energy axis. The left side of the line is the valence band and the right side represents the conduction band. The charge population of valence band is more than that of the conduction band. This hence indicates the semi-conducting behavior of GaAs.

Looking into the density of state in three general regions can be analysed as follows. The left most region has a strong peak arising from the valence band electrons from As 4s states. The next portion shows a flat band with not much energy variations over the square face of the Brillouin zone. The 3rd region extends upto valence band boundary. This should be the focus as it contributes towards the conductivity. The band gap can also be found from the DOS figure. Same is observed in figure 6 for LDA formalism DOS plot. However, the band gaps are slightly different for both the cases. The energy density is continuous in the conduction band in both the figures. The density of states plot showed many peaks with the maximum peak is at -16 eV in case of GGA and -15eV in LDA. A close look at the conduction band shows there is slight difference in the conduction band. It's mainly dependent on the difference in band gap between the calculations. That's why it appears like the peaks are shifted towards right in case of GGA.

CONCLUSIONS

The first principle DFT calculations were done to study the electronic properties of cubic GaAs. The band gap was found out. The band structure and density of states were plotted for GaAs. All these were done for three different formalisms of exchange and correlation potential. The calculations were done using the Dassault system BIOVIA-Material Studio. Our observations can be summarized as follows. The band plot gave us information that GaAs is a direct band gap semiconductor. The same result is obtained from all the methods. The energy band plot and DOS is compared for different formalisms. The band gap found to be different. Out of both the methods, GGA-PW91 calculations gives band gap values closer to experimental value. The distribution of electronic states in the valence and conduction band is found to be same. For electronic and electrical properties calculations both GGA and LDA will produce same results. But if the property under consideration is verymuch affected by band gap value then one can always choose to proceed with GGA-PW91.

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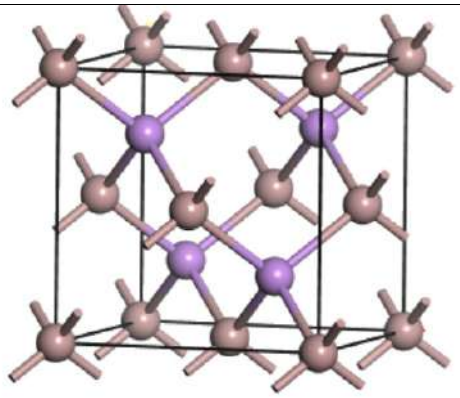

Figure 1: Crystal structure of GaAs

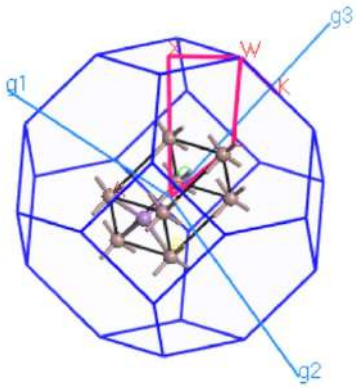

Figure 2: Brillouine zone of GaAs Ga in brown and As in purple.

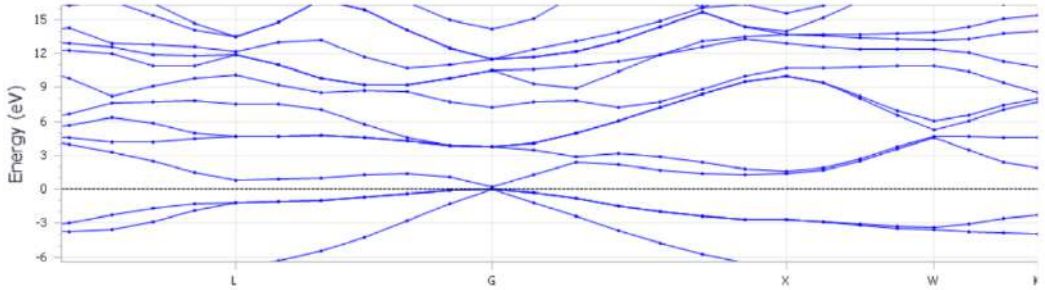

Fig 3: Band structure of GaAs with LDA formalism. The highest value of valence band is taken as zero on the energy axis.

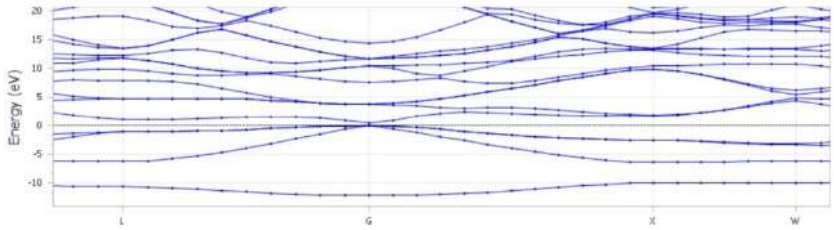

Fig 4: Band structure of GaAs by GGA-PBE formalism. The highest value of valence band is taken as zero on the energy axis.

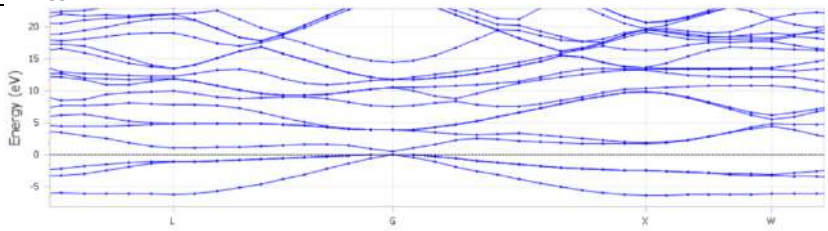

Fig 5: Band structure of GaAs by GGA-PW91 formalism. The highest value of valence band is taken as zero on the energy axis.

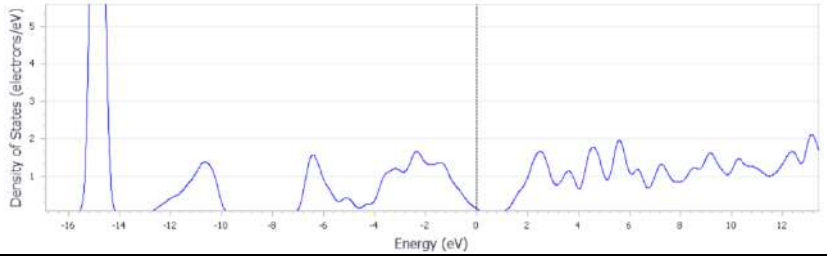

Fig 6: Density of states of GaAs crystal structure with LDA.

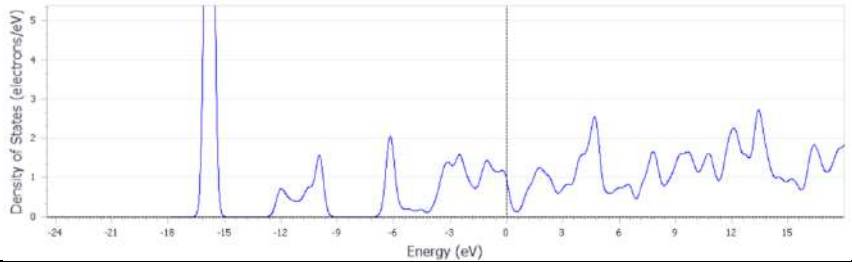

Fig 7: Density of states of SnO2 crystal structure with GGA-PBE.

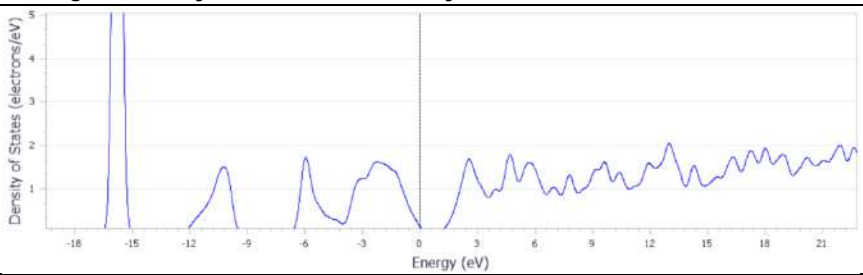

Fig 8: Density of states of SnO2 crystal structure with GGA-PW91.

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RESEARCH ARTICLE

Exploring the Thermophysical Properties of Ionic Liquids for Industrial Applications

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ABSTRACT

Ionic liquids (ILs) are one of the important green solvents and have tremendous application potentials. Thermophysical properties are very much necessary for ionic liquids applications and development. These are the properties that are highly dependent on temperatures, as well as on the cationic and anionic moiety. Furthermore, the carbon chain length, symmetry, attached functional groups, affects significantly the thermophysical properties. Experimental thermophysical property data are also valuable in developing the mathematical model which in turn can be useful to predict the properties without even synthesizing them. Furthermore, accurate thermophysical properties are very much essential in designing industrial equipment of exact dimensions and to develop process control. At this juncture, this article reviewed some of the important thermophysical properties of ionic liquids and highlights how these properties are affected and can be altered by different parameters to get suitable ILs as per the requirements.

Keywords: Ionic liquid, Thermophysical property, Biomass, Synthesis, Viscosity, Surfactant**.**

INTRODUCTION

Since last three decades both academia and industries are focusing on the environmental aspects of their endeavor. As a result, green chemistry is booming and strengthening its footsteps in each passing year. In a quest to develop a green solvent, researchers around the globe found ionic liquid (IL) as one of the suitable candidates. ILs are basically salts that are composed of ions and generally are liquid at room temperature. The exponential growth of ILs publication since the last two decades is the self-testimony and indicates the importance of its potential. ILs have tremendous scope in industrial applications just because they have the capability in tuning the structural moiety. The structural changes eventually provide an opportunity to get a green solvent as per the requirement. High thermal

stability and high liquidous range of ILs provide an enormous opportunity where high temperature plays an inevitable role. Numerous advantages of ILs in terms of thermophysical properties are making scientists consider this as an alternative green solvent. Very low vapor pressure of ILs is also another substantial credibility that favors the industrial applications by generating less toxicity to the environment.[1]Many compounds satisfy the colloquial definition of ILs, however, they are not liquid at room temperature rather melt at a higher temperature and are known as molten salts. As the field evolves, many researchers have named and classified ILs either based on their composition, applications, or behavior. There might be many contradictory views on ILs classifications, but unanimously the scientific and industrial community would admit its potential applications and opportunities. Figure 1 lists some of the commonly used cations and anions in ILs.

Thermophysical Properties of ILs

The potential of any solvents lies with their inherent properties. ILs have the unique ability to modify the structural moiety to meet the required demand of industries and academia. Thermophysical properties are one of the core requirements of ILs before they could be used in any application. These thermophysical properties data are required in designing the industrial equipment to avoid over or under dimensions. Further, thermophysical properties play an essential role in understanding the characteristic behavior of ILs. As ILs are designer solvents and millions of ILs can be possible, it is nearly impossible to synthesize all the ILs, purify them and investigate their properties for required applications. Therefore, a mathematical modeling tool is necessary to predict the properties of ILs. However, some experimental data points are necessary to develop mathematical modeling and then more experimental data points are required to verify the proposed model and to improve it. These are a few important requirements for which thermophysical property investigation is essential. The experimental thermophysical properties can also be used to calculate many other thermophysical properties such as isothermal expansion, isobaric compressibility, fluidity, molar refraction, and so on [2]. Although many thermophysical quantities could be investigated for ILs as depicted in Table 1, few important properties are discussed here.

Density

Density is one of the properties of solvent that is very basic for any type of solvents. Starting from imidazolium ILs to ammonium and pyridinium ILs are investigated at a range of temperatures generally with 5 □ intervals. Most of the literature investigated from 293 K to 343 K and few researchers also have investigated densities beyond these ranges. It has been found that with an increase in temperature, the density of ILs decreases linearly. The experimental densities were also used to fit with empirical equations to validate it. Many researchers also investigated thermophysical properties of pure ILs and the binary mixture made with common organic solvents and water. Calculated properties such as excess molar volume have been determined using density data of the binary system [3–5]. The excess molar volume shows both positive and negative deviation from ideal behavior depending upon the intermolecular interactions between ILs and solvents [6]. The calculated excess molar volume is correlated with the Redlich-Kister polynomial equation and was found to be fitting well by many researchers [7]. The density is a reflection of the molar mass of ILs constituents, a heavier anion such as PF_6 would generate a denser IL as compared to BF_4 containing IL due to the presence of extra heavier atoms in the former anion. Symmetry is also playing an important role in density, more symmetric ions would pack in a tight manner leading to denser ILs.[8,9]Impurities such as halides and moisture/water content in ILs also affect the density measurement [10,11].

Viscosity

Viscosity is the internal resistance of fluids to flow and is considered as one of the transport properties. It has been established that with an increase in carbon chain length of ILs, whether in the cation side or anion side, the viscosity is bound to increase. With increasing carbon chain length, the Van der Waals interactions increases and that eventually leads to an increase in viscosity. Computer simulation investigation shows that the higher viscosity of higher chain length ILs is due to the microstructural arrangement of ILs into non-polar and polar domains [12]. Jacquemin et al. found that for imidazolium cation, bis (trifluoromethane) sulfonimide (NTf2) based ILs have lower

viscosity as compared to BF_4^-, PF_6^-, and $EtSO_4^-$ ions. They also found that ammonium-based ILs are more viscous when compared to their imidazolium counterpart [12]. ILs having symmetrical anions show higher viscosity as compared to similar cations having unsymmetrical anions [13]. Pratap et al. observed that lactam-based ILs have very low viscosity as compared to other reported ammonium and imidazolium ILs. The lower viscosity was attributed to the structure of caprolactam and butyrolactam [14]. Experimental viscosity data are found to be decreasing nonlinearly with increasing temperature and also the experimental data points most of the times fitted well with Vogel–Fulcher–Tamman (VTF) equation[15]. Hydroxyl substituted ILs are generally having more viscosities compared to other substitutions with similar mass and geometry. The reason behind higher viscosity is the tendency of the hydroxyl group to form hydrogen bonding [9]. It also has been observed that anion variation has more influence on viscosity as compared to cation variation. Viscosity is arguably one of the most important properties of ILs from the industrial application point of view. Generally, most of the ILs possess higher viscosity as compared to organic solvents. While low viscous ILs facilitatean easy mass transfer, in some of the applications such as for lubrication purposes high viscous ILs are required [16,17]. However, among the research community, the impression is that the higher viscosity of ILs is one of the most important parameters that is restricting the usage of ILs in some of the applications [18]. Viscosity of ILs is significantly affected by the presence of parts per million (ppm) level of water content in the samples [19,20]. Therefore, utmost care must be taken to keep and make the ILs sample dry before measuring the viscosity.

Surface Tension

Surface tension measures the interactions of constituents that are present on the liquid-air interface. This property is significant as the usage of ILs increasing in interfacial science, colloid chemistry, and biology. The structural non-isotropic nature of ILs contributes significantly to surface tension. Two techniques are most commonly used for the measurement of surface tension of ILs such as the Wilhelmy plate method and the du Nou¨y ring method. Guggenheim's and Eotvos's empirical equations were used to fit experimental surface tension data points [21]. Surface excess energy, surface entropy, and critical temperature were calculated from the experimental data points. It has been observed that by changing both cations and anions the surface tension changes almost equal amounts which indicates that the surface is populated by both the ions. It has also been observed that the hydrocarbon part of ILs tends to populate more at the surface and the ionic/polar part present in the bulk of the liquid. Upon incorporating the hydroxyl group at ILs, the cohesive interaction between the ILs increases that eventually leads to increased surface tension. With an increase in temperature, the surface tension of ILs decreases. Most of the organic solvents possess surface tension below 20 mN m^{-1} at room temperature however, some of the ILs surface tension goes up to 80 mN m^{-1}which is beyond the surface tension of water (72 mN m^{-1}).[22] Similar to other thermophysical properties, many researchers have proposed various methodologies to predict the surface tension of ILs. Among them, QSPR correlation and parachor method is commonly used to predict the surface tension of ILs.[23]

Ionic Conductivity

As ILs are composed of ions, ionic conductivity is one of the inherent properties of ILs by virtue oftheir constituents. Generally, ionic conductivityincreases exponentially with an increase in temperature due to an increase in fluidity. Many researchers have found that ILs composed of smaller ions exhibit higher ionic conductivity compared to longer chain lengths containing ILs [24,25]. Ionic conductivity is the property that is required to define the material as an electrolyte. Tao et al. investigated dicyanamide (DCA) based ILs with different cations. The results show the decrease in ionic conductivity in the following order $[C_4mim] > [C_4m_2im] > N_{4442} > N_{8444}$ and indicates the effect of carbon chain length [26]. Werner et al. found that for $[C_4mim]$ based ILs, the order of ionic conductivity at a given temperature decreases in the order of [DCA] > [TA] > [TfO] > $[PF_6]$ [27]. Forsyth et al. explore the ionic conductivity of different ILs having bis(trifluoromethanesulfonyl)imide as an anion. They found that among the studied ILs, pyrrolidinium-based ILs show higher conductivity followed by imidazolium and ammonium-based ILs [28]. The ionic conductivity trends attributed to the ring and planarity structure of studied ILs. The temperature-dependent conductivity data of ILs is generally fitted well with the VTF equation [29]. Ionic liquid fluidity and conductivity are correlated through the Walden plot. The ILs that are close to the slope of the Walden line indicate the more ionic nature of ILs.If ILs

associate or aggregate themselves, the ionic conductivity decreases as a result of the restricted availability of diffusible ions[28].

Polarity

Polarity is yet another important characteristic property from a solvent point of view and helps to find an optimum solvent for an application. Researchers have investigated the polarity of ILs spectroscopically using different polarity scales such as $E_T(30)$, and E_T^N. Spectroscopic probes/solvatochromic dyes for instance betaine dye and Reichardt's dye are used for determining polarity.[30] Many ILs were found to be less polar than water but more polar than many organic solvents. The complex solvent polarity of ILs was explored by different polarity scales such as di-polarity (π^*), hydrogen bond basicity (β), and hydrogen bond acidity (α) and compared with different solvents. Wilding et al. investigated 12 solvatochromic dyes and measured π^* values for different ILs. The result shows that $[BF_4]$ based ILs possess the highest π^* values and $[NTf_2]$ based ILs possess the lowest π^* values among the studied ILs [31]. Authors also found that for a common anion, the order of π^* values is as follows, morpholinium > imidazolium > pyridinium > pyrrolidinium > phosphonium ionic liquids. Wilding et al. further suggested that β values of ILs are more dependent on anion rather on cation and also strongly affected in the presence of impurities that might come during ILs synthesis.[31] Qingshan et al. found that for acetate anion, the order ofπ^* polarity decreases with increasing the carbon chain length of imidazolium cation in the order of $[C_2mim]$ > $[C_4mim]$ > $[C_5mim]$ > $[C_6mim]$.[32]Ammonium-based protic ILs were found to be more polar than aprotic ILs. Lee et al. showed that the polarity of the ILs decreases upon increasing the temperature. In imidazolium ILs, the C2 position is very crucial in determining the polarity of the medium and reduces the value. Furthermore, the substituted imidazolium ILs have less effect on temperature.

Thermal Stability and Phase Behavior

Thermal stability is one of the marketing values of ILs. Generally, aprotic ILs are more thermally stable as compared to protic ILs as protic ILs contain a loosely bound proton. Thermogravimetric analysis (TGA) determines the melting point of ILs whereas differential scanning calorimetry (DSC) measures the glass transition temperature and phase behavior of ILs. Imidazolium-based ILs are more thermally stable as compared to ammonium-based ILs as investigated by many researchers [33]. Jacob et al. found that anions having more coordinating ability are thermally more stable and bis(trifluoromethylsulfonyl) imide-based ILs are having higher thermal stability [34]. Furthermore, the heat capacities of pyridinium-based ILs linearly dependent on the molar mass of ILs. Many researchers claim that ILs are thermally stable based on TGA data. However, these are short-term thermal stability and the experiment is known as dynamic TGA. To know the actual thermal stability of ILs which can be helpful while doing any industrial-scale operation, a static TGA experiment should be done. In static TGA, the ILs are kept at a particular temperature for a known period of time [35]. Although the mechanism of thermal degradation is complex, in short it may be summed up as the combination of hemolytic cleavage, proton transfer, and autocatalytic effect and also these are depended upon the heating rate while running the dynamic TGA.

Refractive Index

Refractive index is one of the optical properties of ILs and presumably less affected property in presence of water. The refractive index of ILs decreases with an increase in temperature. Molar refraction and free volume can be calculated from refractive index data. Free volume is one of the important parameters and is responsible for gas capture in ILs. As investigated by many researchers with an increase in temperature and molar mass, the refractive index decreases [36]. Dale-Gladstone equation and Lorentz-Lorenz equation were generally used to predict refractive index data points of ILs. Raul et al. studied the refractive index of 14 imidazolium-based ILs [37]. The authors found that the refractive indices increase with increasing carbon chain length on the imidazolium ring for a commonly studied anion. Furthermore, the authors found that for a common imidazolium cation $[C_2mim]$, the refractive indices decrease in the following order $[C_2SO_4]$ > $[C_6SO_4]$ > [OTf] > $[NTf_2]$ > $[BF_4]$.Isabel et al. investigated refractive indices of aminoacid based ILs and found that for common $[C_2mim]$ based ILs, the refractive indices decrease in the following order of [L-Ser] > [L-Pro] > [Gly] > [L-Ala] > [Tau] [38]. The authors also found the decrease in refractive indices

values within the studied temperature range from 293 K to 353 K. Hajime et al. examined 17 different ILs for refractive index measurement between 283 K to 353 K [39]. The results show that for the studied ILs, the refractive indices are more affected by anions rather than the studied imidazolium, pyridinium, and pyrrolidinium ILs. For imidazolium-based cation, the refractive index varies in the following order of SCN ≫ $N(CN)_2$> SO_3OH >SO_3CH_3> $SO_3OC_2H_5$≫ $N(SO_2F)_2$> TCB ≫ $N(SO_2CF_3)_2$> BF_4≫ FAP. The authors also found a good correlation between experimental refractive index and calculated polarizability.

Factors Affecting Thermophysical Properties

Reproducible thermophysical data is very crucial and is necessary for both application points of view and fundamental research. There are some factors we need to be careful about while generating the thermophysical data for ILs. The most important requirement is the purity of ILs. Many times due to the low volatility of ILs, they were difficult to distillate for purification at ambient conditions. Therefore, a sophisticated setup such as the Kugelrohr apparatus is required for distillation at low pressure. Chloride is one of the common impurities that is associated with chloride-based ILs. The small amount of chloride may affect the results of the end product drastically such as hydrogenation reaction, Heck-type reactions, etc.[40] Chloride impurity also affects the thermophysical properties. Water is one of the common impurities present in ILs. Even commercial ILs used to have water as impurity after a few days of opening if not maintained properly. The hygroscopic nature of ILs is the main and presumably the only reason why water is present as one of the common impurities. The purity, chloride, and water content should be monitored each time before thermophysical property determination that would help other researchers to reproduce the data.

Theoretical Calculation of Thermophysical Properties

It has been envisaged that millions of ILs can be possible by combining and altering the cations and anions. Furthermore, it is practically impossible to synthesize all of the ILs and measure their thermophysical properties as per the application requirement. Therefore, it is very much essential to have a predictive model to estimate the thermophysical properties and then synthesize the ILs. Gardas and Coutinho developed a group contribution method to calculate thermophysical properties of ILs by exploring the structure-property relationship [41]. For each of the properties separate equations have been proposed to get the parameters based on the available experimental data points. Then the parameters are optimized for the different moiety of ILs. Based on this group contribution method, several properties such as density, viscosity, surface tension, ionic conductivity, speed of sound, isothermal compressibility, and thermal conductivity, etc. were predicted at different temperatures and found to be in good agreement with the experimental data points. Quantitative structure-property relationship (QSPR) models have also been proposed and investigated for the prediction of thermophysical properties with good accuracy for ILs using different descriptors.[10,11]

Industrial Applications of ILs

Currently, many ILs are being used in several industries in different stages such as in the R&D stage, pilot scale, and commercial scale. Whatever may be the application stages of ILs, the thermophysical properties are inevitable to know and measure. Without proper knowledge of thermophysical properties, equipment design and process development in industrial setup are difficult to make. All of the thermophysical properties have their weightage for a particular application. For instance, the ILs that are used as lubricating fluids need to have high viscosity and those are used in electrochemical devices need to have good electrical conductivity. Some of the industrial usages of ILs areas are listed hereafter. ILs are used as antistatic agents upon mixing with resins. The low volatility and high heat resistant nature of ILs favor the application. Due to the high electrical conductivity and wide electrochemical window of ILs, they are potentially used in capacitors, lithium-ion batteries, dye-sensitized solar cells, electroplating and so on [42–44]. Furthermore, the polarity nature, and low vapor pressure are among the very few properties that make ILs to be used as solvents for various organic synthesis [1]. Furthermore, the solvent nature of ILs makes them a candidate as a potential gas sweetening agent in different industries [45]. The dissolving capacity and solubility nature of ILs make them suitable as a cellulose/biomass dissolving agent and used in the textile industry.[46]The

functional nature of ILs makes them suitable to be used as a catalyst in various reactions.[1]Due to the polarity, solubility, and low toxicity nature of ILs they are used in pharmaceutical industries [47],[48]. The most attractive feature of ILs is their tuning ability and by altering the structural moiety of ILs, their thermophysical properties can be tuned for the desired applications.

CONCLUSIONS

Thermophysical properties are the most important aspects of ILs and are very much necessary to understand the ILs and to make use in any applications. Purity is the foremost requirement for determining accurate thermophysical properties. Impurity such as chlorine plays an important role in deviating from the exact value of ILs. The trace amount of chlorine can influence the thermophysical data in a great way. As most of the ILs are hygroscopic in nature, exposure to the atmosphere can lead to moisture absorption and that eventually reflects in the thermophysical data. As ILs are known to be designer solvents, by changing cation and anion the thermophysical properties are greatly vary as the hydrogen bonding interaction, Van der Waals interaction changes. Temperature variation also affects the thermophysical properties, some are negatively affected and some thermophysical properties are positively affected. In conclusion, ILs are truly designer solvents and we can make the ILs as per our academic and industrial requirements. Furthermore, thermophysical data points are very much essential in developing the modeling that can predict thermophysical properties even without synthesizing them.

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Table 1: Commonly studied thermophysical properties of ILs.

Volumetric Properties			**Transport Properties**		
Sl. No.	Property Name	Symbol	Sl. No.	Property Name	Symbol
1	Density	ϱ	18	Dynamic viscosity	η
2	Free volume	V_f	19	Kinematic viscosity	ν
3	Molar volume	V_m	20	Ionic conductance	λ
4	Excess molar volume	V^E	21	Self-diffusion coefficient	D
5	Partial molar volume	$\tilde{V}_i$	22	Thermal diffusivity	α
6	Apparent molar volume	$\phi\tilde{V}_i$	23	Thermal conductivity	k
7	Adiabatic compressibility	β_T	24	Fluidity	η^{-1}
8	Coefficient of thermal expansion	α			
Other Properties					
9	Glass transition temperature	T_g	25	Speed of sound	u
10	Decomposition temperature	T_d	26	Enthalpy of fusion	ΔH_{fusion}
11	Surface tension (air-liquid)	σ	27	Enthalpy of vaporization	ΔH_{vap}
12	Surface energy	S^s	28	Enthalpy of dilution	ΔH_{dil}
13	Surface entropy	E^s	29	Heat capacity at constant volume	C_v
14	Parachor	P	30	Heat capacity at constant pressure	C_p
15	Osmotic coefficient	ϕ_m	31	Critical pressure	P_c
16	Refractive index	n_D	32	Critical temperature	T_c
17	Relative permittivity	ε	33	Hydration number	N_w

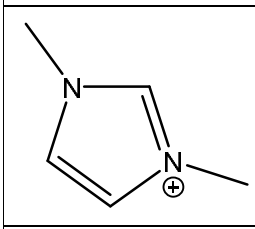
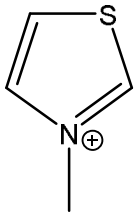
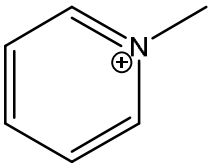
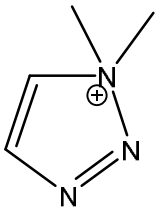
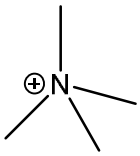
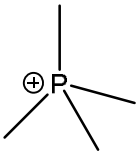
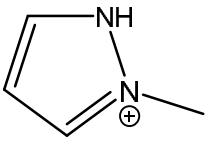
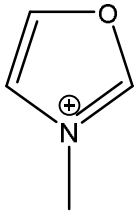
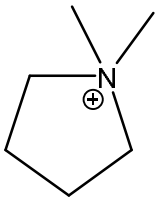
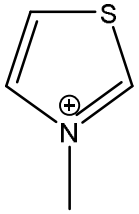
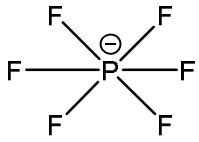
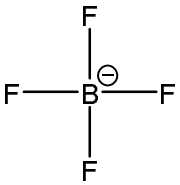
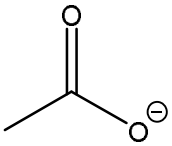
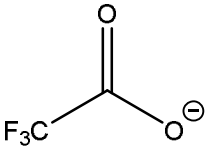
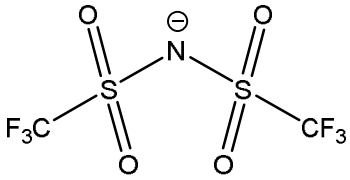
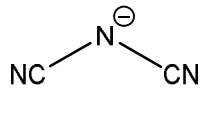
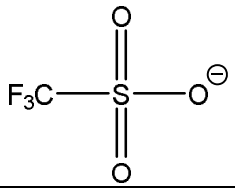
Commonly used Cations				
				
Imidazolium	Thiazolium	Pyridinium	Triazolium	Ammonium
				
Phosphonium	Pyrazolium	Oxazolium	Pyrrolidinium	Thiazolium
Commonly used Anions				
				F^-, Cl^-, Br^-
Hexafluorophosphate	Tetrafluoroborate	Acetate	Trifluoroacetate	Halides
			NO_3^-	
Bis((trifluoromethyl)sulfonyl)imide	Dicyanamide	Trifluoromethanesulfonate	Nitrate	

Figure 1: Examples of commonly used cations and anions in ILs

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RESEARCH ARTICLE

Verification of Suitable Exchange and Correlation Potential for FeO

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ABSTRACT

Density Functional Theory is the most popular method of calculation of material behaviour in Quantum Mechanics. This theory can be used to, successfully predict the structural, electronic, magnetic and many other properties of various materials. The different exchange and correlation functional LDA, GGA-PBE and GGA-PW91 were used to calculate and check for the most suitable one for FeO. The energy plot and Density of states (DOS) were also plotted.

INTRODUCTION

Transition metals oxides are materials having strong electron-electron correlations. Iron oxide (FeO) is one of those transition metal oxide that has potential use in magnetic devices like magnetic tunnel junction [1]. It occurs in 16 different phases [2]. Different phases occurs with different structures and slightly different properties too [3]. This work focuses on using the popular Quantum Mechanics method, Density Functional Theory to calculate the structural and electronic properties of FeO. Computer simulation based on DFT is the most popular method to study various materials and predict their properties under various conditions. DFT has various approximations at different stages of its calculations. One of the most important approximations is applied for exchange and correlation potential of the electrons. In order to find the exact value of exchange and correlation potential, several schemes are developed by different researchers. For example, Local Density Approximation (LDA) [4], Generalized Gradient Approximations (GGA) [5], Perdew–Burke–Ernzerhof (PBE) [6], PW91 [7] etc. In this work the focus is on finding the best fitting exchange and correlation potential approximation for FeO. This study started with calculating the lattice constant, band gap, plotting the band structure and plotting the density of states for cubic FeO. The same calculations were repeated with different schemes like LDA, GGA and PBE. The results were compared and discussed in this work.

Computational Method

To calculate the lattice constant, band structure, band gap and density of states we used Density functional theory. We are using a first principle total energy code available for electronic structure calculations i.e., Biovia. BIOVIA, a

brand of Dassault Systems, is a scientific tool that can be used for research work. Biovia Materials Studio [8] is a tool for modelling and simulation in Materials Science, Physics and Chemistry to study the structure and properties of materials under different conditions. In order to get more clarity on suitability of exchange and correlation potential, we used the functional LDA, GGA-PBE and GGA-PW91to do the calculations with DFT. Before starting the calculations, the structural optimization was done with GGA and LDA. Valence electrons are taken for calculations and the interaction between the valence electron and the cores is handled with the help of ab initio Vanderbilt pseudopotentials [9]. The cutoff values were taken as, kinetic energy cut off of 571.4 eV. A set of 2x2x2 K-points scheme is used for BZ sampling. The exchange and correlation energy per electron is described by Perdew and Zunger [10] parametrization of Monte Carlo calculations of Ceperley and Alder [11] method. The single particle Kohn-Sham [12] equations are solved and the eigen values are taken to interpret bulk band structure and density of states. A face centered cubic FeO crystal is taken for calculations. After the geometry optimization the fcc primitive cell is used for calculations. Psedo atomic calculation are performed for Fe atom $3d^6$ $4s^2$ and Pseudo atomic calculation performed for O 2s2 2p4. The unit cell is shown in figure 1. The primitive cell is shown in figure 2 with the BZ sampling.

RESULTS AND DISCUSSIONS

Density functional theory (DFT) [13] calculation for electronic properties of FeO were done. The first step in calculations were carried out to find out the lattice constant of the primitive unit cell. After geometry optimization of the cell, lattice constant is 4.332 A^0 with cubic structure. Fixing of basic parameters like cutoff energy and K points grid were also done. K point set is 4*4*4 for band plot and 8*8*8 for density of states (DOS). These calculations require the determination of wave function and positions of all the valence electrons. The rest of the calculations were done with FeO crystal with lattice constants as mentioned above.The knowledge of band structure energies is essential for the studying the electronic and optical properties of materials. The energy band calculations were done and plotted for several symmetry points. Non spin polarized calculations were done with energy cutoff value of 590 eV and ultrasoft pseudopotentials were used. The band structure energy of FeO is found out along direction of high symmetry is shown in figure 3, 4 and figure 5. Figure 3 is for the LDA exchange and correlation potential. Figure 4 and 5 are for GGA-PBE and GGA-PW91 exchange and correlation potentials respectively. Energy is plotted in eV along the Y axis in both the figures. The highest value of valence band is taken as ‘zero’ along the energy axis. The maxima of valence band and minima of conduction band do not occur on the same symmetry line. There is no band gap found in any of the calculations. This may be due to the fact that the electron-electron interaction is underestimated for transition metals. The total density of states (DOS) of FeO using first principle DFT with LDA, GGA-PBE and GGA-PW91 are shown in figure 6 to 8. Here the calculated densities of states are plotted against energy. The dotted line represents the Fermi energy. Both figures are plotted taking Fermi energy as zero on energy axis. The left side of the line is the valence band and the right side represents the conduction band. The charge population distribution shows the metallic behaviour of FeO.

CONCLUSIONS

The first principle DFT calculations were done to study the electronic properties of cubic FeO. No band gap was found out and the DOS indicates towards the metallic characteristics. The band structure and density of states were plotted for FeO. All these were done for three different formalisms of exchange and correlation potential. The calculations were done using the Dassault system BIOVIA-Material Studio. Our observations can be summarized as follows. Out of the three methods, the distribution of electronic states in the valence and conduction band is found to be same. For electronic and electrical properties calculations both GGA and LDA will produce exactly same results. But if the property under consideration is verymuch affected by band gap value then one can always choose to proceed with GGA-PW91.

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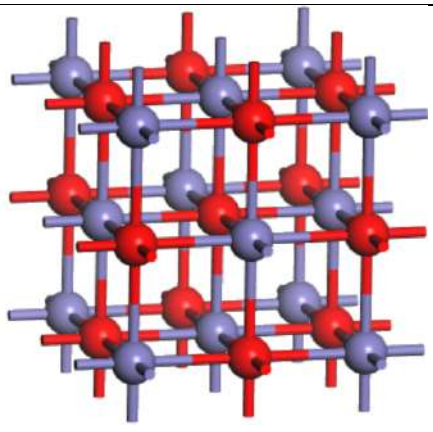

Figure 1: Crystal structure of FeO

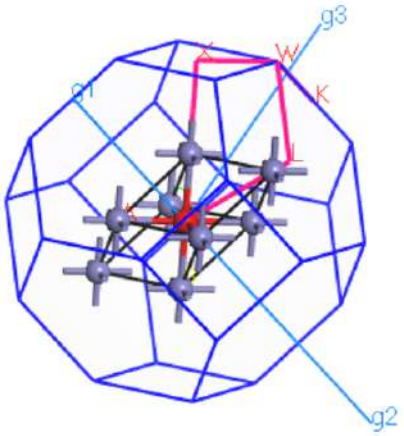

Figure 2: Brillouine zone of FeO
Fe in blue and O in red.

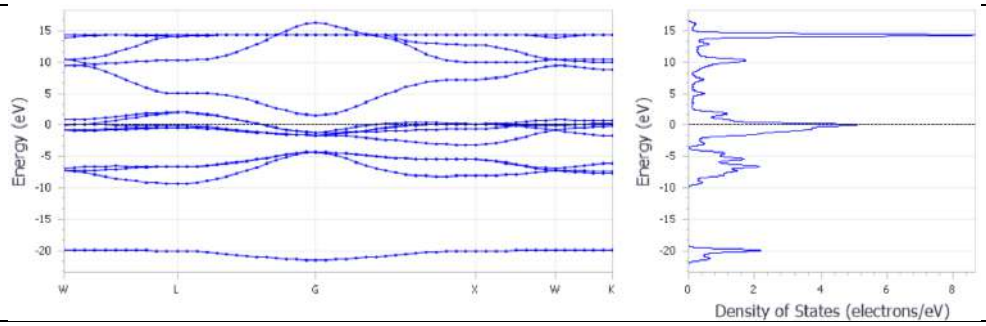

Fig 3: Band structure of FeO with LDA formalism. The highest value of valence band is represented as zero eV of energy axis. Right side shows the density of states.

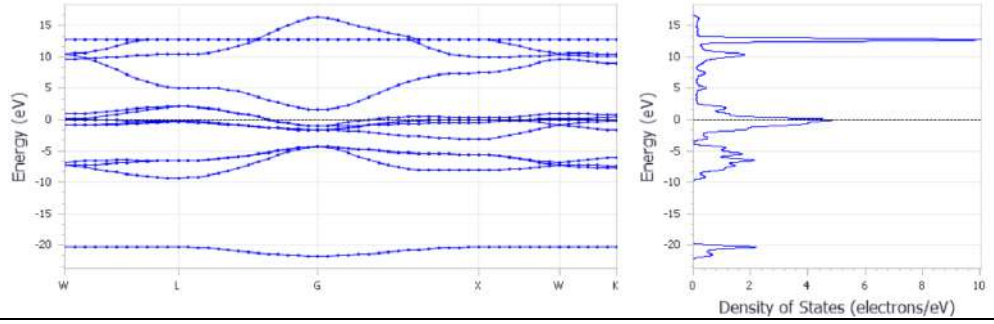

Fig 4: Band structure of FeO by GGA-PBE formalism. The highest value of valence band is represented as zero eV of energy axis.Right side shows the density of states.

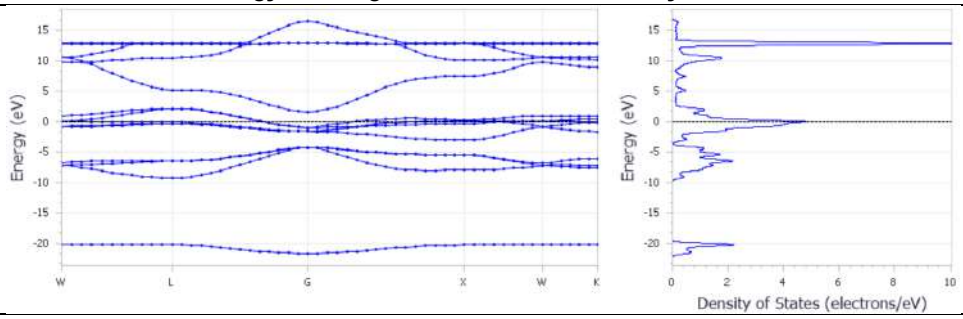

Fig 5: Band structure of FeO by GGA-PW91 formalism. The highest value of valence band is represented as zero eV of energy axis. Right side shows the density of states.

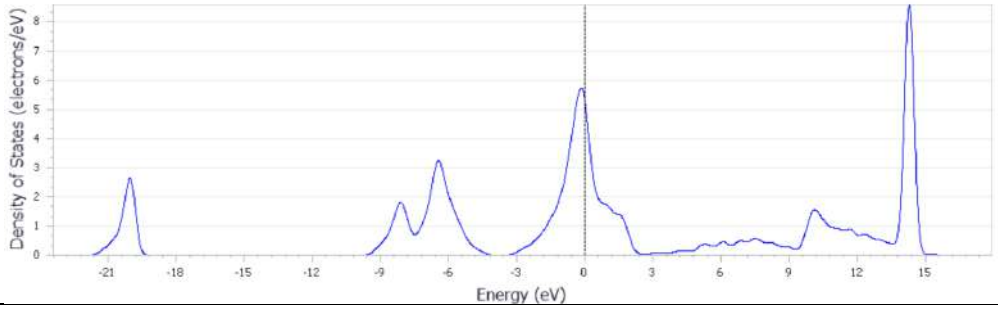

Fig 6: Density of states of FeO crystal structure with LDA.

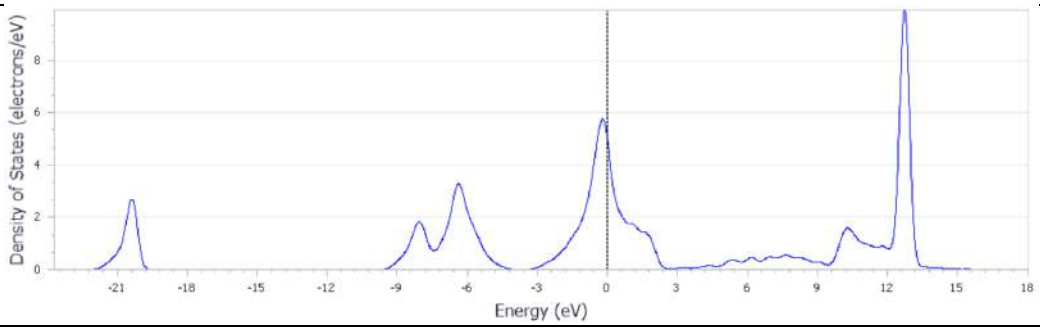

Fig 7: Density of states of FeO crystal structure with GGA-PBE.

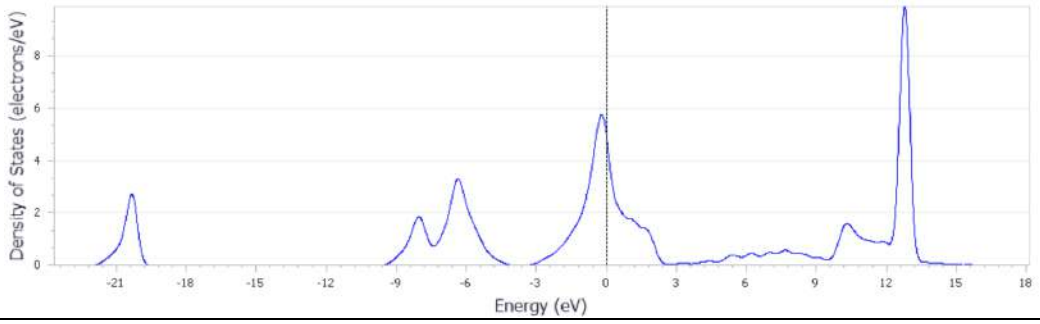

Fig 8: Density of states of FeO crystal structure with GGA-PW91.

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RESEARCH ARTICLE

Investigation of Solubility Parameter of Hexafluoro Phosphate Based Ionic Liquids through Molecular Dynamics Simulation

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ABSTRACT

Ionic liquids being a neoteric solvent were investigated in this work. Solubility is one of the fundamental property of any solvent and is need to understand before it could be used as a potential solvent. To exploit the solvent capacity of ILs for several industrial applications starting from drug solubilization, gas sweetening, and biomass dissolution, the solubility parameter of the ILs need to be exploited for fundamental understanding. Solubility parameter of hexafluorophosphate based ILs were calculated by molecular dynamics simulation using Materials Studio software. Amorphous cell module and Forcite modules were used for the geometry optimization, cohesive energy density calculation, followed by solubility parameter determination. The results were compared with the literature and is found to be under good agreement. The results show that for all the studied ILs, the solubility parameter values decreases with increasing carbon chain length on imidazolium ringof the cations. The results were also analyzed on the basis of structural changes of ILs. These results would be highly beneficial in enhancing the applicability of ILs as a solvent.

Keywords: Ionic liquid, Solubility Parameter, Hildebrand, Hansen, Density.

INTRODUCTION

Ionic liquids (ILs) are being considered as green solvent since past two decades that are composed of organic cations and organic/inorganic anions [1]. Low volatility, high liquidous range, high ionic and thermal conductivity are few properties that make ILs a suitable industrial solvent [2-6]. Furthermore, the tunable ability of ILs make it a suitable entity that can be utilized in a spectrum of applications starting from biomedical science, energy storage device, organic synthesis, gas sweetener and so on. Several thousand research articles have been published so far that are based on fundamental understanding and application perspective of ILs. However, complete understanding of ILs properties are still need to be achieved. In this regard, solubility is one of the very fundamental properties that need to be exploited for ILs as they are mainly regarded as solvent.

In a qualitative sense, solubility refers to the ability of a solvent to separate the solute molecules and make a homogenous solution. To make this happen, solvent molecules should have potential to overcome the energy with which the solute molecules are associated with. The concept "like dissolves like" holds true when the solvent has equal or nearly equal energy with which the solute molecules are combined and also the solvent has the inherent potential to get rid of attraction among the solvent molecules itself.Solubility parameter of a solvent is a numerical value that indicates the relative solvency capacity of a solvent. The solvent and solute having close solubility parameter are readily soluble.Hildebrand solubility parameter take into account the cohesive energy density which in turn is related to enthalpy of vaporization [7,8]. Mathematically Hildebrand solubility parameter is expressed as;

$$\delta = \sqrt{c} = \sqrt{\frac{\Delta H_V - RT}{V_m}} \quad (1)$$

Where, c is the cohesive energy density, ΔH_V is enthalpy of vaporization, V_m is molar volume, R is gas constant, and T is temperature. Hansen solubility parameter is another solubility scale that divide Hildebrand solubility values into three different components such as dispersion force component, a hydrogen bonding component, and a polar component [7,9]. Mathematically, Hansen solubility parameter is given as;

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (2)$$

Where, $\delta_t$is the total Hildebrand parameter,$\delta_d$is the dispersion component,$\delta_p$is the polar component, andδ_h is the hydrogen bonding component. ILs have been successfully used in gas capture, drug carrier agent, biomass dissolution etc [10,11,12]. and in all of the cases solubility play an important role. Therefore, solubility parameter determination of ILs is very much necessary from both fundamental understanding and application point of view. For any particular application, solubility parameter would help in screening ILs as a suitable solvent from the pool of millions of ILs. Solubility parameter can be determined both experimentally and theoretically [13]. In experimental way both direct method and indirect method can be employed to determine the solubility parameter. Direct method measurement of solubility parameter for ILs is difficult as it involves the measurement of enthalpy of vaporization. Indirect method measurement can be performed by using inverse gas chromatography technique [7,8].

As millions of ILs can be possible, it is difficult to measure the solubility parameter of ILs experimentally. Therefore, theoretical investigation of solubility parameter of ILs is a feasible and affordable way out.Many theoretical techniques have been proposed to estimate solubility parameters of ILs that include PC-SAFT and Non-random Hydrogen bonding (NRHB) model, Regular Solution Theory, and Lattice Energy Model [14-17]. However, in all of the above mentioned techniques some experimental data is required to estimate the solubility parameter. Therefore, a complete theoretical solubility parameter calculation is more preferable to save money and time. Molecular dynamic simulation has the capability to estimate the solubility parameter of ILs without any experimental data points. Therefore, in this work we proposed to estimate the solubility parameter of ILs using Accelrys Materials Studio software through molecular simulation.

Computational Details

The ionic liquids used for computational calculation are considered as an equimolar mixture of cations and anions. The name of cations, anions followed by ILs name and abbreviation are illustrated in table 1. The ILs ions were constructed using builder module of Materials Studio. Thereafter, the individual ions were geometry optimized using COMPASS forcefield of Forcite module. The geometry optimized structures of used cations and anions are depicted in Figure 1. Electrostatic potential charges were calculated and assigned to each atoms and the charges were kept constant during follow up calculations. Bulk ionic liquids model were created using Amorphous cell module of Materials Studio software. The density data of ILs as mentioned in Table 1, were used from literature to construct the Amorphous cell [18,19]. However, the density of ILs can also be calculated by molecular dynamics (MD) simulation using NPT ensemble of Materials Studio [9,20]. For all the ILs, 20 cations and 20 anions were used to construct the

simulation model. The systems were equilibrated for 100 ps using NVE ensemble where number of particles (N), system's volume (V), and total energy of the system (E) were conserved. To determine cohesive energy density followed by solubility parameter, NVT molecular dynamics were performed for another 100 ps where number of particles (N), system's volume (V), and temperature (T) were kept constant.The simulation was runt at 298.15 K and using Andersen thermostat. Electrostatic and van der Waals terms were calculated using Ewald, and Atom based summation method. Cohesive energy density was then calculated using the same Forcite module and solubility parameters of ILs were extracted from the resulting trajectories. As ILs were treated as a combination of cations and anions, the intramolecular interaction energies (arises due to interaction among same cations/anions) were not included for cohesive energy density calculation as mentioned above. Therefore, blend module was used to calculate the intramolecular interaction energies and added to the cohesive energy density for correction. The solubility parameter was then calculated using the equation 1 from the corrected cohesive energy density that includes intramolecular interaction energies of ILs. A representative example of Amorphous Cell containing 1-Butyl-3-methylimidazolium hexafluorophosphate (BMIMPF6) IL is depicted in Figure 2.

RESULTS AND DISCUSSION

To validate our methodology, we have calculated solubility parameters for imidazolium based ILs using similar methodologies as described above. As can be seen from Table 2, the solubility parameters values show little deviations from that of literatures probably due to some differences in selected parameters during simulations. The total solubility parameter as calculated for ILs is the combination of two components such as van der Waals (dispersive) and electrostatic component. The electrostatic component again is the combination of hydrogen interaction component and coulombic interaction component. As can be seen from Table 2, the total solubility parameter for hexafluorophosphate based ILs goes on decreasing slowly with increasing the carbon chain length on imidazolium ring. This decreasing trend of solubility parameter is consistent with literature data as well for other ILs. The decreasing trend of solubility parameter with increasing carbon chain length could be attributed to the decreasing contribution from electrostatic components. Furthoremore, on an average per carbon chain length there is an increment of approximately 0.26 in solubility parameter values among the studied ILs. The plausible explanation for this behavior could be decrease in electrostatic interactions with increasing size of imidazolium cation.The results indicate that with increasing size of the solvent ILs, the solvency capacity decreases presumably due to increase in van der Waals interactions among ILs. However, it can be observed from the Table 2, that with increasing carbon chain length from ethyl to octyl on imidazolium ring the solubility parameter does not increase significantly. Therefore, we may infer that the solubility parameter and size of ILs are not proportionately related. The results obtained from this investigation is corrobarted with the experimental evidences from several literature that suggest 1-Ethyl-3-methylimidazolium based ILs are more suitable as a solvent either for biomass dissolution or gas capturing when compared to their higher chain length counterpart [22].

CONCLUSION

Hexafluorophosphate based imidazolium based ILs are considered for solubility parameter determination using Materials Studio software. Amorphous Cell module was used to construct ILs in bulk phase and Forcite module was used for geometry optimization, and MD simulation. Using NVT ensemble and experimental density from literature, the cohesive energy density and solubility parameters were calculated. Correction terms also included for ILs considering all the interaction energies. To validate our methodologies, we have compared the solubility parameters data with literature for imidazolium based ILs and the result shows good agreement with the literature. The results for studied ILs shows that with increasing chain length of cation, the solubility parameter value decreases. These obtained solubility parameter results would be highly valuable while considering ILs as solvent whether in drug solubilization, gas absorption, biomass dissolution, or similar kinds of work.

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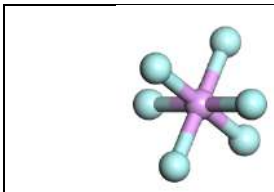
Table 1: The cations, anions, ionic liquids, abbreviations and density of ILs at 298.15 K.

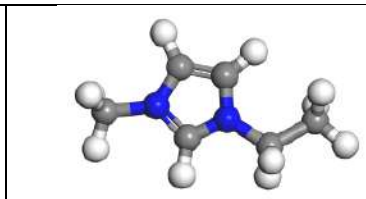
Cation	Anion	Ionic Liquid	Abbreviation	Density [18,19] / g.cm^{-3}
1-Ethyl-3-methylimidazolium	hexafluorophosphate	1-Ethyl-3-methylimidazolium hexafluorophosphate	EMIMPF6	1.492
1-Butyl-3-methylimidazolium	hexafluorophosphate	1-Butyl-3-methylimidazolium hexafluorophosphate	BMIMPF6	1.389
1-Hexyl-3-methylimidazolium	hexafluorophosphate	1-Hexyl-3-methylimidazolium hexafluorophosphate	HMIMPF6	1.287
1-Octyl-3-methylimidazolium	hexafluorophosphate	1-Octyl-3-methylimidazolium hexafluorophosphate	OMIMPF6	1.24

Table 2: Solubility parameters of Imidazolium ILs.

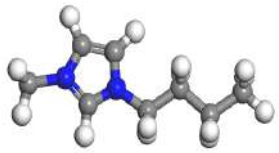
Sl. No.	Ionic Liquid	δ (this work)	δ (Literature)
1	1-Ethyl-3-methylimidazolium hexafluorophosphate	20.22	20.78 [17]
2	1-Butyl-3-methylimidazolium hexafluorophosphate	19.58	19.11 [14,21]
3	1-Hexyl-3-methylimidazolium hexafluorophosphate	19.10	19.98 [16,7]
4	1-Octyl-3-methylimidazolium hexafluorophosphate	18.64	18.25 [22]



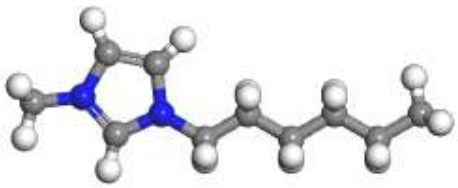
hexafluorophosphate



1-Ethyl-3-methylimidazolium



1-Butyl-3-methylimidazolium



1-Hexyl-3-methylimidazolium

1-Octyl-3-methylimidazolium

Figure 1: Geometry optimized structures of cations and anions of ILs used for calculation.

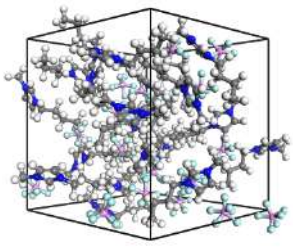


Figure 2: Bulk IL 1-Butyl-3-methylimidazolium hexafluorophosphate (BMIMPF6) prepared in Amorphous cell module of Materials Studio software.

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RESEARCH ARTICLE

Suitable Exchange and Correlation Potential for ZnSe - A DFT Study

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ABSTRACT

This work reports the theoretical study of the popular quantum method for electronic structure calculation, Density Functional Theory to study zinc blend ZnSe crystal. Its structural and electronic properties are studied computationally. In order to check the best fitted exchange and correlation functional for ZnSe, we have used LDA, GGA-PBE and GGA-PW91. The electronic structure calculation using the three approximations are done. Lattice constant and band gap were calculated. Band energy and density of states were plotted too.

INTRODUCTION

The advancement in computational simulations have made it possible to study the structural and electronic properties of compounds with excellent accuracy. This helps in prediction of material properties and hence their uses. Zinc selenide (ZnSe) is an II–VI compound semiconductor which is in focus of researchers because of its technological importance [1,2]. ZnSecrystallizes in two different forms, zinc-blende and Wurtzite. Of these forms, zinc-blende is the most stable, and as such, the most suitable for studying their electronic properties. ZnSe is used for LED and diode lasers too. Because of its wide transmission wavelength range, it is used in infrared optical materials too. Density Functional Theory (DFT)[3] is the most popular quantum mechanical method totheoretical prediction of material properties. This includes different approximations to produce accurate values. But variation in results has been observed and to minimize it the best fit approximation can always be a good finding.

One of the most important factors to be calculated accurately is the exchange and correlation potential of the electrons. Different schemes available for this are Local Density Approximation (LDA) [4], Generalized Gradient Approximations (GGA) [5], Perdew–Burke–Ernzerhof (PBE) [6], PW91 [7] etc. In this work the focus is on finding the suitable exchange and correlation potential for ZnSe which will lead to more exact values for other related properties. This study started with calculating the lattice constant, band gap, plotting the band structure and plotting the density of states for cubic ZnSe. The same calculations were repeated with different schemes like LDA, GGA-PBE and GGA-PW91 and compared.

Computational Method

First principle methods of calculations using density functional theory is used for this study. Here the calculations are done using DFT with any one of the functional LDA, GGA-PBE and GGA-PW91are done. The calculations here are done using BIOVIA material studio. BIOVIA, a brand of Dassault Systems, is a scientific tool that can be used for research work. Biovia Materials Studio [8] is a tool for modelling and simulation in Materials Science, Physics and Chemistry to study the structure and properties of materials under different conditions. The zinc blend ZnSe crystal with two atoms per unit cell belongs to group F-43M. The crystal structure is shown in figure 1. The primitive cell is shown in figure 2 with the BZ sampling. Optimized K-point grid chosen for the calculations is 4*4*4 for BZ sampling. Norm-conserving pseudopotentials were used for the calculations considering the valence electrons of Zn and Se. The cutoff energy value taken as 353.699eV.The single particle Kohn-Sham [9] equations are solved and the eigen values are taken to interpret bulk band structure and density of states. The unit cell is shown in figure 1. The primitive cell is shown in figure 2 with the BZ sampling. The optimized structures were obtained for each scheme.

RESULTS AND DISCUSSIONS

In this study the main concentration is on structural, band energy plot, calculating band gap and total density of states by using DFT. To begin with the zinc blend crystal of ZnSe was considered and geometry optimization was done. Thus, generated primitive cell was taken for all calculations. The lattice constant is found to be 4.065 A^0. Slightly different K-point sets are taken in different cases like 4*4*4 for band plot and 8*8*8 for density of states (DOS).The valence electrons involved in calculations are 3d10 4s2 orbitals of Zn and 4p4 orbitals of Se. The minimum energy of bulk ZnSe calculated at the equilibrium lattice constant. The energy band structure calculations were done for ZnSe with different schemes. The band energy plots are shown in figure 3, 4 and figure 5 along with the respective DOS plot. Figure 3 is for the LDA exchange and correlation potential. Figure 4 and 5 are for GGA-PBE and GGA-PW91 cases respectively. Energy is plotted in eV along the Y axis in all the figures. The energy calculations are done for 16 electrons and plotted for different high symmetry directions.

From the energy band plot, it can be seen that the maximum energy of valence band is occurring at high symmetry point Γ. The minimum energy value in conduction band also occur on the same symmetry line, Γ. This shows that ZnSe has a direct band gap which is obtained in all the three figures and also same as experimental observation [10]. Shape of energy bands are similar in all the three cases. However, the obtained value of band gap is different. It is 0.865 eV obtained from LDA, 1.121 eV for GGA-PBE and 1.063 eV for GGA-PW91. The experimental value is 2.82 eV.So, it can be easily observed that GGA-PBE gives the closest results to experiment. The total density of states plots are shown in the same figures. Here the calculated densities of states are plotted against energy. The valence band has two parts with some gap inbetween. One sharp peak can be seen inside the valence band. The position of the peak is almost same in all the three figures. The occupation in the conduction band is also same. This indicates most of the properties can be studied with any of the three schemes. But optical properties and electronic properties can be better predicted with GGA-PBE.

CONCLUSIONS

From the first principle DFT calculations were done for ZnSe bulk crystal in zincblend phase and the followings were concluded.DFT calculations were done with LDA, GGA_PBE and GGA-PW91 for exchange and correlation potential. The band plot shows that ZnSe is a direct band gap semiconductor. The lattice constant is in good agreement with experimental value. Calculated band gap was found to differ for different exchange and correlation potential. However, the shape of the overall band plot remains the same in all the cases. The DOS curve reflects that the distribution of electronic states in the valence and conduction band is found to be same from all the three formalisms LDA, GGA_PBE and GGA_PW91. The band gap value obtained from GGA-PBE is closest to the experimental value. This helped us to conclude that amongst these three formalisms considered here, GGA-PBE is the most preferred one especially for electronic and optical properties verification of ZnSe.

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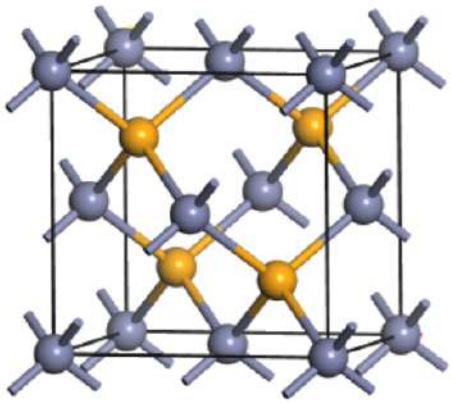

Figure 1: Crystal structure of ZnSe

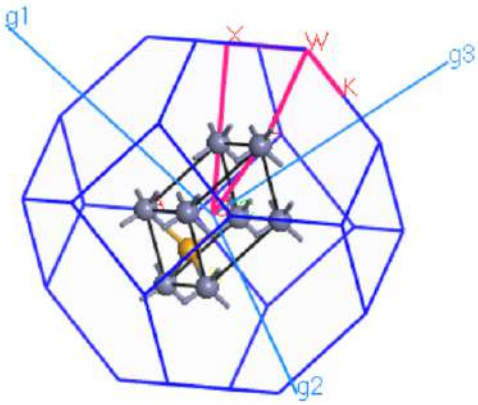

Figure 2: Brillouine zone of ZnSe primitive cell. Zn atoms are in grey color and Se are in yellow.

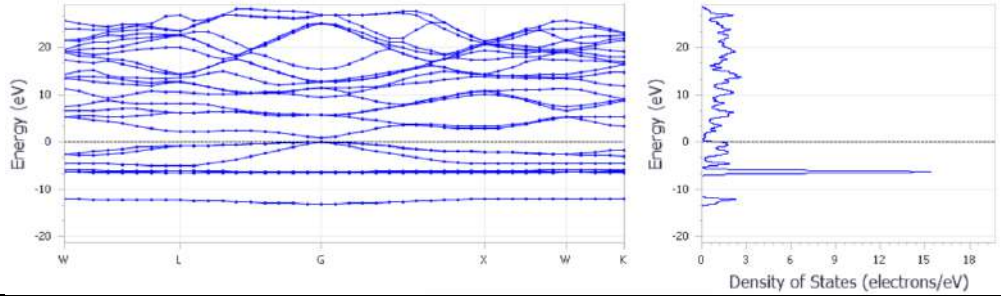

Fig 3: Band structure of ZnSe with LDA formalism. The highest value of valence band is taken as zero on the energy axis.

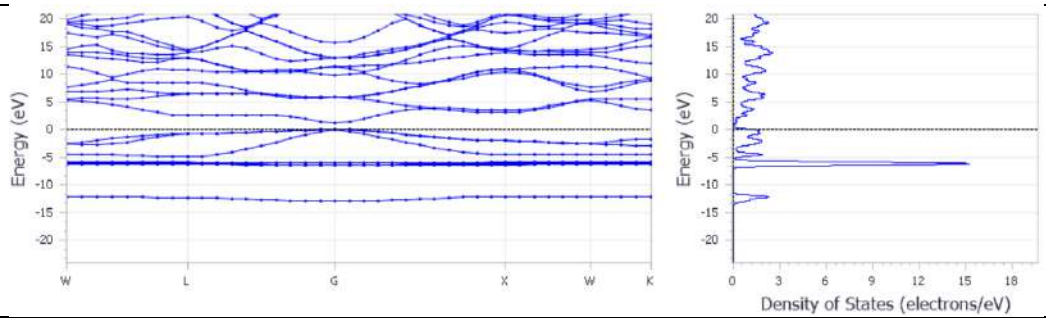

Fig 4: Band structure of GaAs by GGA-PBE formalism. The highest value of valence band is taken as zero on the energy axis.

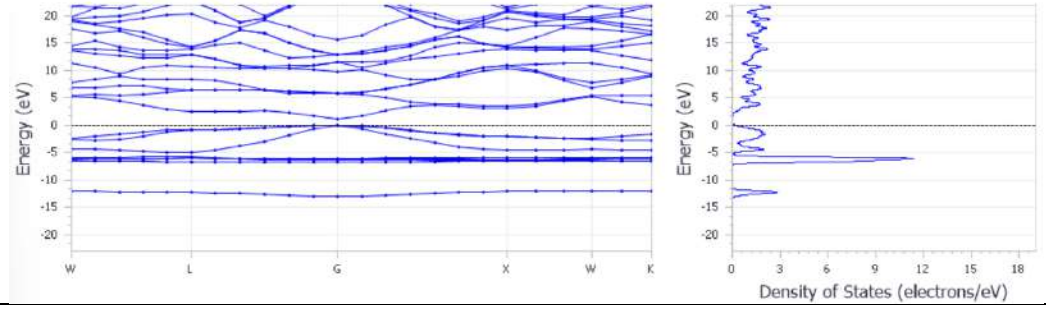

Fig 5: Band structure of GaAs by GGA-PW91 formalism. The highest value of valence band is taken as zero on the energy axis.

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RESEARCH ARTICLE

Effect of Potassium Solubilizing Bacteria on Growth, Phytochemical Character and Yield of Turmeric (*Curcuma longa.* L.)

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ABSTRACT

Soil microbes are supportive agents to the translocation of soil potassium (K). It is an important component in the soil potassium cycle. The presence of potassium in the soil is usually very low and more than 90% of potassium is found in the form of insoluble rocks and silicate minerals. Rhizosphere inhabiting bacteria act to dissolve potassium from insoluble potassium bearing rocks. In this study four bacterial isolates were obtained from rhizosphere soil on Aleksandrov medium (KSB1, KSB2, KSB3 and KSB4). In this study four promising bacterial cultures of KSB1, KSB2, KSB3 and KSB4 were evaluated and it was compared with other inoculants and chemical fertilizer. The role of Potassium on turmeric crop investigated under the field study. During this study plants biometric were investigated and phytochemical characters as well as Saponins content in rhizomes. A significant increase rate of plant length, leaf length, leaf width and rhizome length were observed which were found in respective treated and untreated control.

Keywords: *Bacillus sp.*, Potassium Solubilizing Bacteria, *Curcuma longa*

INTRODUCTION

Potassium (K) is a vital macronutrient and most abundantly absorbed cation that play an important role in growth, metabolism and development of plants. Mineral potassium solublization by microbes which enhances crop growth and yield, when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than insoluble form of potassium (Rajan *et al,* 1996). Free-living beneficial bacteria imparting health benefits to crop plants are collectively called as plant growth-promoting rhizobacteria (PGPR)

comprising of different genera like *Azospirillum, Pseudomonas, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthrobacter, Burkholderia, Bacillus* and several others (Bashan *et al*. 2014). The various direct and indirect mechanisms of plant growth promotion by Bacillus spp. are nitrogen fixation, solubilization and mineralization of phosphorus and other nutrients, phytohormone production, production of siderophores, antimicrobial compounds and hydrolytic enzymes, induced systemic resistance (ISR) and tolerance to abiotic stresses (Goswami *et al.* 2016). Potassium Solubilizing Bacteria (KSB) can dissolve K-minerals such as mica, illite and orthoclase in the soil through the production and excretion of organic acids or production of capsular polysaccharide (Friedrich *et al.,* 1991). Several strains of potassium solubilizing bacteria, such as *Pseudomonas, Burkholderia, Acidothiobacillus ferroxidans, Bacillus mucilogenosus, B. edaphicus, B. circulans and Paenibacillus sp*. (Liu *et al*., 2012) can be used as biofertilizer. The potassium is made available to plants, when the minerals are slowly weathered or solublized microbes are the major soil groups in the land. In general block soils are high, red soils are medium and laterite soils are low in available potassium. Laterite, shallow red and black soils have been found to show decline in potassium fertility over the years under intensive cultivation and imbalanced fertilizer application currently very little information is available on minerals potassium solublization by bacteria, their mechanisms of solublization and its effect on growth, potassium uptake and yield of several crops. In this view a present study were under taken and observe the effect of potassium solublizing bacteria on growth, phytochemical characters and yield of *Curcuma longa*.

MATERIALS AND METHODS

Isolation and Characterization of potassium solubilizers

Soil Samples were collected from seven different sites of Rhizosphere region in Turmeric crop at Sivagiri Village in Kodumudi Taluk, Erode District, Tamilnadu, India. The soil samples were serially diluted upto10^{-8} and inoculated on enriched with KCl and K_2SO_4 Aleksandrov medium and incubated at 28± 2°C for seven days. After seven days of inoculation released level of potassium was determined by measuring zone of solublization in culture plate (Prajapati and Modi, 2012).

Identification of Isolated Bacteria

The Identification of purified KSB culture were done by the basis of its biochemical study and its morphological characters due to observed by using Bergey's (1993).

Observation of Morphological and Biochemical Characters of KSB

In order to study the cultural morphology and biochemical characteristics of isolated bacterial cultures on Aleksandrov Agar medium. A colony characteristic such as Size, Shape, texture, colony and motility were examined. Similarly Gram Staining, Indole test, Methyl red test, Starch hydrolysis test, Citrate utilization test, Triple Sugar Iron Test, voges proskauer test, Casein hydrolysis test and gas production test were determined.

Experimental Design

A plot culture experiment was conducted in three different types of treatments

1. Seed Application (Rhizome treatment): Seeds of Rhizome were treated about 30 minutes by microbial inoculants before showing the plot.
2. Soil Application: Microbial inoculants were inoculated directly in the soil
3. Seed of Rhizome treated with microbial inoculants for 30 minutes and soil application in known volumes of microbial inoculants.

The soil was prepared and divided into eight plots, each of 3 × 3 m size and rhizomes were placed on the respective place. All agriculture recommendation was carried out as required during the growing seasons. The Tamilnadu agricultural university recommended dose of N, P, K chemicals were used in the experiment for control as well as biofertilizer treatment. Biofertilizers were added as a drench to the rhizome as well as soil twice (after germination of rhizome and after one month of germination). *Curcuma longa* plants were treated with different bacterial inoculums as follows: 100% bio-fertilizers T1 - KSB1 (*Bacillus subtilis*), T2 – KSB2 (*Bacillus circulans*), T3 – KSB3 (*Bacillus edaphicus*), T4 – KSB4 (*Pseudomonas striata*), T5 – *Azotobacter*, T6 – *Azospirillum,* T7 – 100% mineral fertilizers, T8

without fertilizer (control). A random sample of few plants was taken from each plot after 60, 90 and 120 days from sowing date (vegetative growth stage). The following data were recorded during the period like plant height, leaf length, leaf diameter, rhizome length and quantify the Chlorophyll content, protein, lipids and saponins in rhizome.

RESULTS AND DISCUSSION

Nitrogen, phosphorus and potassium are major essential macronutrients for plant growth and development. To enhance the crop yield, nitrogenous and phosphatic fertilizers are applied at high rates, which cause environmental and economic problems. Therefore, direct application in soluble form of rock phosphate and rock potassium may be agronomically more useful and environmentally safer than synthetic form of P and K chemical fertilizers. In this study potassium solublizing bacteria were isolated from rhizophere soil of turmeric crop. The serial dilutions of rhizosphere soil samples were made upto 10^{-8} and diluted samples were placed on Aleksandrov medium. Totally 6 Rhizobacterial cultures were isolated based on morphological, Biochemical and potassium solublization efficiency. Out of six isolates four efficient potassium solublizers such as KSB, (*Bacillus subtilis*), KSB2 (*Bacillus circulans*) KSB3 (*Bacillus edaphicus*) and KSB4 (*Pseudomonas striata*) were screened and observed their potassium solublization activity. A four number of distinct bacterial cultures were isolated exhibiting cleared zone of potassium solublization on Aleksandrov agar medium. The efficient strains of KSB1 – *Bacillus subtilis*, KSB2 – *Bacillus circulans*, KSB3 – *Bacillus edaphicus*, and KSB4 – *Pseudomonas striata.*

The plant biometric results were recorded for plant height, Leaf Length, Leaf width, Rhizome Length and biochemical characters such as total chlorophyll, protein and saponins content of Rhizome were observed in the 60th, 90thand 120th days of intervals. A Significant level of increasing leaf length, leaf diameter and rhizome length was recorded in soil application and rhizome treatment of KSB4, KSB3, KSB2 and KSB1. The treatment of potassium solublizing isolates of KSB4 in soil and Rhizome application showed increased rate of plant height (16.54, 96.28 and 107.28 cm). Similarly the promising level of increased nature of plant height, leaf length, leaf diameter and rhizome length were recorded the *Azotobacter* and *Azospirillum* soil treated and rhizome treated *Curcuma longa.* L. While maximum rhizome length was recorded in 120th day of soil inoculated and rhizome treated with KSB4 shows (6.36 cm) than the other treatment respectively. Phytochemical compounds distribution in leaf and rhizome region can be observed 60th, 90th and 120th days of intervals. A highest rate of total chlorophyll (1.76 mg/g) in KSB4 Bacteria in both soil application and rhizome treated *curcuma* leaf than the control. The increased rate of protein and lipids were found in KSB4 Bacteria treated *curcuma* leaf than the other treatments. Similarly, highest rate of saponin content (0.48%) were recorded in the rhizome, both soil inoculation and Rhizome treated with KSB4 in the turmeric crop.

Potassium solubilization has been reported in various species of Bacillus like *B. velezensis, B. cereus, B. circulans, B. coagulans, B. edaphicus, B. megaterium, B. subtilis, B. firmus, B. mycoides, B. decolorationis and B. horikoshii* (Verma *et al*. 2015). KSB when co-inoculated with N-fixers and P-solubilizers can enhance the effect of N-fixers and P solubilizers (Basak and Biswas 2010). *Bacillus licheniformis* BHU18 was shown to solubilize potassium and produce IAA. The strain was found to tolerate various regimes of pH and hence can be an effective bioinoculant in both acidic and alkaline soils (Saha *et al*. 2016). *Pseudomonas sp.* are member of bacteria group having capability in solubilizing P and K by producing organic acids (Archana *et al.,* 2013) and promoting plant growth through the production of IAA (Patten and Glick, 2002). *Pseudomonas sp.* has ability in protecting plants from pathogens and potentially by useful as biocontrol agent applicable in green house and field (Arshad and Frankenberger Jr., 1993). Anjanadevi *et al*., 2016 reported the inoculation of seeds and seedlings of different plants with KSB generally showed significant enhancement of germination percentage, seedling vigor, plant growth, yield, and K uptake by plants under greenhouse and field conditions.

CONCLUSION

Previous researches well showed that KSB were able to dissolve K from different insoluble K bearing minerals by excreting organic acids. Application of KSB as bioinputs not only enhance plant growth and yield but also can lessen the use of chemicals fertilizer and support eco-friendly crop production. Further field studies should be performed to assess the potential of crop production and evaluate their long term benefits on plant growth and soil behavior.

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Table 1: Morphological Characters of *Curcuma Longa. L* in Rhizome Application and soil inoculation of Potassium Solubilizing bacteria (KSB).

S.No	Days of Study	Kinds of Treatment	Plant Height (CM)			Leaf Length (CM)			Leaf Diameter (CM)			Rhizome Length (CM)		
			RA	SA	RA+SA	RA	SA	RA+SA	RA	SA	RA+SA	RA	SA	RA+SA
1	60th DAYS	T1 - KSB1	16.42	16.40	16.52	42.30	42.38	42.40	10.92	10.90	10.96	4.24	4.26	4.60
		T2 - KSB2	16.26	16.28	16.30	42.26	42.28	42.32	10.84	10.82	10.88	4.20	4.22	4.56
		T3 - KSB3	16.29	16.32	16.34	42.34	42.36	42.38	10.80	10.84	10.86	4.22	4.24	4.30
		T4 - KSB4	16.48	16.46	16.54	42.32	42.40	42.46	10.93	10.96	10.98	4.50	4.58	4.74
		T5 - *Azotobacter*	15.10	15.12	15.14	42.01	42.12	42.18	9.18	9.24	9.22	4.32	4.34	4.36
		T6 - *Azospirillum*	15.12	15.14	15.16	41.38	41.40	41.42	9.16	9.20	9.26	4.38	4.39	4.40
		T7 - Chemical fertilizer(K2O)	14.22	14.30	14.36	41.30	41.32	41.36	9.14	9.18	9.22	4.40	4.42	4.44
		T8 - Control	14.21	14.26	14.28	26.02	26.04	26.08	9.12	9.14	9.20	4.01	4.04	4.20
2	90th DAYS	T1 - KSB1	94.40	92.42	96.20	52.04	51.02	51.12	11.27	11.30	11.34	5.02	5.04	5.12
		T2 - KSB2	94.38	94.30	94.32	52.02	51.04	51.20	11.24	11.26	11.28	5.04	5.06	5.08
		T3 - KSB3	94.42	94.48	94.50	52.04	52.06	52.08	11.26	11.28	11.30	5.06	5.08	5.10
		T4 - KSB4	96.20	96.24	96.28	54.02	54.06	54.06	11.28	11.40	11.46	5.62	5.64	5.68
		T5 - *Azotobacter*	93.12	93.14	93.16	53.04	53.06	53.08	11.02	11.04	11.14	5.02	5.04	5.06
		T6 - *Azospirillum*	93.20	93.22	93.24	49.12	49.14	49.20	10.26	10.28	10.30	5.10	5.12	5.14
		T7 - Chemical fertilizer(K2O)	92.22	92.26	92.28	48.36	48.38	48.40	10.18	10.20	10.22	5.04	5.08	5.10
		T8 - Control	90.20	90.24	90.22	46.42	48.44	48.46	10.12	10.16	10.18	5.20	5.24	5.26
3	120th DAYS	T1 - KSB1	100.60	102.40	104.42	54.12	54.42	54.44	12.32	12.34	12.36	5.40	5.68	5.70
		T2 - KSB2	100.62	102.42	104.48	54.14	54.16	54.18	12.12	12.16	12.18	5.12	5.36	5.38
		T3 - KSB3	101.18	102.54	104.50	54.16	54.20	54.22	12.18	12.20	12.22	5.38	5.40	5.42
		T4 - KSB4	107.20	107.24	107.28	55.46	55.48	55.50	12.38	12.40	12.42	6.31	6.34	6.36
		T5 - *Azotobacter*	96.18	96.20	96.22	54.26	54.28	54.30	11.36	11.32	11.38	6.20	6.18	6.22
		T6 - *Azospirillum*	92.14	92.24	92.25	53.24	53.26	53.28	11.32	11.30	11.36	6.22	6.24	6.24
		T7 - Chemical fertilizer(K2O)	90.12	90.18	90.20	48.22	48.20	48.28	11.30	11.28	11.32	6.24	6.26	6.23
		T8 - Control	88.10	88.14	88.16	46.20	46.22	46.26	10.11	10.12	10.14	6.20	6.22	6.24

Table 2: Biochemical Characters in leaf of *Curcuma longa. L* in Rhizome Application and Soil Application of potassium solubilizing bacteria

S.No	Days of Study	Kinds of Treatment	Total chlorophyll mg/g			Protein (%)			Lipids (%)			Rhizome Saponins (%)		
			RA	SA	RA+SA	RA	SA	RA+SA	RA	SA	RA+SA	RA	SA	RA+SA
1	60th DAYS	T1 - KSB1	1.65	1.64	1.66	7.2	7.0	7.4	6.21	6.23	6.12	0.38	0.36	0.38
		T2 - KSB2	1.66	1.68	1.68	7.4	7.6	7.8	6.32	6.36	6.38	0.40	0.42	0.43
		T3 - KSB3	1.68	1.64	1.69	8.6	7.10	8.12	6.34	6.38	6.40	0.42	0.44	0.43
		T4 - KSB4	1.70	1.71	1.74	9.01	9.02	9.04	6.58	6.56	6.54	0.45	0.44	0.45
		T5 - *Azotobacter*	1.64	1.62	1.60	8.4	8.2	8.01	6.22	6.20	6.18	0.40	0.41	0.42
		T6 - *Azospirillum*	1.62	1.60	1.61	7.6	7.4	7.8	6.10	6.12	6.14	0.42	0.40	0.41
		T7 - Chemical fertilizer	1.60	1.58	1.56	7.2	7.3	7.4	6.08	6.06	6.10	0.38	0.36	0.39

		T8 - Control	1.50	1.52	1.54	6.8	6.6	6.4	5.02	5.04	5.08	0.32	0.36	0.38
2	**90th DAYS**	T1 - KSB1	1.68	1.66	1.67	7.3	7.2	7.4	5.01	5.00	5.02	0.30	0.32	0.34
		T2 - KSB2	1.58	1.60	1.62	7.8	7.10	7.12	6.2	6.4	6.8	0.40	0.42	0.44
		T3 - KSB3	1.54	1.52	1.50	8.2	8.4	8.6	6.0	6.01	6.02	0.38	0.36	0.34
		T4 - KSB4	1.72	1.74	1.76	10.6	10.4	10.2	6.58	6.60	6.62	0.44	0.46	0.48
		T5 - Azotobacter	1.68	1.70	1.72	7.6	7.8	7.10	1.62	1.64	1.66	0.42	0.43	0.42
		T6 - Azospirillum	1.52	1.54	1.56	7.2	7.8	7.6	1.58	1.60	1.62	0.43	0.42	0.43
		T7 - Chemical fertilizer	1.54	1.58	1.60	7.0	7.2	7.4	6.02	6.04	6.06	0.40	0.41	0.42
		T8 - Control	1.52	1.54	1.56	6.8	6.6	6.4	6.11	6.14	6.16	0.36	0.38	0.32
3	**120th DAYS**	T1 - KSB1	1.62	1.68	1.70	7.4	7.6	7.8	6.04	6.02	6.03	0.38	0.40	0.41
		T2 - KSB2	1.60	1.62	1.64	7.8	7.6	7.2	6.22	6.24	6.26	0.42	0.44	0.43
		T3 - KSB3	1.66	1.68	1.70	7.2	7.4	7.6	6.20	6.24	6.26	0.44	0.46	0.45
		T4 - KSB4	1.78	1.76	1.74	11.4	11.2	11.6	6.59	7.10	7.21	0.46	0.48	0.49
		T5 - Azotobacter	1.64	1.66	1.68	8.2	8.4	8.6	6.22	6.20	6.24	0.42	0.43	0.42
		T6 - Azospirillum	1.62	1.61	1.64	7.4	8.6	7.4	6.01	6.02	6.04	0.41	0.42	0.43
		T7 - Chemical fertilizer	1.54	1.58	1.60	7.2	8.4	7.0	6.12	6.14	6.16	0.38	0.36	0.40
		T8 - Control	1.54	1.58	1.60	6.8	8.0	7.2	6.10	6.08	6.06	0.32	0.30	0.34

[RA - Rhizome Application, SA – Soil Application and RA+SA - Rhizome Application+ Soil Application]

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RESEARCH ARTICLE

Performance of Hybrid Electric Vehicles Based on Switched-Capacitor Voltage Boost Converter

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ABSTRACT

This work provides a switched-capacitor (SC) voltage raise inverter and it's manipulating techniques for imposing DC to DC and AC to DC strength adaptation. The SC converter employ a switched capacitor circuit improved with the principal converter circuit of strength source, for that reason supplying special points that can't be attained with the aid of the usual voltage-source inverter (VSI) or increase VSI. The extra points encompass doubling the location of the linear modulation place and doing away with each the giant inductor in the enhance DC to DC stage and the massive filtering capacitor, which escort to a greater strength density and decrease cost. The SC converter notion can be utilized to all dc-ac, ac-dc, ac-ac, and dc-dc electricity conversions. To express the running precept and the control, we focal point on one example bidirectional SC converter for dc-ac and ac-dc energy conversion in electric powered and hybrid electric powered vehicles.

Keywords: switched-capacitor, voltage-source inverter, DC-DC converter.

INTRODUCTION

A Switched Capacitor (SC) of digital circuit aspect imposing of filter. It workings with the aid of shifting prices of indicators are used to manage in switches, so that no longer all of switches in are closed concurrently [1-2]. Filters applied with these factors are termed "switched-capacitor filters", and rely solely on this is useful for extra appropriate for use inside built-in circuits, the place precisely certain resistors and capacitors are now not comparatively cheap to construct of [3]. SC of circuits are generally applied the use of Metal–Oxide–Semiconductor (MOS) technology, and they are typically fabricated the usage of the complementary MOS (CMOS) process.

Common purposes of MOS SC circuits encompass mixed-signal built-in circuits, Digital-to-Analog Converter (DAC) chips, Analog-Digital Converter (ADC) chips, pulse been encouraged with boosting ability. However, it requires back-end H-bridge for the terrible polarity era throughout the load. This will increase the usual voltage stress of the topology. The multiplied topology of has been introduced in, which gets rid of the H-bridge, leads to the reduce of the voltage stress projected [4].

Conventional inverter/converter topology & proposed switched-capacitor

In Fig.1, the battery of a strength supply of consisting one/ greater electro-chemical cells with exterior relations for powering in electrical gadgets such as an flashlights, cell phones, and electric powered cars [5]. If the battery is presenting electric powered power, its fantastic battery of the it's terrible if the control.

SC Converter based Space Vector Pulse Width Modulation

The battery is linked to an exterior electric powered load in the a response converts of high-the "switched-capacitor filters", and rely solely on this an awful lot extra appropriate for use inside built-in circuits, the place precisely certain resistors and capacitors are now not comparatively cheap to construct of [6]. SC of circuits are generally applied the use of Metal–Oxide–Semiconductor (MOS) technology, and they are typically fabricated the usage of the Complementary MOS (CMOS) process. Common purposes of MOS SC circuits encompass mixed-signal built-in circuits, Digital-to-Analog converter (DAC) chips, Analog-Digital Converter (ADC) chips, pulse been encouraged with boosting ability. In an electrical engineering, 3-phase electric powered energy structures contain at least 3-conductors carrying flashing voltages that are into are offset of in time by means of one-third of the period. A three-phase device can also be organized in delta or star [7].

SIMULATION RESULTS

In Fig.2 and Fig.3, substantiate the projected design the equation of control, a simulation representation of the SC converter is build using matlab/simulink software

CONCLUSION

This work has introduced a novel switched capacitor strength converter for imposing DC to DC and AC to DC strength conversion. The SC-converter make use of a switched capacitor circuit amplified with the foremost converter circuit to the energy source, hence offering special facets that can't be attain via the typical VSI or improve VSI. One of these special aspects is replication the location of the linear modulation area. The SC converters reduce the want for the unwieldy and pricey inductor to increase the voltage. As an alternative, it depends on solely the capacitors to reap voltage boost, which approves greater energy concentration.

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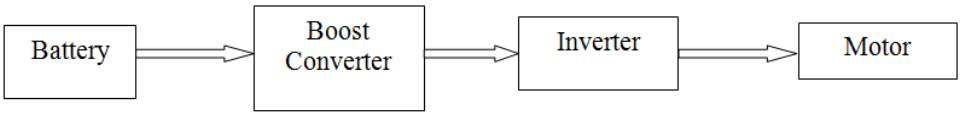

Fig 1: Block diagram of Proposed Model

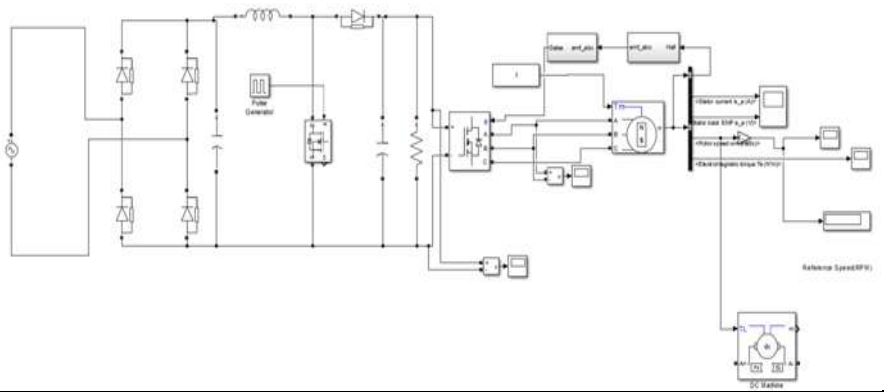

Fig 2: Simulation of sub system

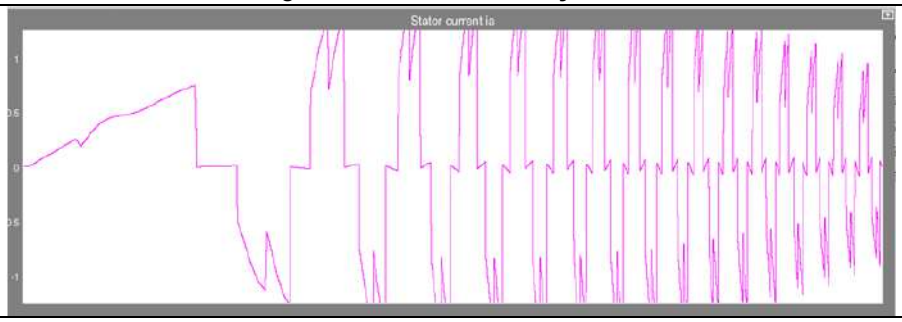

Fig 3: (a) Stator output current

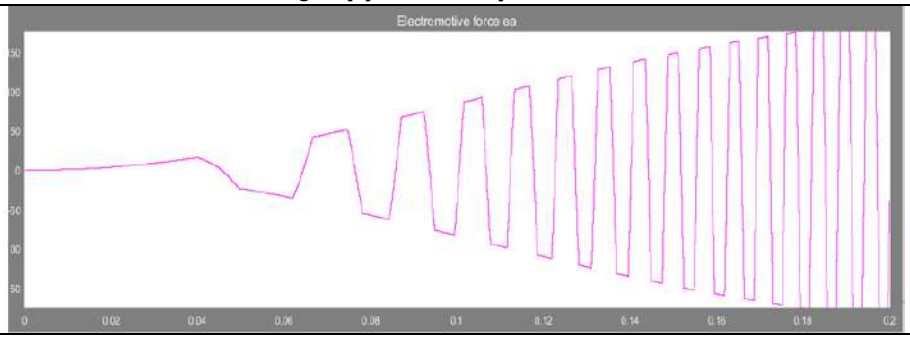

Fig 3: (b) Electromotive force output

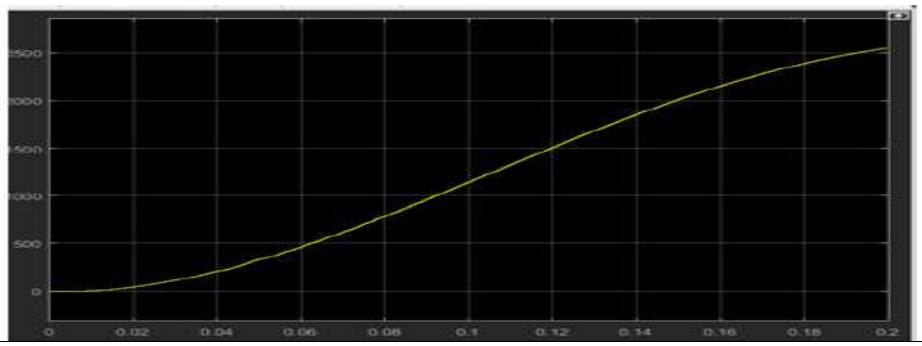

Fig 3: (c) Rotor speed

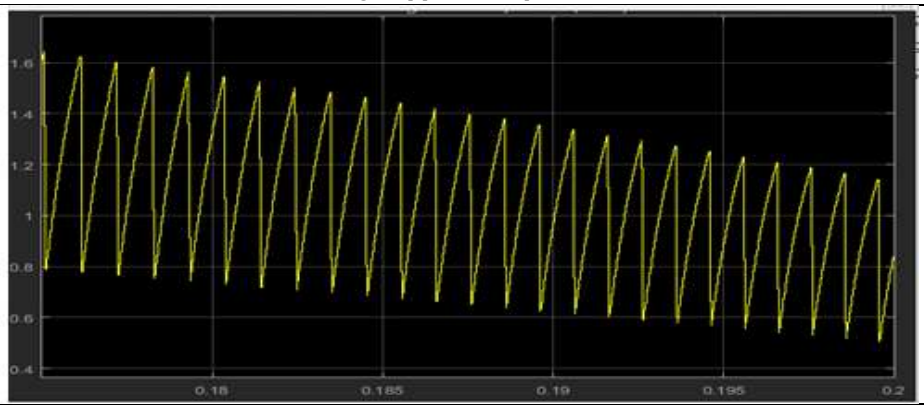

Fig 3: (d) Torque

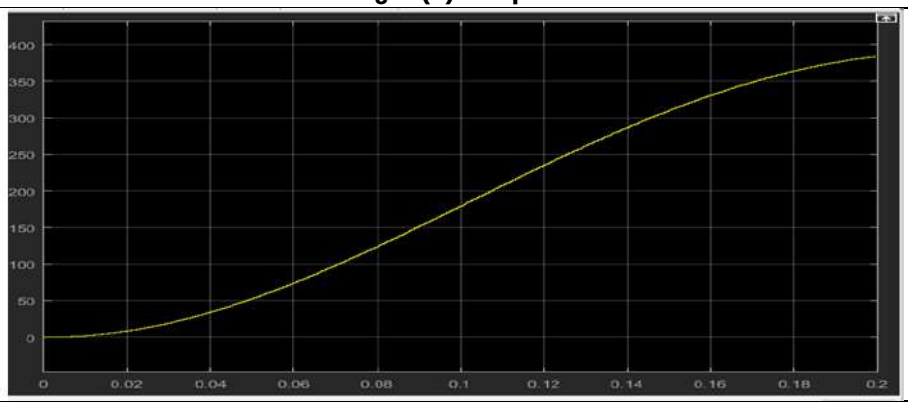

Fig 3: (e) Rotor output voltage

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RESEARCH ARTICLE

Character Association and Path-Coefficient Studies in Brinjal (*Solanum melongena* L.) for Fruit Yield per Plant and its Component Characters

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ABSTRACT

The present investigation was carried out at the Horticultural Farm, Assam Agricultural University, Jorhat in Rabi, 2016-17 with twenty-seven genotypes comprising nine brinjal lines, their eighteen F1's developed through line x tester mating scheme. The results highlighted that the genotypic correlation coefficient is greater than the corresponding phenotypic correlation coefficient for all the traits under studied. Trait association at genotypic and phenotypic levels for fruit yield per plant and its component characters revealed that fruit yield per plant had highly significant positive phenotypic association with fruit girth (0.639**), and fruit weight (0.595**).The traits like, fruit length had significant positive phenotypic association with plant height (0.265*) and number of branches (0.226*); fruit weight had significant to highly significant positive phenotypic association with fruit length (0.279*) and fruit girth (0.788*); and number of fruits per plant had significant to highly significant negative phenotypic association with days to 50 per cent flowering (-0.495**) and fruit weight (-0.262*).Path coefficient analysis at phenotypic level revealed that the trait, fruit girth (0.481) had the highest positive direct effect on fruit yield per plant followed by fruit weight (0.209), fruit length (0.178), and number of fruits per plant (0.086). Hence, direct selection all for these traits will be effective.

Keywords: Brinjal, character association, and path-coefficient analysis

INTRODUCTION

Brinjal is one of the most important vegetable crops of subtropics and tropics with nutritional and ayurvedic medicinal value. It is popularly known as poor man's vegetable. It is a hardy crop grown commercially under diverse agro-climatic environments. The fruits contain low in calories and fats, mostly water, some protein, fibre and

carbohydrates. It is with ayurvedic medicinal property, useful to diabetic patients and also excellent remedy for liver complaints. Fruit yield is a complex trait influenced by several yield attributing traits and sole selection for fruit yield is not much effective. Character association and path-coefficient analysis is widely used in plant breeding to determine the nature and magnitude of relationship between yield and its component traits and to classify certain components with profound impacts on yield as selection criteria for potential use. A path coefficient is a standardized partial regression coefficient, which determines a predictor variable's direct influence on the response variable. This allows for the division of the coefficient of association into direct effect and indirect effects. Hence a sound knowledge of traits association and path coefficient is necessary for selection of traits for developing high grain yielding varieties

MATERIALS AND METHODS

Twenty-seven genotypes consisting of nine brinjal genotypes, their eighteen F1's developed through line x tester mating design. All these were evaluated for their agronomic performance during the rabi, 2016-17 at Horticultural Farm, Assam Agricultural University, Jorhat, Assam. The experiment was carried out in randomized block design with three replications and 3m row length having row to row distance 70 cm and plant to plant distance 60cm. The single seedling per hill of one moth old was transplanted in the experimental area. The recommended package of practices was followed for raising healthy crops stand. The data were recorded on eight quantitative traits on five competitive plants from each replication viz., days to 50 per cent flowering, plant height (cm), number of branches per plant, fruit length (cm), fruit girth (cm), average fruit weight (g), number of fruits per plant, fruit yield per plant (g/p).The mean values on different traits were analysed using online software "OPSTAT" developed by Chaudhary Charan Singh Agricultural University, Hissar. The correlation coefficients for different traits were calculated by the method suggested by Panse and Sukhatme (1967). The path coefficient analysis was performed as per the procedure suggested by Wright (1921) and adopted by Dewey and Lu (1959).

RESULTS AND DISCUSSION

Character association

The phenotypic and genotypic correlation coefficients of eight quantitative traits highlighted the presence of several statistically significant relationships and are presented in Table 1(a) and (b).The results indicated that the genotypic correlation coefficient is greater than the corresponding phenotypic correlation coefficient for all the traits studied indicating that the interrelationships were strongly inherent and low phenotypic expression was triggered by environmental factors. This result was in close conformity to Niranjan *et. al*., (2020). Character association studied at phenotypic level indicated that fruit yield per plant had highly significant and positive phenotypic association with fruit girth (0.639**) and average fruit weight (0.595**) Table 1(a). These results are in close conformity to the findings of Dhaka and Soni (2014) for average weight of fruit; Kustagi *et. al.,* (2019) for fruit width; Nikitha (2020) for fruit diameter and fruit weight; Sakriya *et. al.,* (2020) for fruit girth. The fruit yield attributing characters showing positive and significant association with fruit yield per plant indicated that fruit yield per plant can be enhanced by selecting for these traits at the same time. Interrelationship among the other traits revealed the existence of significant to highly significant and positive phenotypic association by fruit length with plant height (0.265*) and number of branches per plant (0.226*); by fruit weight with fruit length (0.279*) and fruit girth (0.788**). These findings are in the close conformity with the finding of Nikitha (2020) for fruit length with plant height; Sakriya *et. al.,* (2020) for fruit length with plant height, fruit weight with fruit length and fruit girth. The character number of fruits per plant exhibited significant to highly significant negative phenotypic association with days to 50 per cent flowering (-0.495**) and average fruit weight (-0.262*). These results are in close conformity with the finding of Nikitha (2020) for days to 50 per cent flowering; Sakriya *et. al.,* (2020) for days to 50 per cent flowering. Character association studied at genotypic level indicated that fruit yield per plant had highly significant and positive genotypic association with fruit girth (0.665**), average fruit weight (0.605**), and number of branches per plant (0.333**) Table 1(b).These results are in close conformity with the findings of Dhaka and Soni (2014) for average weight of fruit and branches

per plant; Kustagi *et. al.,* (2019) for fruit width; Nikitha (2020) for number of branches per plant, fruit weight, fruit diameter; Sakriya *et. al.,* (2020) for fruit weight. Interrelationship among the other traits revealed the existence of significant to highly significant and positive genotypic association by fruit length with number of branches per plant (0.431**), plant height (0.327**) and days to 50 per cent flowering (0.230*); by fruit weight with fruit girth (0.830**), fruit length (0.291*), and days to 50 per cent flowering (0.257*); by number of branches per plant with plant height (0.265*). These results are in close conformity with the findings of Dhaka and Soni (2014) for average weight of fruit; Sakriya *et. al.,* (2020) for plant height, fruit girth, and fruit length.

Path coefficient analysis

The coefficient of correlation which determines the association between any two characters does not necessarily be the assurance of a direct causal relationship since it does not indicate the contribution of variation in one trait in relation to observed variation in another. Correlation coefficients are useful in deciding the attributes of a complex trait such as fruit yield but it doesn't provide an accurate image of the relative worth of direct and indirect effects of each and every component trait on the fruit yield. Analysis of the path coefficient showing the cause and effect of various yield attributes will provide a stronger index for selection instead of the coefficients for correlation. Therefore, the studies on the path coefficient were conducted to know the direct and indirect effects of yield attributes on fruit yield per plant and are presented in Table 2. The path coefficient analysis at phenotypic level revealed that the character, fruit girth (0.481) had the highest positive direct effect on fruit yield per plant followed by average fruit weight (0.209), fruit length (0.178) and number of fruits per plant (0.079). Kustagi *et. al.,* (2019) reported positive direct effect of fruit width, fruit length, number of fruits per plant on fruit yield per plant. Dash *et. al.,* (2020) reported positive direct effect of number of fruits per plant, fruit length and fruit girth at genotypic level. The high direct effects of these traits seemed to be the key explanation for their powerful association with fruit yield per plant. Thus, direct selection will be effective for all these traits. Number of branches per plant had highest negative phenotypic direct effects on fruit yield per plant followed by days to 50 per cent flowering and plant height.

CONCLUSION

Present study highlighted that the traits like fruit yield per plant had highly significant positive phenotypic association with fruit girth, and average fruit weight. Hence, these traits can be given an importance in selecting superior genotypes for higher fruit yield per plant. Path coefficient analysis at phenotypic level revealed that the character, fruit girth had the highest positive direct effect on fruit yield per plant followed by average fruit weight, fruit length, and number of fruits per plant. Hence, Hence, direct selection for the genotypes with higher fruit yield per plant, the weightage of all these traits would be given.

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CONFLICT OF INTEREST

Authors have not declared any conflict of interest.

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Table 1(a): Phenotypic correlation coefficients for eight quantitative characters in brinjal

Characters		1	2	3	4	5	6	7	8
		Days to 50% flowering	Plant height (cm)	No of branches/ plant	Fruit length (cm)	Fruit girth(cm)	Average fruit weight (gm)	No. of fruit per plant	Fruit yield/plant
1	Days to 50% flowering	1							
2	Plant height(cm)	0.121^{NS}	1						
3	No of branches/plant	0.020^{NS}	-0.060^{NS}	1					
4	Fruit length (cm)	0.197^{NS}	0.265*	0.226*	1				
5	Fruit girth(cm)	0.048^{NS}	-0.026^{NS}	-0.172^{NS}	-0.063^{NS}	1			
6	Average fruit weight (gm)	0.210^{NS}	0.189^{NS}	-0.073^{NS}	0.279*	0.788**	1		
7	No. of fruit per plant	-0.495**	0.213^{NS}	0.075^{NS}	-0.130^{NS}	-0.162^{NS}	-0.262*	1	
8	Fruit yield/plant	-0.068^{NS}	0.059^{NS}	-0.187^{NS}	0.134^{NS}	0.639**	0.595**	-0.030^{NS}	1

*&**: 5% and 1% level of significance, respectively.

Table 1(b): Genotypic correlation coefficients for eight quantitative characters in brinjal

Characters		1	2	3	4	5	6	7	8
		Days to 50% flowering	Plant height(cm)	No of branches/plant	Fruit length (cm)	Fruit girth(cm)	Average fruit weight (gm)	No. of fruit per plant	Fruit yield/plant
1	Days to 50% flowering	1							
2	Plant height(cm)	0.136^{NS}	1						
3	No of branches/plant	0.119^{NS}	-0.077^{NS}	1					
4	Fruit length (cm)	0.230*	0.327**	0.431**	1				
5	Fruit girth(cm)	0.085^{NS}	-0.003^{NS}	-0.284*	-0.078^{NS}	1			
6	Average fruit weight (gm)	0.257*	0.206^{NS}	-0.112^{NS}	0.291**	0.830**	1		
7	No. of fruit per plant	-0.748**	0.265*	0.161^{NS}	-0.169^{NS}	-0.182^{NS}	-0.312**	1	
8	Fruit yield/plant	-0.068^{NS}	0.069^{NS}	0.333**	0.132^{NS}	0.665**	0.605**	-0.036^{NS}	1

Table 2: Direct (diagonal) and Indirect (both side of diagonal) phenotypic and genotypic effects for eight quantitative characters in brinjal

Characters		1 Days to 50% flowering	2 Plant height (cm)	3 No of branches/ plant	4 Fruit length (cm)	5 Fruit girth (cm)	6 Average fruit weight (gm)	7 No. of fruit per plant
1	Days to 50% flowering	-0.126 (0.315)	-0.003 (-0.054)	-0.003 (-0.069)	0.035 (0.118)	0.023 (0.038)	0.044 (0.057)	-0.039 (-0.474)
2	Plant height(cm)	-0.015 (0.043)	-0.024 (-0.397)	0.008 (0.044)	0.047 (0.168)	-0.013 (-0.001)	0.039 (0.045)	0.017 (0.168)
3	No of branches/plant	-0.003 (0.038)	0.002 (0.030)	-0.134 (-0.573)	0.040 (0.221)	-0.083 (-0.127)	-0.015 (-0.025)	0.006 (0.102)
4	Fruit length (cm)	-0.025 (0.073)	-0.006 (-0.130)	-0.030 (-0.247)	0.178 (0.514)	-0.030 (-0.035)	0.058 (0.064)	-0.010 (-0.107)
5	Fruit girth(cm)	-0.006 (0.027)	0.001 (0.002)	0.023 (0.163)	-0.011 (-0.040)	0.481 (0.446)	0.164 (0.183)	-0.013 (-0.115)
6	Average fruit weight (gm)	-0.026 (0.081)	-0.005 (-0.082)	0.010 (0.064)	0.050 (0.150)	0.379 (0.370)	0.209 (0.220)	-0.021 (-0.198)
7	No. of fruit per plant	0.062 (-0.236)	-0.005 (-0.105)	-0.010 (-0.092)	-0.023 (-0.087)	-0.078 (-0.081)	-0.055 (-0.069)	0.079 (0.634)

Figures given in parentheses are genotypic effects; Residual are 0.383

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RESEARCH ARTICLE

Transformerless Inverter Designed for Solar PV Applications

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ABSTRACT

This paper presents relations of single phase transformer less inverter designed used for solar PV application. The different kind of inverter unit comprises and accomplished of justifying the current leakage with have constant grid frequency voltage on PV parasitic capacitor in spite of allowing for switch terminal capacitances. A typical feature of the proposed designed inverter be with the intention of DC bus consumption, symmetrical action and steady Total common-Mode-Voltage (TCMV) are achieve simultaneously. The alternate of the planned inverter relations is considered briefly. The control approach used for the gate pulses utilizes valid function. Switching purpose based TCMV study, SC proposes with loss study be offered. Simulation outcome of H8 alternate be presented. Planned topology is in addition comparing with the presented five-level transformerless inverters toward establishing its different advantages. MATLAB/Simulink and a model is carried out in the force hardware research facility which the reproduction.

Keywords : Multilevel inverter, Transformer Less Inverter, Switched-Capacitor (SC), Total common mode voltage

INTRODUCTION

Transformers less design are used now a day's many applications, mainly used in solar PV application. This inverted used for the reason that removal of transformer, which be huge element, lead to decrease of the range of mass, and rate of the classification. Additionally structure efficiency improves at the same time; this design is important quantity of copper and iron losses [1-3]. Still, the presence of transformer imply present be no galvanic isolation connecting the basis and the load, whichresults in a bulky leak current to surge in the scheme [4]. This deteriorate the classification performance, power value and in addition compromise protection [5-7]. In Figure-1 , the time reliable voltage to appear across the PV scrounging capacitance (*CPV*), Since the inverter switching performance [8], outcome

into current leakage. Several researchers contain address the problem of leakage current by means of an ample choice of technique. However, all these technique turn around building TCMV constant or remove/ attenuating the large frequency ripple component of TCMV. In this, a number of transformers less MLI topologies are planned to design by means of the matter of TCMV. In [9], and flying- capacitor base topologies be well recognized into which the time unstable TCMV is maintain steady through clamp to partly the enter voltage. However the problem of the topologies is half utilization of DC bus. Proposed model of Different Modules, This problem is stylishly address in adopt a only one of its kind result in crate of the topologies planned in in Fig. 1.

PROPOSED MODULE

The common configuration of the planned TSC5LI, it includes switched capacitor (SC)block; a usual H bridge and an possible four quadrant switch (4QS) [10-11]. The proposed design of the SC block, pass on toward to SCB-I and SCB-II. Based the condition of reactive power potential, single of these SC blocks be capable of the preferred. Using SCB-I, the model, is activated to feed reactive power to the load, which is especially critical as for each the recent worldwide values [12]. In the design, two of the switches of the SCB-I can survive replace with diodes to attain SCB-II building block which is unable of feed reactive power. In Figure 2, on the other hand H8-variant is analyzed and discussed. Further models are not incorporated. The H8 variant is accomplished of feed power switches, reactive power by means of minimum number of switches The H8 variant consists of eight controlled one diode, and two capacitors. H8 variant have two various methods to produce zero output voltage. All through positive voltage, half cycle mode 3a is use to obtain zero output voltage and mode-4a is used during the negative.

SIMULATIONS RESULTS

Fig 3. The Mat lab circuit diagram sub system of switching pattern. Fig 4: Output current waveform of switches. Fig 5.The Mat lab circuit diagram sub system of switching pattern. Fig 6; Output current waveform of power switches. Fig 7: Output voltage waveform of power switches and Simulation parameter of the proposed system as shown in the figures below.

CONCLUSION

The transformer less inverter design is proposed in this paper for the solar PV applications. The future different converters can be used to improve the performance, depend on the arrangement of the switched capacitor building block and possible four quadrant switch. The variant of the planned is calculated generally with involved TCMV study in conditions of switching function. It is exposed that the far above the ground frequency ripple from TCMV is eliminate level behind in view of the switch incurable capacitances, which outcome in slight leakage current. The present design cannot be completed to achieve optimum solutions and future many methods can be implemented to improve the performance of the proposed methods. MATLAB/Simulink and a model is carried out in the force hardware research facility concluded and analyzed.

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Table: 1 Simulation parameter of the proposed system

S.No	Simulation parameters	Symbol	values
1	Voltage source	Vdc	100
2	Capacitance	C	200mF
3	Inductance	L	1200mH
4	Load resistance	R	1Ω
5	Frequency	f	50Hz

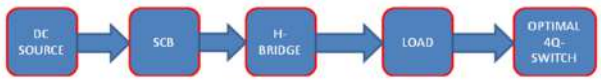

Fig. 1. Proposed design model

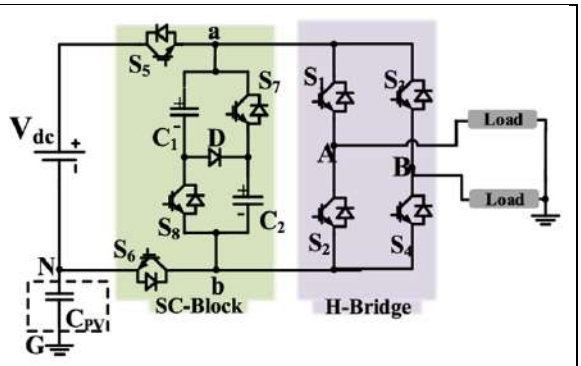

Fig. 2: The diagram of the H8 alternate of the planned five level transformerless

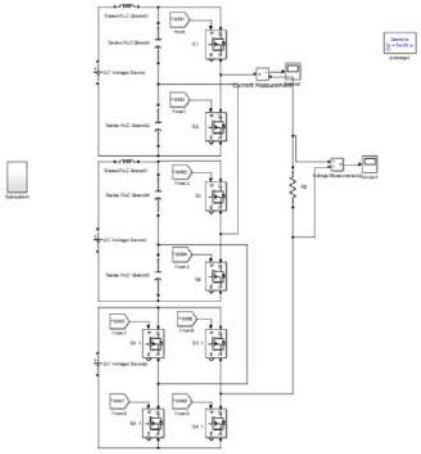

Fig 3 : The Matlab circuit diagram sub system of switching pattern

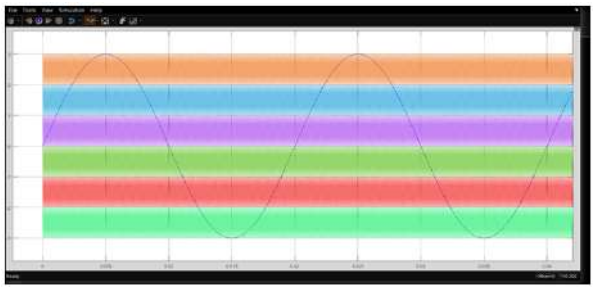

Fig 4: Output current waveform of switches

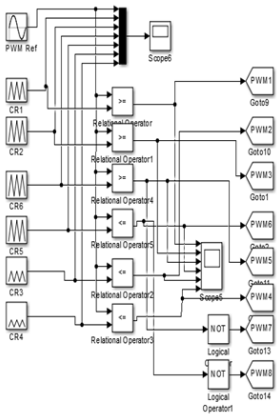

Fig 5 : The Mat lab circuit diagram sub system of switching pattern

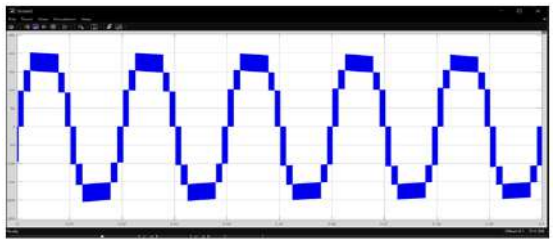

Fig 6: Output current waveform of power switches

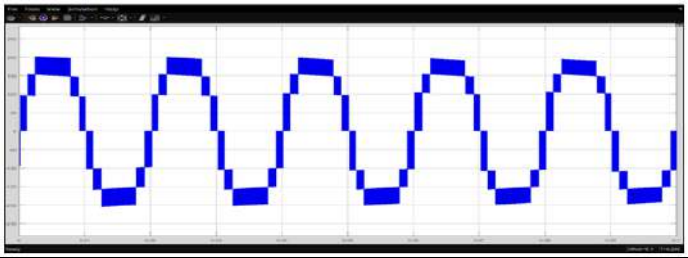

Fig 7: Output voltage waveform of power switches

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RESEARCH ARTICLE

Innovative Cultural Practices and Cluster Front Line Demonstration by Krishi Vigyan Kendra, Angul, on Yield of Green gram (*Vigna radiata* L.)

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ABSTRACT

Implementation of innovative cultural practices to study the performance of green gram cultivar IPM 02-03 through Cluster Front Line Demonstration (FLDs) was carried out by Krishi Vigyan Kendra, Angul, on seed production and profit of green gram (*Vigna radiata* L.). In this study two villages namely Sankhapur and Partara have been selected by KVK, Angul. Twenty hectares of land area was sanctioned for FLDs with sanctioned budget of Rs. 1, 50,000 in FLDs, with actual expenses of Rs. 1, 16,503/. Fifty villagers were participated in the FLDs and seeds of IPM 02-03 variety were distributed to the farmers. Extension activities was also carried including weekly field visit in the initial period of cultivation and then once in a fortnight till date of harvesting. Cultivar IPM 02-03 was subjected to seed treatment, foliar fertilization and pest management through integrated pest management to control the disease and pest infestation and also to observe the response of this improved cultivar including its yield. The potential yield was 12q /ha and duration of harvesting is of 62-70 days. This variety has large seed, suitable for Rabi and summer season and is resistant to mung bean yellow mosaic virus (MYMV).The demonstrated variety has shown promising result of increase in yield of 43.12% over the control variety.

Keywords: cultivar IPM 02-03, integrated pest management, green gram, *Vigna radiata*, yield.

INTRODUCTION

Vigna radita (L.) R. Wilczekcan be cultivated both as Kharif as well as summer crop and commonly known as moong bean. This plant belongs to Fabaceae family. This crop is mainly cultivated in East Asia and Indian sub-continent. India is reported in having largest cultivated area in the world under grain legumes, and this crop occupies the third among the pulse crop of India in terms of cultivated area and production next to gram (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*). Low productivity of green gram is because of cultivation of this crop with the non-adoption of

appropriate management practices. This indicates that there is a scope for increasing the green gram yield by suitable management practice. Grain legumes in general do not require high amount of N since the N requirement is met with by the N fixed by them. Even though, some amount of N as starter dose has to be applied initially to take care of the early requirement of N for the growth and development. Ayub *et. al*., (1999) reported that application of 40 kg N/ ha was optimum for harvesting higher yield with maximum protein content (26.18%). It can be inferred that green gram responds well up to an amount of 30-40 kg N/ ha which could be the optimum dose. Khan et al. (1999) stated that application of P_2O_5 from 60 – 90 kg ha-1 was optimum for realizing better yield of green gram. Similarly, maximum green gram yield was realized at 75 kg P_2O_5 ha-1 (Ayub *et. al*., 1998) and 100 kg P_2O_5per ha (Sharar *et. al*., 1999). Maximum yield of 962 kg/ha was harvested with 85 kg P_2O_5/ ha under irrigated condition (Ali *et. al*., 1999). There is wide variation in the quantity of P to be applied. However, a quantity of 40 kg P_2O_5 would be optimum. Maximum seed yield (753 Kg/ ha) of green gram was harvested with application of 90 Kg K_2O/ ha (Hussain *et. al*., 2011). Chaudhry and Mahmood (1999) reported that fertilization of 50 Kg K_2O/ ha resulted in higher seed yield (832 kg/ ha) and increased protein content in green gram. Sadeghipour et al. (2010) found that application of 90 kg N and 120 kg P_2O_5 per hectare was optimum for obtaining the highest yield of green gram. Oad *et. al*., (2003) stated that fertilization of green gram with 100 kg P and K each helped to achieve maximum seed yield.

Highest grain yield of 876 kg ha-1 was recorded in sandy clay loam soil of irrigated green gram with addition of 30 kg N and 70 kg of P_2O_5 ha-1 in combination with K (Tariq *et. al*., 2001) Sulphur deficiency induces chlorosis in young leaves and sulphur application is essential in soil deficient in sulphur in order to get higher yield. Impact of foliar fortification and different insecticides and fungicides application was reported to reduce pest and disease infestation with increase in net return of 88.56% in IPM 02-03 variety (Das and Parida, 2020). Foliar feeding of micronutrients and seed yield was also recorded and application of as 1 per cent $FeSO_4$, $MnSO_4$ and $ZnSO_4$ solution and 0.1 percent H_2SO_4 solution to green gram foliage significantly increased the N and sulphur contents of leaves and also increase in grain yields (Mehta and Singh, 1979). Among KCl and K_2SO_4 @ one per cent foliar spray, K_2SO_4 significantly increased the seed yield by 12.2 per cent (Chandra Babu *et. al*., 1985). Radhamani *et. al*., (2003) revealed an increase in number of pods per plant, pod and seed yield (874 kg/ ha) by spraying of DAP (2%) in combination with NAA (40 ppm) at 50 per cent in flowering stage. All the growth characters and yield of green gram were better when applied with 125 per cent NP with two per cent DAP and one per cent SOP twice foliar spray (Sathyamoorthi *et. al.,* 2008). Maximum growth and yield (529 kg ha-1) was recorded under combined application of 20 kg N, 50 kg P_2O_5 and 10 t FYM/ ha in green gram (Singh, 2007). Maximum growth was recorded in humic liquid fertilizer and NPK (20:20:20) in controlled condition. Maximum nodulation was reported in foliar feed spray and MAP sprayed cultivated fields (Nayak et al., 2020). Qureshi *et. al*., (2011) reported that *R. phaseoli* and *B. megaterium* inoculated with 20 kg N and 50 kg P/ ha enhanced the green gram growth, root mass of about 231.3 g, 50.54 cm root length, 78 numbers of nodules, nodular mass (0.216 g), pod (24.3 g pot-1) and straw (32.07 g pot-1) yield. Raising of high yielding short duration varieties throughout the year is the only method to increase the productivity. To achieve this, development of location specific management technology is of great importance. The objective of the study is implementation of innovative cultural practices to study the performance of green gram cultivar IPM 02-03through Cluster Front Line Demonstration by Krishi Vigyan Kendra, Angul, on seed production and profit of green gram (*Vigna radiata* L.). Response of improved green gram cultivar to seed treatment, foliar fertilization and pest management through integrated pest management to control the disease and pest infestation and increase in yield/ production rate was estimated in this study.

MATERIALS AND METHODS

Krishi Vigyan Kendra (KVK), Angul, is located in Panchmahala and was established on 25th March, 1995 under administrative control of CRRI (Now known as NRRI), Cuttack with the financial assistance of Indian Council of Agriculture and Research, New Delhi for overall development of Agriculture and allied sector in Angul district. On 3rd August 2001, it was transferred to the administrative control of Orissa University of Agriculture and Technology, Bhubaneswar. The experimental work was conducted in Sankhapur and Partara village of Angul district (Fig. 1).

Site Selection for cluster front line demonstration (FLDs)

Two villages viz. Sankhapur and Partara of Angul block were selected by the authority of KVK, Angul for cluster frontline demonstration (FLDs) because to attract large number of farmers as the audience because of easy accessibility. The selection of the sites was decided by Krishi Vigyan Kendra, Angul after consulting Odisha University of Agriculture and Technology (OUAT).The field experiments were conducted to study in implementing innovative cultural practices to study the performance of green gram cultivar IPM 02-03 through Cluster Front Line Demonstration.

Field preparation

Fields were prepared before sowing of the seeds. Soil testing is an important practise to know the soil in which the crop will be grown. Soil samples of different lands from different villages were collected and tested in soil lab. The samples were collected before the addition of manure to the soil. Soil sample was collected from a depth of 6 inches by cutting the land in a 'V' shape. Then the further soil testing was done in soil lab.

Sowing of seed

Sowing of green gram seeds was done in mild winter. Dry land was required for germination. Due to heavy rains before the sowing period, there was a delay in sowing. Sowing was done in different dates in different villages of Anguldistrict i.e. Partara, Sankhapur and Athamalik. Seed variety shown was IPM 02-03. Seeds sown were done in small lands using traditional hand sowing method. Seed sowing in large lands were done by using tractors. Seeds were both sown in random traditional way and in lines. Total 26 hectares were used for cultivation of green gram in Sankhapur village and 4 hectares in Partara village.

Foliar spray

Oregon 80 was sprayed by adding 2ml of Oregon 80 to 1litre of water. Spraying was done in early morning or late afternoon for preventing evaporation. Spraying was done in one acre of crop in Sankhapur and the results were noted after one week. Immidacloprid was sprayed in all lands supplied by K.V.K, Angul to treat aphids. Immidacloprid was added 3ml in 10litres of water. After 7 days Thiomethoxam was sprayed on the plants by mixing 5gms in 15litres of water to treat white fly. Then after one week profenophos was sprayed to treat pod bug by mixing 2ml in 1lit of water. Spraying of nutrients and plant protection measures was carried on in regular intervals.

Benefit- Cost ratio (BCR) and Yield Gap

Benefit cost ratio (B: C) is the ratio of gross returns with total expenditure incurred and yield indicated the promising effect of the demonstrated variety.

$$\text{Yield gap} - \text{I}\ (\%) = \frac{\text{Potential yield} - \text{Demo yield}}{\text{Potential yield}} \times 100$$

$$\text{Yield gap} - \text{II}\ (\%) = \frac{\text{Demo yield} - \text{Check yield}}{\text{Demo yield}} \times 100$$

RESULTS AND DISCUSSION

Soil fertility Status of Sankhapur and Partara village of Angul District

Green gram cultivated field of Sankhapur and Partara villages were chosen to collect soil samples. Six samples were collected from two villages (Fig.2A- Fig.2F). Different parameters of soil were measured and depicted.

Cluster Frontline Demonstrations on green gram under NFSM 2018-19

Data in Table 2 revealed about the cluster front line demonstration (FLDs) in two villagesi. e., Sankhapur and Partara were conducted by KVK, of Angul district. One frontline demonstration was sanctioned and conducted for rabi crops for the year 2018-2019. Twenty hectares of land area was sanctioned for FLDs with sanctioned budget of Rs. 1, 50,000. In FLDs, with actual expenses of Rs. 1, 16,503/. Fifty villagers were participated in the FLDs. The cultivated area is rain fed and the crop is rain fed crop. The seeds of t he variety IPM 02-03 were distributed to the farmers (Table 1).

Practices demonstrated for plant protection measures in each cluster

During the demonstration seeds of High Yield Variety (HYV), IPM 02-03 were taken and seed treatment was done using mixture of carboxinand thiram@ 3gm/ kg of seed before sowing to control seed borne diseases and pest. Herbicide spraying was done at 20 days of sowing with imazethapyr at the rate of one litre per hectare to control the growth of the weeds. Timely plant protection measures were implemented at various stages of growth of the plant. Spraying of prophenophos at the rate of one litre per hectare was done against foliage beetles during vegetative stage. Thiamethoxam @125g/hectare was done against aphids during vegetative stage and dimethoate at the rate of one litre per hectare against white fly(YMV) during maturity stage. Different techniques were demonstrated in each cluster to adopt in the cultivation practices to get higher yield. Initially before sowing seed treatments were done. The data in Table 2 showed that the seeds were subjected to *Rhizobium* culture @20g per kilogram of seed before sowing. Initial treatment of Imazethapyr at the rate of one litre per hectare was sprayed at 20 days after sowing (DAS) to control the weeds. Spraying of prophenophosat the rate of one litre per hectare was sprayed against foliage beetles during vegetative stage, thiamethoxamat the rate of 125gper hectare was sprayed against aphids during vegetative stage and dimethoate at the rate of one litreper hectare was sprayed against white fly (YMV) during maturity stage. Data depicted in Table 3 revealed that for weeding practices imazethapyr was used to control weeds in the field. After emergence and establishment of seedlings urea was applied as top dressing as manure. Prophenophos and cypermethrine was used as plant protection measure to control lepidopteran insects and pests. In both the villages viz. Sankhapur and Partara, 50 farmers were selected in each cluster. Table 4 revealed that out of 50 farmers 04 farmers have personal land area of 0.8 ha and 46 farmers have 0.4 hectares of land. In Partara village 6 farmers have 0.8 hectares of land while 44 farmers have 0.4 hectares of land area.

Data depicted in Table 5 showed that four quintal seeds of green gram variety IPM 02-03were distributed to the farmers of both Sankhapur and Partara villages during demonstration held together in one cluster i.e. in Sankhapur village. Seeds were sown in different plots without initial application of both organic or inorganic fertilizers and micronutrients. Different weedicides, pesticides and fungicides were supplied to 63 numbers of farmers present and participated in cluster demonstration held in Sankhapur village. Seventy five litres of Imazethapyr (10% SL) having a cost of Rs. 32302.5 was given to 63 farmers of both the villages present in cluster demonstration for using to control the pests. Thirty litres of Carboxin (37.5%) with a cost of Rs. 41064 and 1200 gms of Thiram (37.5%) costing around Rs. 2194.8 and 20 litres of both Profenophos (40%) and Cypermethrin (4%) costing Rs. 13452 was supplied to the farmers in the cluster demonstration. No bio-agents, bio-products and nutrient complex were supplied to the farmers in frontline demonstration An off campus training programme was organised on “Improved agronomic practices in green gram cultivation”. The participant farmers were from general caste as well as from SC and ST communities. Total 28 farmers from general caste were participated where as30 participants were from ST and ST categories. Total participants were 55 in the training programme. It was observed from the Table 6 that from general caste men participant farmers were more with 16 participant farmers than women farmers with 12 numbers of participants. In SC/ST community only 9 men and 18 women farmer participants were participated. Total 25 men and 30 women farmer participants were present in the training programme.

Extension activities was also carried including field visit once in a week in the initial period of cultivation and then once in a fortnight till date of harvesting. In the field day a total of 80 farmers were present along with 8 extension personnel (Table 7).HYV greengram (IPM 02-03) was released on 2011 by PDKV, Akola. The potential yield was 12q

/ha and duration of harvesting is of 62-70 days. This variety has large seed, suitable for Rabiand summer season and is resistant tomungbean yellow mosaic virus (MYMV). Table 8 revealed that Kalamuga variety was taken as check or control and IPM 02-03 was taken as demonstrated variety. National average yield was found to be 5.18 q/ha and state average yield and district average yield was 4.80 and 4.34 q/ha respectively. Potential yield of the demonstrated variety IPM 02-03was 12 q/ha with a yield gap of 36.33 and 30.61% respectively. From Table 9 it was shown that maximum yield obtained from check variety was of 6.28 q/ha and minimum 4.15 q/ha while in demonstrated variety maximum of 8.92 and minimum of 6.45 q/ha yield was obtained. The average yield of check and demonstrated variety was 5.26 and 7.58 q/ha respectively. Therefore the demonstrated variety has shown promising result of increase in yield of 43.12% (Fig. 3). It was shown that gross expenses cost/ ha for check variety was Rs.18850 with gross return of Rs. 29324.5 and net returns of Rs. 10474.5 with a benefit cost (B:C) ratio of 1.56. But in case of demonstrated variety gross expenses cost/ ha for check variety was Rs. 22508 with gross return of Rs. 42258.5and net returns of Rs. 19750.5 with a benefit cost (B:C) ratio of 1.88(Das and Parida, 2020).

Specific technology demonstrated for Protection Measures

The data depicted in Table 10 revealed that seed treatment with the mixture of carboxin and thiram @ 2g/kg of seed showed no YMV symptoms. These results indicated that carboxin and thiram with the recommended dose of 40g/ha protected the seed and early emerging seedlings from seed and early seed borne diseases like YMV. Weed management was done by application of Imazethapyr @1.0 l/ha at 15-20 days after sowing (DAS) just after post emergence. Recommendation dose/ha is 2.5 litres. Weed density/m^2 was 28 and percentage of weed control efficiency was recorded as 65. This weedicide was found to be effective for control of broadleaf and grass weeds. Application of mixture of prophenophos and cypermethrine @ 1 lit/ha was found to be 90% effective to control the foliage beetle. Aphid and white flies was found to be controlled with application of thiamethoxam @ 125g/ha against aphids during vegetative stage and dimethoate @ 1 litre/ha against white fly during maturity stage. The data in Table 11 revealed about the socio-economic impact parameters on cultivation of demonstrated variety. It was observed that 7.58 q/ha green gram was obtained and total produce was estimated to be 151.6 quintals 115 quintals produce was sold per cluster with a selling price of Rs. 5575. Three quintals produce was retained as seed purpose per cluster and 45 quintals were distributed to the farmers as seed. This CLFD programme generated 54 man days per cluster. This income gained by the farmers were utilised for education of their children and to fulfilment of other house hold activities.

Observations and feed-back of farmers

Observations under CFLD programme resulted higher yield and income of the farmers owing to high yielding variety with efficient weed and pest management practices (Fig. 4; Table 12). Farmers' opinion and feedback was taken and the farmers reported that cultivation of improved variety with seed treatment, weed management by herbicides application in right time and following proper plant protection measures resulted higher yield and profit. Higher grain yield was reported with the application of 80 kg P_2O_5/ ha (Sharma *et. al*., 1984). Luo *et. al.* (1995) reported that the proportions of nutrients returned from green gram straw were 43.6 – 55.7% of N, 41.8 – 50.3% of P_2O_5 and 55.7 – 61% of K_2O and concluded that green gram is a potassium enriched plant when applied with recommended NPK schedule.

CONCLUSION

One cluster front line demonstration (FLDs) in two village i. e. Sankhapur and Partara was conducted by KVK, of Angul district for rabi crops for the year 2018-2019. Twenty hectares of rain fed land area was sanctioned for FLDs with sanctioned budget of Rs. 1, 50,000 in FLDs, with actual expenses of Rs. 1, 16,503/. Fifty villagers were participated in the FLDs. The seeds of the variety IPM 02-03 were distributed to the farmers. During the demonstration seeds of HYV (IPM 02-03) variety were taken and seed treatment was done using mixture of carboxinand thiram at the rate of 3gm per kg of seed before sowing to control seed borne diseases and pest.

Herbicide spraying was done at 20 days of sowing with imazethapyr at the rate of one litre per hectare to control the growth of the weeds. Timely plant protection measures were implemented at various stages of growth of the plant.Spraying of prophenophos at the rate of one litre per hectare was done against foliage beetles during vegetative stage. Thiamethoxamat the rate of 125g/hectare was done against aphids during vegetative stage and dimethoateat the rate of one litre per hectare against white fly (YMV) during maturity stage. Different techniques were demonstrated in each cluster to adopt in the cultivation practices to get higher yield. Initial treatment of imazethapyr at the rate of one litre per hectare was sprayed at 20 days after sowing (DAS) for effective control of weeds.

Seed germination, protein content, photosynthetic pigments and proline content in the leaves of *Macrotylama uniflorum* leading to yield loss was also reported by the application of extracts of *Mikaniamicrantha* (Jali et al., 2021). Spraying of prophenophosat the rate of one litre per hectare was sprayed against foliage beetles during vegetative stage, thiamethoxamat the rate of 125g per hectare was sprayed against aphids during vegetative stage and dimethoateat the rate of one litre/ hectare was sprayed against white fly(YMV) during maturity stage. Different weedicides, pesticides and fungicides were supplied to 63 numbers of farmers. Seventy five litres of imazethapyr (10% SL) having a cost of Rs. 32302.5 was given to 63 farmers touse to control the pests. Thirty litres of carboxin (37.5%) with a cost of Rs. 41064 and 1200 gms of thiram (37.5%) costing around Rs. 2194.8 and 20 litres of both profenophos (40%) and cypermethrin (4%) costing Rs. 13452 was supplied to the farmers in the cluster demonstration. No bio-agents, bio-products and nutrient complex were supplied to the farmers in frontline demonstration. It was observed that 7.58 q/ha greengram was obtained and total produce was estimated to be 151.6 quintals 115 quintals produce was sold per cluster with a selling price of Rs. 5575. Three quintals produce was retained as seed purpose per cluster and 45 quintals produce distributed/sold to other farmers as seed. This CLFD programme generated 54 man days per cluster. This income gained by the farmers were utilised for education of their children and to fulfilment of other house hold activities.

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Table 1. Cluster FLDs on pulses under NFSM

Sl.No.	Details for cluster demonstration	Attributes
1	Season and year	Rabi'2018-19
2	No. of FLDs (farmers) sanctioned	1
3	No. of FLDs (farmers) conducted	1
4	Area (ha) sanctioned	20
5	Area (ha) actually conducted	20
6	Sanctioned budget (Rs.)	Rs.1,50,000/
7	Budget received actually (Rs.)	Rs.1,50,000/
8	Actual expenditure (Rs.)	Rs.1,16,503/
9	Balance amount (Rs.)	33497
10	Number of clusters implemented in FLDs	1
11	No. of villages and farmers in each cluster	Cluster-I(Village-1; Farmers-50)
12	Land situation (irrigated, rainfed, others specify)	Rainfed

Table 2. Technologies/Practices demonstrated in each cluster in cultivation of HYV (IPM 02-03)

IPM Techniques				
Initial treatment before sowing	**Initial treatment after 20 DAS (days after sowing)**	**Control of foliage beetles**	**Control of aphids**	**Control of white fly(YMV)**
Carboxin + Thiram @ 2g/kg of seed	Imazethapyr@1 litre/ha at 20 DAS	Prophenophos @1 litre/ha against foliage beetles during vegetative stage	Thiamethoxam @ 125g/ha against aphids during vegetative stage	Dimethoate @ 1 litre/ha against white fly(YMV) during maturity stage

Table 3. Post emergence application (manuring, weeding, irrigation)

Post emergence application/IPM Techniques		
Weeding	**Manuring**	**Application of Plant protection measure**
Imazethapyr	Top dressing of Urea	(Prophenophos + Cypermethrine)

Table 4. Farmers strength and land area for Cluster Demonstration

Villages	Farmers Strength	Land Area (ha) and Number of Farmers			
Sankhapur	50	Land area (ha)	Number of farmers	Land area (ha)	Number of farmers having land area of 0.4 ha.
		0.8	04	0.4	46
Partara	50	0.8	06	0.4	44

Table 5. Critical Inputs Provided for Demonstration

Sl. No.	Critical inputs	Name of critical input	Quantity	Value (Rs.)	No. of farmers	No. of villages	No. of clusters
1	Seeds (name variety)	Greengram: IPM 02-14;	4 q		50	1	1
2	Fertilizers (Organic and inorganic)	-	-	-	-	-	-
3	Micro-nutrients	-	-	-	-	-	-
4	Pesticides	Imazethapyr (10% SL)	75 litre	32302.5	63	2	2
5	Fungicides	Carboxin (37.5%) or	30 litre	41064	63	2	2
		Thiram(37.5%)	-	-	-	-	-
6	Insecticides	Profenophos(40%) or	1200 g	2194.8	63	2	2
		Cypermethrin-(4%)	20 litre	13452	63	2	2
7	Bio-agents	-	-	-	-	-	-
8	Bio-products	-	-	-	-	-	-
9	Nutrient complex	-	-	-	-	-	-

Table 6.Training programmes organized on 'Improved Agronomic practices in Greengram cultivation'

Sl.No	Type of training (on/off campus)	Participant farmers (general)-A			Participant farmers (SC/ST)-B			Total participants (A+B)		
		Men	Women	Total	Men	Women	Total	Men	Women	Total
1	Off campus	16	12	28	9	18	27	25	30	55

Table 7. Extension activities including field visits organized

Sl.No.	Name of extension activity	Participant farmers			Participant extension personnel		
		Men	Women	Total	Men	Women	Total
1	Field day	37	43	80	3	5	8

Table 8. Performance of the demonstrations

Name of the crop	Demos (No.)	Variety		National average yield (q/ha)	State average yield (q/ha)	District average yield (q/ha)	Potential yield of the demo variety (q/ha)	Yield gap I (%)	Yield gap II (%)
		Check	Demo						
Greengram	1	Greenmuga	IPM 02-03	5.18	4.80	4.34	12	36.33	30.61

Table 9. Yield and Net Returns

Yield obtained (q/ha)						Yield increase (%)
Check			Demo			
Max.	Min.	Avg.	Max	Min.	Avg.	
6.28	4.15	5.26	8.92	6.45	7.58	**43.12**

Table 10.Results on specific technologies other than variety

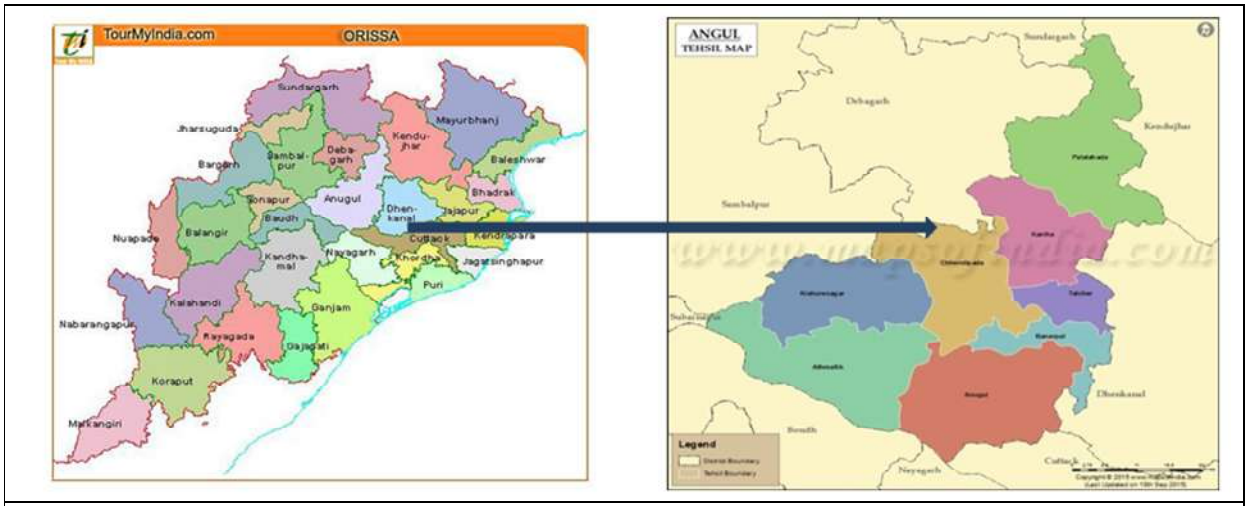
Crop	Pesticides/Insecticides /Weedicides	Specific technology demonstrated	Recommendation /ha	Observations taken	Results
Seed treatment	Carboxin + thiram	@2g/kg of seed	40g	Yellow Mosaic Virus	Protected the seed and early emerging seedlings from YMV
Seedling of 15-20 DAS	Imazethapyr	@1.0 l/ha at	2.5 litre	Weed density/m^2	28% effective control of broadleaf
Seedling of 15-20 DAS	Imazethapyr	@1.0 l/ha at 15-20 DAS	2.5 litre	Weed control efficiency (%)	655 control of grasses
Seedling of 25-30 DAS	Prophenophos + Cypermethrine	@ 1 lit/ha	1.0 litre	% of reduction	90% control of foliage beetel
Vegetative stage	Thiamethoxam	@ 125g/ha	1.0 litre	% of reduction	90% aphid controlled during vegetative stage
Plants at maturity stage before flowering	Dimethoate	@ 1 litre/ha	1.0 litre	% of reduction	Above 90% white flies controlled during maturity stage

Table 11. Socio-economic impact parameters

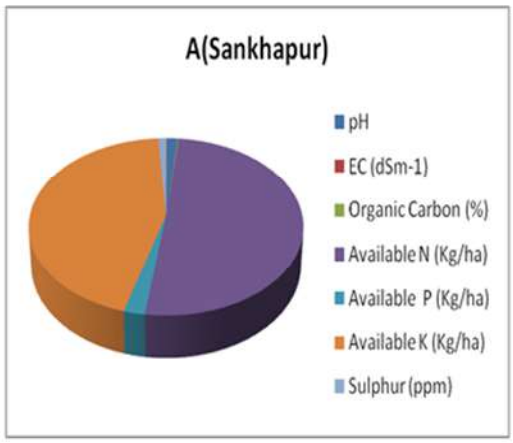
Sl. No.	Parameters	Crop-1
1	Name of the crop	Greengram
2	Variety	IPM 02-14
	No. of clusters	1
3	No. of farmers	50
4	Total area (ha)	20
5	Yield obtained (q/ha)	7.58
6	Total Produce Obtained (q)	151.6
7	Produce sold (q/cluster)	115
8	Selling price (Rs./q)	5575
9	Produce retained as seed purpose (q/cluster)	3.0
10	Produce distributed/sold to other farmers as seed (q/cluster)	45.0
11	Employment Generated (Man days/ cluster)	54
12	Purpose for which income gained was utilized by the farmers	Social function; Education of children; Repairing of house; Purchase of household assets

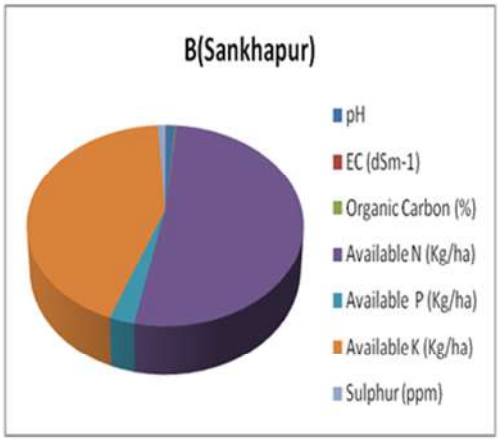
Table 12. Farmer's perception of the intervention demonstrated

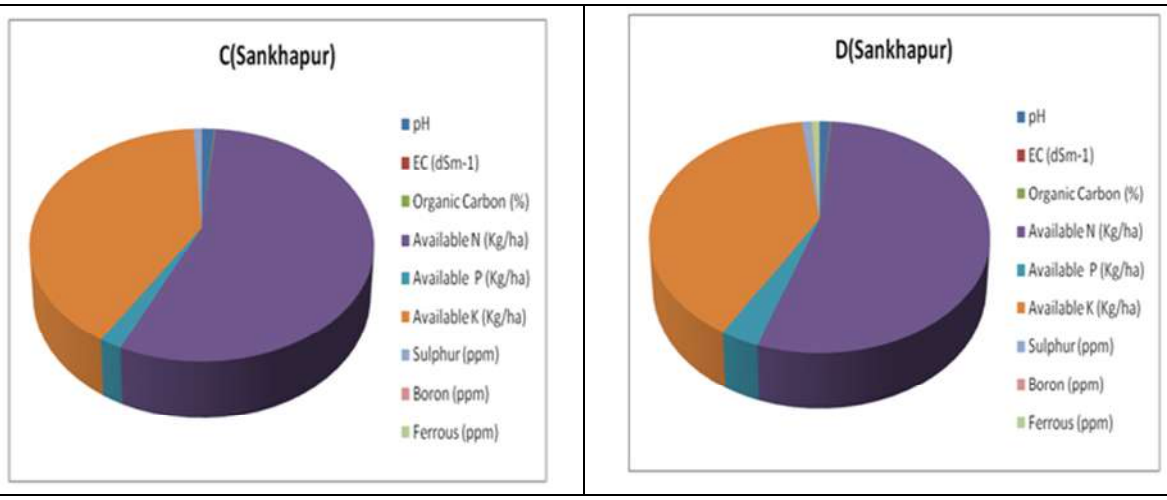
Technology attributes	FARMERS PERCEPTION								
	Technology-1 (Seed treatment)			Technology -2 (Weed management)			Technology -3 (Plant protection)		
	High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
Problem solving		>			>		>		
Understand ability			>		>			>	
Practicability		>			>		>		
Cost effectiveness	>			>				>	
Profitability	>			>			>		
Sustainability		>			>			>	
Compatibility		>			>			>	
Accessibility			>		>		>		
Acceptability		>		>				>	
Preference		>		>				>	



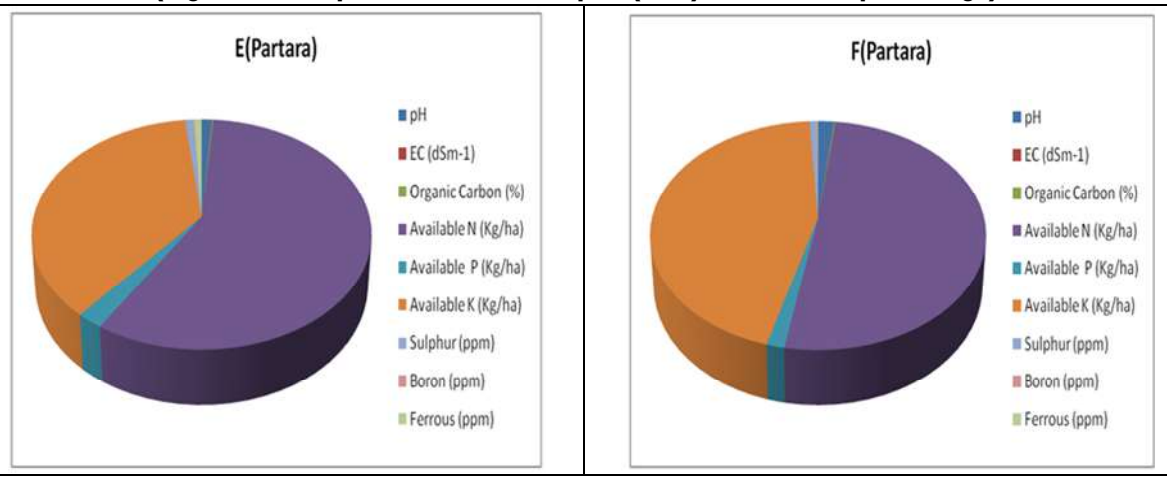
(Fig 1. Odisha map showing Angul District Map).



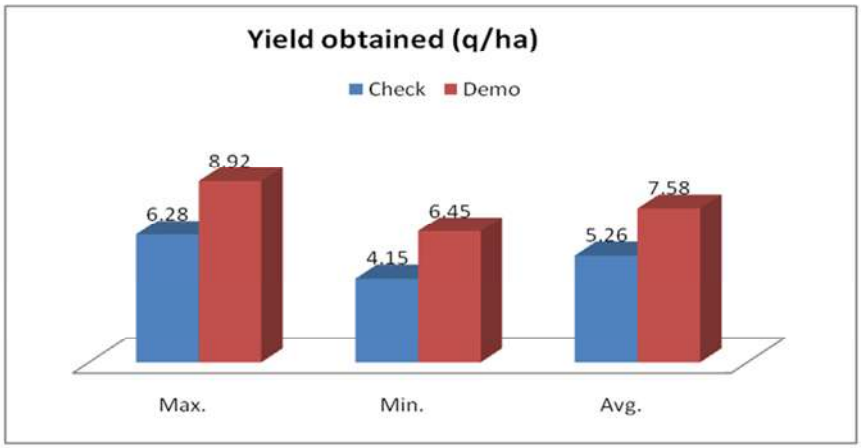




(Fig 2. Soil composition of four samples (A-D) from Sankhapur village)



(Fig 2. Soil composition of two samples (E & F) from Partara village)



(Fig 3.Yield (q/ha) in Check and Demonstrated variety)

(Fig 4. Cultivation field, harvesting of green gram and Farmers' training photographs)

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RESEARCH ARTICLE

Design and Analysis of High Frequency Optimization of Bidirectional, Grid-Connected Converter

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ABSTRACT

A high frequency, interleaved, double-buck, bidirectional converter topology linked to the grid is suggested in this study. Unless there are direct and deadly problems, you can obtain higher frequency of switching and power density. Due to the approach interleaved, the current rip and stress may be decreased efficiently for inductors and other power equipment. In addition, a new design method is proposed for filter parameters. The approach is optimized with less inductance, greater filtering capacity and improved stability. Firstly, the performance requirements for the two inverters and converter states are fully taken into account. Another aspect is the relation between the performance indexes and the filter settings. The results, however, demonstrate that there are inconsistent links between performance indexes. The priority of the filter performance index was set to acquire a number of optimisation parameters. The total harmonic distortions (THDs) in grid current at 2.7 percent, 1.2 percent and 4.5 percent, and the power density was 36 W/in^3 accordingly, among the grid-connected inverters, disconnected inverters and full-load rectifiers.

Keywords: dual-buck; bidirectional; grid-connected converter; parameter design

INTRODUCTION

In distributed new power, grid connected power generation systems, energy storage units are commonly used. They lessen the power fluctuation in grid systems, on the one hand. Instead, the energy connection between the users and the grid improves the ease. The energy storage device must however be further improved with its efficiency and power density [1–3]. Thus, the grid-connected converter needs to emphasize two characteristics of performance as an interface circuit of distributed energy storage devices: (1) The bidirectional energy flow is highly efficient. (2) Lower in weight and smaller volume. The ways for increasing power density are as follows, according to the research of the

relevant scholars: (1) To increase the frequency of operation and minimize the filter size. (2) Suitably designing and reducing the filter element nominal value to produce a greater power density filter parameters [4-10]. Increasing the converter operating frequency can considerably decrease the inductors and condensers. This improves the converter's power density. However, the classic bridge-based transformer must inject dead time, which restricts operating frequency increases. In addition, the dead period leads to additional distortion in waveform. Consequently, the new form of grid-connected converter (i.e. no dead zone) does have considerable research value in comparison with the classic bridge circuit. The approach of design is more intuitive, but the process is more complex. The design of the filter is based on the properties of the circuit in the previous literature, with clear design objectives and a feasible design process. However, most of the material mentioned above takes individual performance as the goal and does not take into account the restrictions between different performances. Multiple performance requirements are therefore difficult to take into account simultaneously. Moreover, the converter's power density is not the optimization objective. A high frequency, cross-leaved, dual-buck, two-way, grid-connected converter is presented in this document on the basis of SiC power devices. The topology contains two states: Inverter and Corrector.

Topology Description

Figure 1, which includes the inverter state and the corrective state, shows the topology of the proposed interleaved, dual-buck, bidirectional, grid-connected converter. The circuit consists of four identical Buck circuits in the inverter state: S_1, S_3, D_1, and L_{i1} form $Buck_1$. The circuit includes S_1, S_4, D_2, and L_{i2} create $Buck_2$. The driving signal of switch S_3 on $Buck_1$ results in a 180-degree drive signal of switch S_4 on $Buck_2$. The pull signal on switch S5 from $Buck_3$ is 180 degrees S_6 from $Buck_4$, which is the interleaving unit 2 of the inverter. The filter performance is directly determined by the parameters of the filter. Thus, the filter settings should be properly designed to achieve the desired results. The filter includes L_{i1}, L_{i2}, L_{i3}, L_{i4}, L_g, C_f and Co for the proposed converter. The inverter LCL filters include L_{i1}, L_{i2}, L_{i3}, L_{i4}, C_f and L_g and the corrective LCL filters in L_{i1}, L_{i2}, L_{i3}, L_{i4}, L_g and C_o. The architecture of the filter in both states are obviously different. Furthermore, the connection link between the filter parameters leads to parameter interactions.
This research proposes a new way of designing parameters that considers the performance demands of both countrie s. The major objective is to get high power, strong filtering performance and good continuous performance. The proc edure can be separated into three different parts. To begin with, the operation principle and circuit process are follow ed by a generalised range of filter settings. Secondly, by studying the connection between the parameters, the range o f filter parameters is optimised. Finally, the priority of the filter performance index is determined to achieve a set of s uitable parameters.

This method will be described in full in the specific design process. The inductance values of L_{i1}, L_{i2}, L_{i3}, and L_{i4} are assumed to be the same as those indicated by L_i in order to simplify the analysis process. Furthermore, the total inductance equivalent in the inverter state L_b total inductance in the rectifier state is defined in (1) as follows:

$$L_a = L_i + L_g$$

$$L_b = 1.5L_i + L_g$$

SIMULATION RESULTS

The simulation diagram is shown below and results are carried out in MATLAB/Simulink.

CONCLUSION

This work offers an interleaved, two-dimensional, grid-connected topology converter, and a corresponding approach for optimizing filter settings. This approach has numerous design aims for optimization, such as filter induction, filtering capacity and system stability. Firstly: simultaneous consideration of the filter's performance requirements in inverter and rectifier states. In addition, the converter's total inductance is low sufficiently and good performance in

the constant condition. This is the unique design process: (1) The link between the circuit performance and filter parameters is taken into account and the filter range of parameters is constantly decreased. (2) Set the filter performance priority and optimize the filter parameters' reasonable value. This produces positive features such as higher density of power, better filtration and higher stable status.

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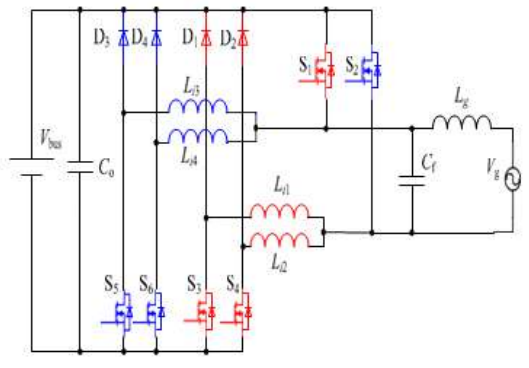
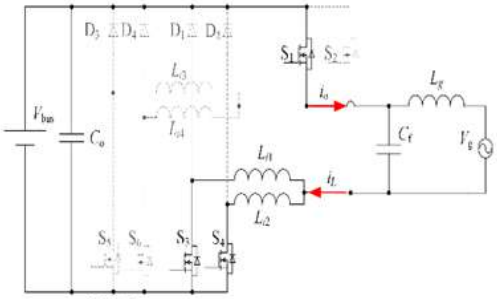
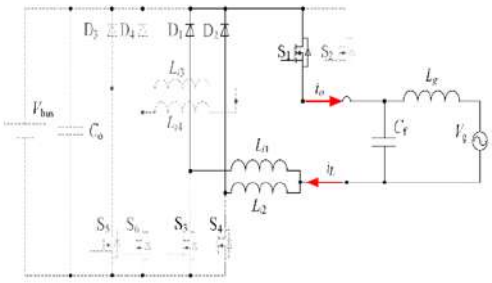


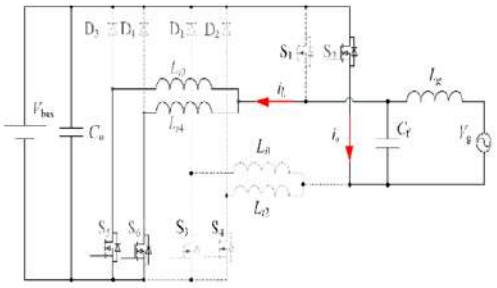
Figure 1: Circuit Diagram



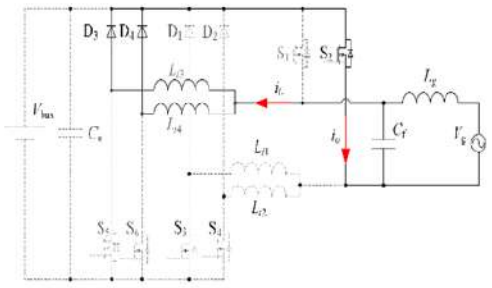
(a) Inverter a



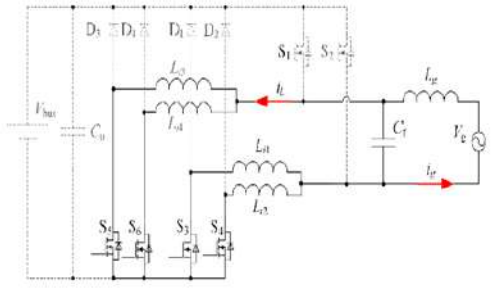
(b) Inverter b



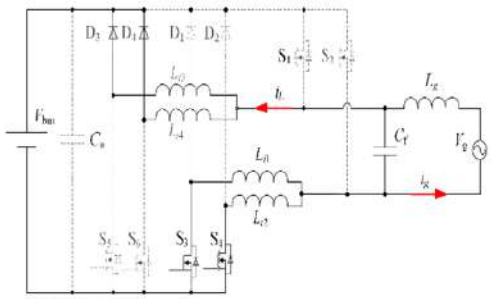
(c) Inverter c



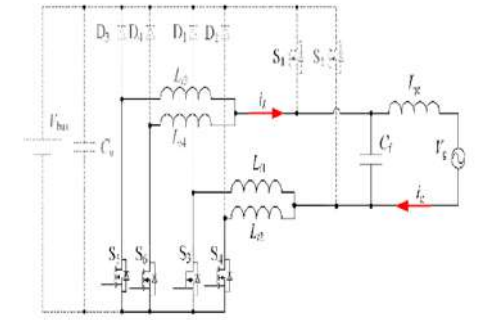
(d) Inverter d



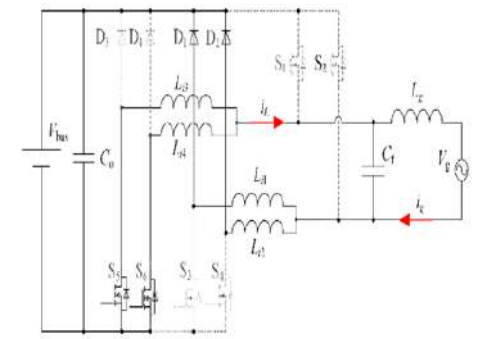
(a) Rectifier a



(b) Rectifier b



(c) Rectifier c



(d) Rectifier d

Modes of Operation

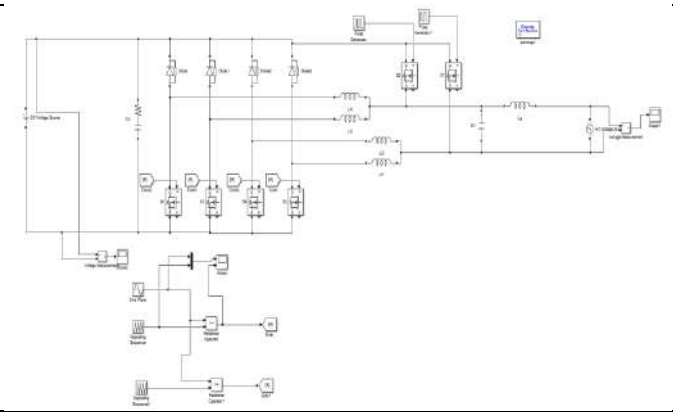


Figure 3: Simulation Diagram of Inverter

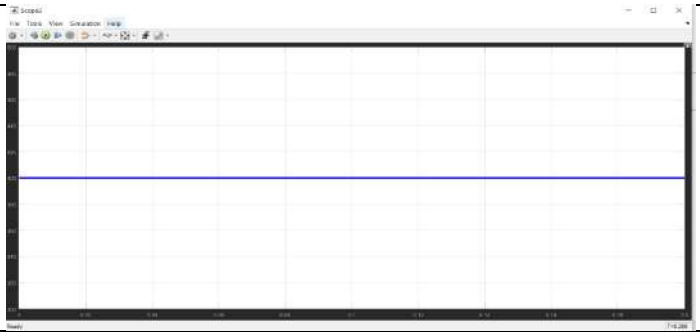


Figure 4: DC Voltage input to the Inverter

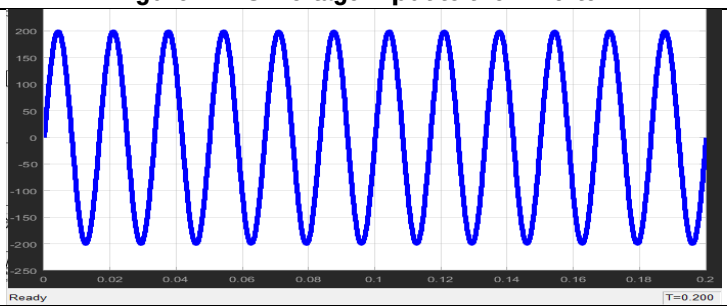


Figure 5: Output Voltage Waveform of Inverter

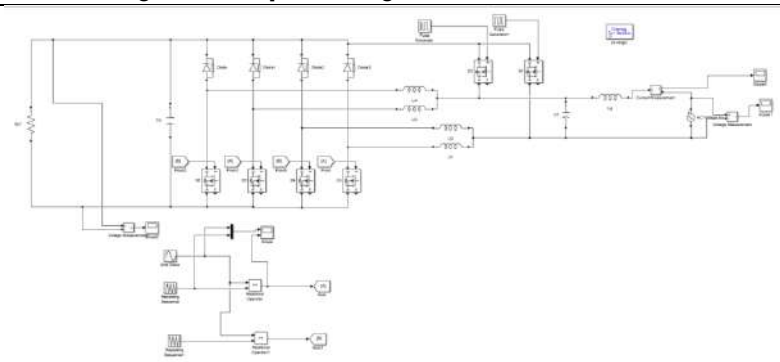


Figure 6: Simulation Diagram of Rectifier

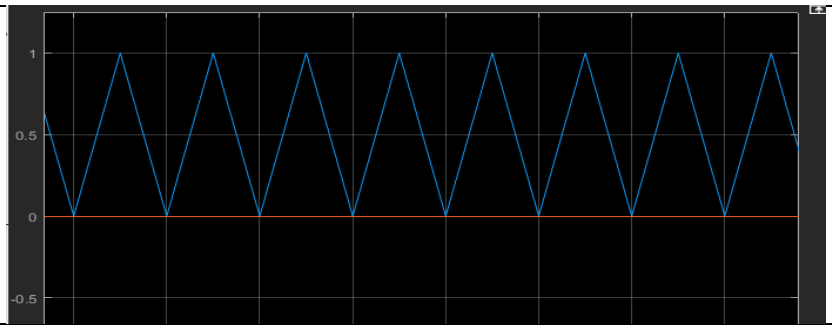


Figure 7: Input to the rectifier

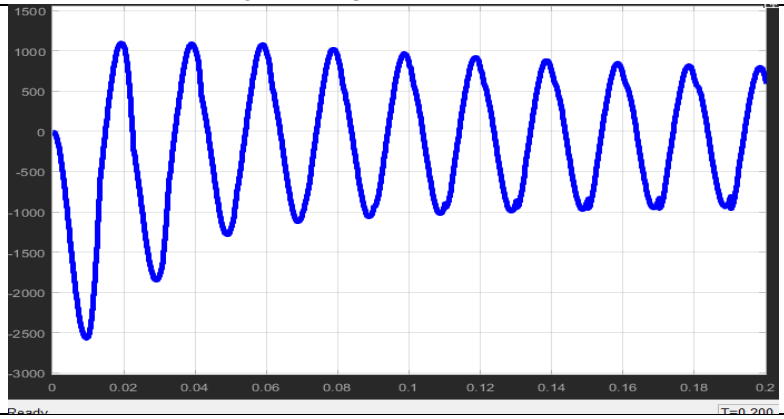


Figure 8: Output waveforms of currents

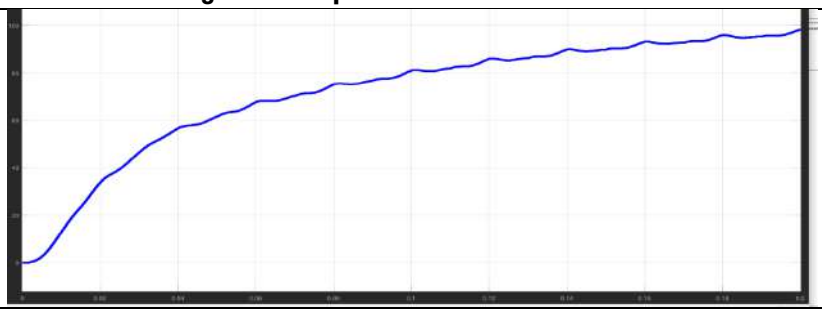


Figure 9: Output voltage waveform of rectifier

Waveforms

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REVIEW ARTICLE

Diversification of Fungus Associated with Skin and Nail Infection: A Review

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ABSTRACT

Fungal skin infections are common worldwide and are burden to patients. In this review article, focus has been given to different fungal and bacterial diseases associated with skin and nail. Six fungal pathogen such as *Cutaneous candidiasis, Tinea versicolor, T. capitis, T.cruris, T. pedis* and *T. corporis* was found to be associated with skin infection and *Tinea unguium,* distal subungual infection caused by *Pseudomonas,* white superficial infection, proximal subungual infection, infection caused by *Candida* species and bacterial skin infections such as cellulites, boils, leprosy and bacterial associated nail infections i.e., Paronychia was discussed.

Keywords: Bacterial diseases, dermatophytosis, skin infections, nail infections

INTRODUCTION

Microorganisms are associated with all living organisms including humans and show both beneficial and harmful effects on human health. Some microbes are the cause of many deadly diseases in humans. The microorganisms like fungi, bacteria, virus and even the parasitic protozoans are able to cause various epidermal diseases on mammals. Fungal and bacterial infections over the skin, nail and hair is common all over the world. The skin, nail and hair diseases are caused by common fungal and bacterial species. Nearly one billion people suffer from dermatomycoses or fungal infections of skin, hair and nails (Bongomin et al., 2017). Dermatomycoses are in warm and humid climates and in tropical countries (Havlickova et. al., 2008). *Candida glabrata*, *C. auris*, etc. are the major fungal disease-causing organisms. There is a huge development on the field of fungal treatment with the application of specific antifungal and antimicrobial compounds. Earlier the fungal infections can be easily cured by normal treatments but now with the increased disease complexity and resistance of the pathogenic organisms, the involvement of modern healthcare is included. Due to the drug application, some disease-causing organisms also developed some in build resistance power and in such case the organism becomes drug resistance and sustain in normal antifungal treatment. Overall,

the fungal infection is having a greater impact on human health and the ailments can be controlled by well diagnosis and proper treatment by natural drugs without any side-effects. Botanicals also reported to have effects in curing skin diseases (Sahoo et al., 2020).

Fungus associated with skin infections

Dermatophytosis is the study of fungal infections caused by dermatophytes over skin. Dermatophytes involves a group of fungi maintains their growth over dead keratin. Some species from genera such as *Epidermophyton*, *Microsporum* and *Trichophyton* are commonly invade human keratin. Other genera like *Trichophyton* also grow over the skin producing a ring like structure which is termed as 'Ringworm'. This is very common and impacts different parts of body (Gupta et.al., 2005). Out of millions of species under fungal group, only 300 are well known of causing human skin diseases. Mostly the moist and warm environments and the areas having less airflow together influence the development of fungus resulting fungal skin diseases like scaly rash and skin discoloration (Owen, 2020). A minute and very common fungus can have the impact over the skin and scalp easily. Some of the skin and nail infections are described below.

Tinea corporis: It is the most common human skin disease identified as ringworm and is caused by the fungal genus like *Trichophyton*, *Microsporum* and *Epidermophyton* belongs to the family Arthrodermataceae and the order Onygenales. The identified body parts by this disease are torso and limbs, whereas over other areas of body the rain worm is having different names such as athlete's and jock itch. The symptoms include the edges with circular ring shaped rashes. The inside skin quite looks healthy. However the rashes are generally itchy and can easily spread. These rashes have harmful effect over human skin but commonly treated by antifungal cream.

Tinea pedis: *Tinea pedis* infection is commonly known as Athlete's Foot caused by the fungus *Trichophyton rubrum* belongs to the Family Arthrodermataceae and the order Onygenales. It generally attacks the skin of foot and identified between toes. In case of this infection the skin became red and dry and in case of high infection sometimes it looks like scaly and flaky. A stinging sensation on the soles of feet, burning and itching between the toes are the most typical symptoms of this fungal infection. It can also be identified from blistered and cracked skin. If the infection gradually increases on the lack of treatment, then it can be spread to other skin parts of human body such as groin. Hands and nail can also be affected and is termed as *Tinea manuum*.

Tinea cruris: *Tinea cruris*is commonly called as jock itch, a fungal infection caused by the fungus *Trichophyton tonsurans, T. Mentagophytes* and *T. rubrum* belongs to the Family Arthrodermataceae and order Onygenales. The infection is commonly identified over the area of groin and thighs, which is always found in men and adolescent boys. In case of jock itch the skin looks cracked and flaky and some cases it also looks scaly. The rashes can be identified by a darker border which is slightly raised and well identified. Itchy red rashes are the very first symptoms of *Tinea cruris*. The infection generally starts from the upper inner thighs and the groin area of human body. With increase in time the infection can grow and the rashes can be spread to abdomen and buttocks after heavy physical activity or exercise.

Tinea capitis: It is the fungal infection called as ringworm of scalp and hair shafts, which is the result of contact with the fungus *Microsporum*, *Trichophyton* belongs to the family Arthrodermataceae and order Onygenales. The infection is mainly located over the scalp of young children and looks like red patches. In the very first stage the infected parts become red and started itching and after some days the bald patches look scaly. It results tenderness over that areas with substantial pain because of rashes over the patches. The only treatment is the application of antifungal herbal medicines and the use of antifungal shampoo.

Tinea versicolor: *Tinea versicolo r*is also called as *Pityriasis versicolor*. It is a fungal/yeast skin infection causes by the overgrowth of a specific type of fungus i.e. *Malassezia* belonging to the Family Malasseziaceae and the order Malasseziales. This infection develops small oval colourless patches over the skin and infects about 90 % of adult all

over the world. In this infection the body parts like chest, upper arms and back get affected by discoloured patches over skin. Sometimes the patches can be brown, red and tan or blue however in general they might be darker or lighter. These are scaly or itchy. The diagnosis is little difficult in this infection because of only itching symptom. The warm and wet climate can impact the infection.

Cutaneous candidiasis: This is a very normal skin infection caused by the fungus *Candida* belongs to the family Saccharomycetaceae and order Saccharomycetales. This fungus is generally found inside human body hence the overgrowth can cause infection. The infection generally identified over the skin area such as the folds of buttocks and under the breasts. So it basically located to the areas which are moist and warm or improperly ventilated areas. The infection can be identified by the symptoms like red rashes or dark rashes, itching and red pustules can be identified.

Fungus Associated with Nail infections

Fungus can infect any part of the body and it's over growth causes harmful effect to human body. Fungal nail infections are normally located over 50 % in case of nail abnormalities (Tim Newman, 2017). Fungal nail infection is a transmitted disease hence it can be transmitted from others; also the fungus which is already present on human body can cause fungal nail infections (Elaineand Luo, 2019). Some of the important fungal diseases of nail are discussed here.

Tinea unguium: *Tinea unguium or* onchomycosis is the term identifies the fungal infection over the nail. It is caused by the fungus *Trichophyton rubrum* and *T. interdigitale* belonging to the family Arthrodermataceae and order Onygenales. It mostly infects the nails of humans as well as Animals. However, the infection over toenails is much more effective than the fingernails. This infection is more specific over men than the female. In case of this infection the nails break easily and become thickened, typically the nails turn yellow, or brown and some white patches located over the nail. The treatment includes the involvement of doctors however the infected nails can be removed or prescribed medicines should be taken to prevent this infection.

***Distal subungual* infection**

The most common fungal nail infection is distal lateral subungual nail infection caused by the fungus *Pseudomonas* belonging to the family Pseudomonadaceae and order Pseudomonadales. In distal subungual onychomycosis, the nail area is jagged with yellow-white spikes projecting into the proximal nail plate. Yellow patches and green discoloration indicates the growth of fungus along the proximal area. Longitudinal streaks are produced as a result of over growth of melanin producing fungus which shows homogeneous brownish pigmentation. (Natalia Mendoza, et al., 2009).The treatment includes oral antifungal medicines for finger and toe nails. Also medicated nail lacquers have been developed for the infection. The disease shows less toxicity and short treatment period.

White superficial infection

White superficial Onychomycosis is a primary nail infection caused by the organism *Leukonychia trichophytica*. Some other species are also partially included such as *T. mentagrophytes, C. albicans* and the non-dermatophyte mode by *Aspergillus terreus, Fusarium oxysporum*, *Acremonium* species. In-case of child, the species *T. rubrum* has also been identified. The infection occurs in the nail bed and the hyponychium, generally located in the dorsal side of toe nails by *T. Mentagrophytes* which secrets enzyme that helps to cause this particular disease. The symptoms include speckled or punctate porcelain-white lesions randomly distributed along the surface of nail plate and it gradually spread over the whole surface of nail with the increase in fungal growth. The white patches may acquire yellow shade. (Jenny and Elewski, 2005).This can be treated by tropical medications and depends upon the rate of recurrence. Surgery and laser therapy is also included for easy and early treatment.

Proximal subungual infection

Sometimes the fungal infection begins from the under surface of proximal nail fold and then progress distally and is identified as proximal subungual onychomycosis. It is the result of the fungus *Trichophyton rubrum* belonging to the

family Arthrodermataceae. Other than this also the involving organisms are *Candida, Fusarium and Aspergillus*. The nail changes are either diffused patches or transverse striate patterns that may occur like superficial onychomycosis. The over growth of the fungus affects the entire nail and toe nails get infected more than the finger nails. Males get affected more than females and adults affected more than children. This disease is reported in higher frequency with the patience of acquired immunodeficiency syndrome (ADIS). The infected persons with HIV virus showed this disease (Jenny and Elewski, 2005). Topical antifungals are applied over the skin and the nail area to kill the fungus. Newer oral prescribed medicines are also involved to cure this disease in an earlier manner. Surgery involves removing the nails when the infection mode is high and harmful. Leaser treatment is one of the most advance methods to treat the fungal nail infection with the penetration of laser beam.

Candida Infection

This is the infection caused by the yeast *Candida albicans* belongs to the family Saccharomycetaceae and order Saccharomycetales. In this disease the nail fold becomes inflamed and that is erythematous, otherwise the nail plate separates from its bed which is called as onycholysis. Here the nail blade thickens and hardens and inflammation of the nail blade becomes folded. This infection results some unpleasant odour might be the involvement of chemicals with the result of colonization of bacteria over fungal infected, damp or damp areas. It changes the shape and size of nails, the sides or corners of nail causes pain and the over growth of bacteria and the bacteria causing chemical results unpleasant odour. It results discoloration, thickening or brittle. The disease can be a result of genetic factors. Synthetic and traditional drugs both are useful against this type of nail infection. Prescribed medicines and advance treatment mechanisms can be used for the betterment of Candida Infection.

Bacterial Skin and Nail Infections

Skin is the largest and one of the most sensitive organs in the body, hence it simply gets infected by various minute involvement of bacterial species and with the over growth of causal organism it becomes huge and harmful. Bacterial skin infection or nail infection are slow growing and effective processes. The proper diagnosis and proper treatment within the time is most important to prevent the disease. Some infections are easy to treat with tropical medication only the involvement of antibiotics and other require proper oral medication, sometimes with a high dose.

Bacteria associated with skin infections

Cellulitis: It is a bacterial infection mostly located over the inner layer of skin and also located over the fat and soft tissue underneath by the organism *Streptococcus* and *Staphylococcus* belongs to the family Streptococcaceae and order Lactobacillus. The bacterial generally present over the surface of skin and does not harm the body however when they enter into the inner part of skin through any cut or bites or grazes. The infection is highly painful with redness with the infected area and the rapid growth of infection may cause sepsis. The symptoms include swelling and redness over the area and tenderness. In some cases chilled and cold sweating, fever and nausea has been identified. The disease mostly located during or after middle age. And this is more common among people having excess weight and mostly found in let skin. People with low blood circulation show high risk. The effect of treatment depends upon the immunity of human body, as with strong immune system the treatment contains a short period and easy impact and beneficial. It can be responded by oral antibiotic treatment of 7 to 14 days. In more severe cases the treatment can be led to hospital for synthetic drug process.

Boils: It is a bacterial infection having painful pus-filled bump under the skin with the involvement of bacteria *Staphylococcus aureus* belongs to the family Staphylococcaceae and order Bacillales. It generally found inside the skin of nose and bump forms as pus collects under the skin. It appears mainly on your skin of face, buttocks, armpits, thighs and back of the neck and hair bearing areas with the cause of sweating. The symptoms include red bump with heavy pain and it enlarges up to or more than 2 inches. Swollen skin located around the bump. The size of bump increases when fills with pus. The tip of the bump becomes yellow-white when ready to ruptures. It can impact the vision when located over the face. Diabetes can help it to impact more and it became difficult to overcome. Insect bite

and a minute injury can lead to boils. Cleaning of cuts and use of antiseptic can prevent this infection, whereas after high infection proper use of antibacterial ointments and oral medication is much more important.

Leprosy: Leprosy is one of the chronic diseases caused by nerve damage and skin lesions through bacterial involvement such as *Mycobacterium leprae* which belongs to the family Mycobacteriaceae and order Mycobacteriales. The disease mainly located in eye, nose, skin and peripheral nerves. The patches may be numb or looked faded, growth of nodule like structures over the infected part. It forms the skin area stiff or dry. Loss of eyelashes can be located. Initially it looks lightly coloured or darker than the normal skin or red skin patches are generally located. Also the symptoms include blister, rashes, redness and ulcers. Eye problem may cause blindness. Nosebleeds and stuffy nose can be observed. It can be treated with the high dose of Antibiotics and can be cured with 6 to 12 months of multi drug therapy. Doctor prescribed synthetic medicines can be needed to cure.

Bacteria associated with Nail Infections

Paronychia: It is the bacterial nail infection commonly caused by the pseudomonas species belongs to the family Pseudomonadaceae and order Pseudomonadales. Looks like a red, swollen parts over nail skin. Incase of toe nail the entire nail become destroyed by this disease (Bologinia, et al., 2003). Inflammation of the region on the origin of finger and toe finger. It is generally located on one side of skin joined with the nail containing pus and the area becomes yellowish white and result heavy mail over that particular area only. This may lead to abnormal nail growth. To treat this infection self-care guideline can be followed such as soaking of nails in warm water. Avoid of chemicals over this section to prevent infection. Use of prescribed antibiotics for easy and fast treatment and an antiviral medication for a herpes infection (Freed berg, et al., 2003; Keisha Findley et al., 2013).

CONCLUSION

Various fungal and bacterial diseases associated with skin and nail was discussed. Various symptoms of the diseases and their attributes are discussed which will help the future researcher to investigate in finding biological methods to cure this skin and nail diseases. This also provides a framework for future investigation between pathogenic and disease pathogenesis.

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RESEARCH ARTICLE

Magnesium Content in *Cucumis sativus* L in the Various Agroclimatic Zones of Maharashtra –A Comparative Study

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ABSTRACT

Family Cucurbitaceae is nutraceutically very important as many members of this family have chemical derivatives which are helpful in diabetes type I and type II (Murtaza 2017). Members of family Cucurbitaceae include fruit vegetables which primarily comprise of food having high nutraceutical value. *Cucumis sativus* L. is an excellent fruit vegetable with good amount of magnesium, which is an essential constituent required for good health of humans. In the present investigation the fruit vegetables were collected from various places from the nine Agroclimatic zones of Maharashtra (which is classified based on rainfall, soil type and vegetation) and they were analysed for Magnesium content in summer and winte. It was observed that there was a slight variation in the magnesium content of fruits of *Cucumis sativus* L. collected during the two seasons. Comparatively higher Magnesium content was observed in the fruits collected in winter compared to the fruits collected in summer. Out of the nine agroclimatic zones of Maharashtra, the fruits collected from Nagpur which is in MH8 Central Vidarbha (Moderate rainfall) zone showed maximum Magnesium content in the fruits collected in summer. The fruits collected in winter showed maximum magnesium content in the fruits collected from Aurangabad which is in MH 7 Central Maharashtra Plateau (Assured rainfall) zone.

Keywords: Nutraceuticals, *Cucumis sativus* L. Environmental factors, Magnesium content, Agroclimatic zones of Maharashtra.

INTRODUCTION

The nutraceutically important compounds such as vitamins, minerals, anti-oxidants, dietary fiber and omega-3 poly unsaturated fatty acids are recommended for prevention of cardio vascular diseases. The biochemical compounds such as flavonoids, proteins, vitamins and minerals are recommended as anti-oxidants and anti-inflammatory treatments. Lycopene, beta carotene, vitamin C, sulphur compounds and glycosides such as Cucurbitacin are

recommended as an anti-cancer, anti-diabetic, blood purifier drug (Alghasham*et.al.* 2013). The juice and extract of *Cucumis sativus* L. (Cucumber) and the leaves and roots of *Cucumis sativus* L. have been highly regarded for their blood sugar-lowering attributes. However, the general belief that the bitter substance (Cucurbitacin) from Cucurbitaceae is a potent blood sugar-lowering agent has not been substantiated. Leaves and tuberous roots of pointed gourd are also used in Ayurvedic medicine (Chandrasekar *et. al.* 1988). The Geography of Maharashtra is characterized by a narrow coastal plain that separates the Arabian Sea from the Western Ghat Mountains. The western coastal plains have high rainfall, followed eastward by the ghat mountain zone, the transition zone and the drought prone zones. The eastern zones are again characterized by moderate to high rainfall patterns. On the eastern side of the mountains the climate is drier. In the present investigation an attempt was made to find out if there is seasonal variation in the magnesium content in the fruits of *Cucumis sativus* L collected from various places collected from the nine Agroclimatic zones of Maharashtra.

MATERIAL AND METHODS

Plant selected

Cucumis sativus L. is a common fruit vegetable found in a climate that is warm and humid. It is found in almost all over India in the wild and eaten by common man. These plants were collected from the nine Agroclimatic zones of Maharashtra, in the summer months of April and May and winter months of November and December, they were then analysed for Magnesium content.

Sites selected

Plants from the following regions were collected.
MH-1 South Konkan Coastal: Vengurla, Ratnagiri (Chiplun and Rajapur).
MH-2 North Konkan Coastal Zone: Thane, Karjat, (Kolad) Raigad
MH-3 Western Ghat Zone: Lonawala, Igatpuri, Trimbak
MH-4 Sub-montane (Transition 1) Zone: Surgana, Peth, Patan
MH-5 Western Maharashtra Plain Ganeshkhind (Transaction 2) zone: Dhule, Nashik, Kopargaon
MH-6 Scarcity Zone: East Dhule (Songir), East Nashik (Malegaon), Nevasa (Ahmednagar)
MH-7 Central Maharashtra Plateau (Assured rainfall) zone: Aurangabad, Amravati, Akola
MH-8 Central Vidarbha (Moderate rainfall) zone: Wardha, Yavatmal, Nagpur
MH-9 Eastern Vidarbha Zone: Gondia, Bhandara, Chandrapur (Agriculture statistical information 2002)

Method

The Mineral content, magnesium was analysed using EDTA titration method.

Observation

In the fruit samples of *Cucumis sativus* L. collected during the summer season from nine Agro climatic zones of Maharashtra (Agriculture statistical information, 2002). Magnesium content was found to be maximum i.e. 5.6 mg/100g dry weight (Table No. 1) in the samples collected from Nagpur which is in MH8 Central Vidarbha (Moderate rainfall) zone. The p value was found to be 0.75 and the f calculated value 0.29. which was lower than f critical value of 3.63 hence all the results were significant, and the null hypothesis was accepted (Table No. 3). Honest significance of difference (HSD) value was calculated as 1.49. The q value was in between 0 to 1.49 (Table No. 2). The fruit samples of *Cucumis sativus* L. collected during winter season from nine Agro climatic zones of Maharashtra showed an increase magnesium content in most of the samples collected. Maximum magnesium content of 5.1 mg/100g dry weight (Table No. 1) was observed in the samples collected from Aurangabad which is in MH7 Central Maharashtra Plateau (Assured rainfall) zone. The p value was found to be 0.84 and f calculated value 0.18 which was lower than f critical value 3.63. All the results were significant and hence null hypothesis was accepted (Table No. 3). Honest significance of difference (HSD) value was calculated as 1.36. The q value was in between 0 to 1.36 (Jones & Tukey 2000) (Table No. 2).

RESULTS AND DISCUSSION

Magnesium content of *Cucumis sativus* L. fruits collected during winter from the nine Agroclimatic zones of Maharashtra was comparatively higher than the Magnesium content level observed in the fruits collected during summer season. In the summer it was found to range between 2.2-5.6 mg/100g while in the fruits collected during winter it was observed in the range between 3.3-5.1 mg/100g. Essein et. al (2016) reported magnesium content of 13mg/kg, Karanja *et.al*. (2013) reported magnesium content in the range of 33.86 mg/100g. Similar work was reported in other countries (Agatemor Uzuazokaro 2018).

CONCLUSION

Magnesium occurs naturally in many Cucurbitaceae fruits. *Cucumis sativus* L. fruits are important source of magnesium. Magnesium is necessary for maintaining normal nerve and muscle function. Its helps in keeping healthy immune system and steady heartbeat. It helps in maintaining blood sugar level. Magnesium deficiency may lead to gastrointestinal movement and cardiovascular function. Magnesium content in the fruits of *Cucumis sativus* L. collected from Nagpur which is in MH8 Central Vidarbha (Moderate rainfall) zone was found to be comparatively higher than the fruits collected from the other places in the agroclimatic Zones of Maharashtra. The magnesium content in the fruits also showed a slight variation season wise, with a comparatively higher amount present in the fruits collected during winter months. These observations can be useful for nutraceutical companies while selecting fruits with high nutraceutical value.

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Table 1. Magnesium content of *Cucumis sativus* L. fruits (mg /100g fresh wt)

Places of Collection	Magnesium content summer	Magnesium content winter
Vengurla	4.2±0.4	3.8±0.11
Chiplun	4.1±0.3	3.7±0.2
Rajapur	3.5±0.26	3.5±0.35
Thane	3.5±0.23	3.5±0.21
Karjat	3±0.31	3.4±3
Kolad	2.9±0.11	4.4±0.21
Lonawala	2.9±0.25	4.1±0.66
Igatpuri	3.2±0.15	4.4±0.31
Trimbak	3.1±0.31	4.5±0.2
Surgana	2.5±0.31	4.6±0.2
Peth	2.6±0.15	4.2±0.64
Patan	3.1±0.15	3.7±0.1
Dhule	3.4±5.2	3.3±0.15
Nashik	2.9±0.17	3.6±0.26
Kopargaon	2.5±0.26	3.5±0.3
Songir	2.7±0.23	3.9±0.2
Malegaon	4.1±0.6	4.5±0.25
Nevasa	4.3±0.1	5±0.21
Aurangabad	2.3±0.15	5.1±0.81
Amravati	2.2±0.2	3.9±0.45
Akola	2.7±0.11	3.3±0.32
Wardha	5.5±0.21	3.7±0.2
Yavatmal	5.3±0.15	3.3±0.17
Nagpur	5.6±0.26	3.6±0.41
Gondia	3.6±0.2	4.5±0.32
Bhandara	4.6±0.17	4.4±0.21
Chandrapur	2.8±0.11	4.9±0.15

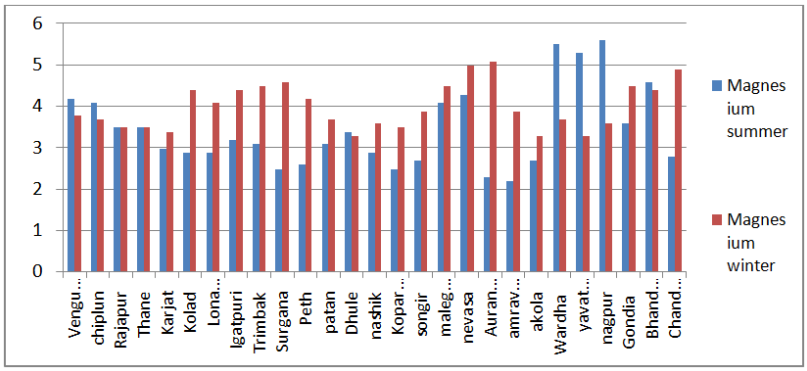
Table 2. Magnesium content of *Cucumis sativus* L. fruits collected in summer Honest significance of difference (HSD) by Tukey test.

Comparison between agro climatic region	HSD Value 1.49 Null hypothesis to be accepted or rejected (summer)	HSD Value 1.36 Null hypothesis to be accepted or rejected (winter)
MH 1 & MH 2	2.66	0.36
MH 1 & MH 3	2.89	2.43
MH 1 & MH 4	4	1.82
MH 1 & MH 5	3.33	0.73
MH 1 & MH 6	0.78	2.91
MH 1 & MH 7	5.1	1.58
MH 1 & MH 8	5.1	0.49
MH 1 & MH 9	0.89	3.4
MH 2 & MH 3	0.22	2.06
MH 2 & MH 4	1.33	1.46
MH 2 & MH 5	0.67	1.09

MH 2 & MH 6	1.89	2.55
MH 2 & MH 7	2.44	1.21
MH 2 & MH 8	7.77	0.85
MH 2 & MH 9	1.78	3.03
MH 3 & MH 4	1.11	0.61
MH 3 & MH 5	0.44	3.15
MH 3 & MH 6	2.11	0.49
MH 3 & MH 7	2.22	0.85
MH 3 & MH 8	7.99	2.91
MH 3 & MH 9	2	0.97
MH 4 & MH 5	0.67	2.55
MH 4 & MH 6	3.22	1.09
MH 4 & MH 7	1.11	0.24
MH 4 & MH 8	9.1	2.3
MH 4 & MH 9	3.11	1.58
MH 5 & MH 6	2.55	3.64
MH 5 & MH 7	1.78	2.3
MH 5 & MH 8	8.43	0.24
MH 5 & MH 9	2.44	4.12
MH 6 & MH 7	4.33	1.33
MH 6 & MH 8	5.88	3.4
MH 6 & MH 9	0.11	0.49
MH 7 & MH 8	10.2	2.06
MH 7 & MH 9	4.22	1.82
MH 8 & MH 9	5.99	3.88

Table 3. ANOVA Magnesium content of *Cucumis sativus* L. fruits collected in summer

Statistical Parameter	Value (summer)	Value (winter)
P value	0.75	0.84
F value	0.29	0.18
F. critical	3.63	3.63
SE value	0.3	0.27



Magnesium content mg/100 g fresh weight in the nine Agroclimatic zones of Maharashtra

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RESEARCH ARTICLE

Survey on Predominant Diseases of *Vigna mungo* (L.) Hepper. in Jajpur District of Odisha

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ABSTRACT

Survey was carried out in three villages namely Kamalpur, Lalbagh and Tentulidhia of Jajpur district of Odish of Jajpur district to know the severity of the predominant diseases of *Vigna mungo* (L.) R. Wilczek. Powdery mildew, anthracnose, leaf spot disease, ascochyta leaf spot, mung bean yellow mosaic disease, leaf crinkle disease and leaf curl/necrosis were the predominant diseases found in these three villages during the survey period. The results of the present study revealed that powdery mildew was found in all the surveyed black gram cultivated fields.

Keywords: Black gram, predominant diseases, powdery mildew, *Vigna mungo*

INTRODUCTION

Black gram is one of the important pulse crops in India. Due to its nutritional richness and cropping system adaptability, it is an ancient and well-known leguminous crop in Asia. It is known as "poor man's meat" and is a primary source of dietary protein for a substantial portion of the world's vegetarian population. It is known for its rich source of protein, iron and high fiber content. Black gram is a dicotyledonous plant with the scientific name *Vigna mungo* (L.) R. Wilczek, commonly known as “Biri” in Odia and belongs to family Fabaceae. This crop can be cultivated as Kharif as well as summer crop and is mainly cultivated in East Asia and Indian sub-continent. Pulse crops are vitally pertinent for our country's vegetarian population because pulses are the main source of protein. However, per capita availability of pulses is steadily diminishing due to population growth and low productivity of pulse crops. The daily availability of pulses per capita is only 47 gram, compared to the minimal requirement of 104 grams advised by World Health Organization/Food and Agriculture Organization nutritional experts. Black gram is one of the most significant pulse crops since it can be grown at any time of year. Jajpur district of Odisha, is known for the production of black gram. It is under cultivation in indie is about 3.25 million hectares and annual production

is 1.45 million tons. Black gram is high in proteins, potassium, calcium, iron, niacin (B3), thymine, and riboflavin, making it a very nutritious food (B2). Blackgram is used in the treatment of liver diseases, diabetes, heart diseases and central nervous system infection. Low genetic yield potentiality, indeterminate growth habit, canopy design, low partitioning efficiency, farming on marginal land, and biotic and abiotic stressors all contribute to lower productivity of black gram. Foliar disease management was studied to increase the productivity of black gram (Madhuri & Sagar, 2020). Ravinder et al., 2005 studied the influence of plant age on establishment of leaf crinkle viral infection. Host range and disease assessment of black gram was studied by Mayee et al., 1986. Integrated foliar disease management of legumes was evaluated to increase the seed yield (Pandey et al., 2009). This survey was conducted in three villages of Jajpur district of Odisha to assess the severity of different fungal and viral diseases of different black gram varieties. Crinkle virus disease of black gram was also studied (Ashfaq et al., 2021). Black gram seed yield assessment was also studied (Sood et al., 2021). In order to know the severity of diseases in black gram the survey was carried out to know the severity of different diseases in Kamalpur, Lalbagh and Tentulidhia of Jajpur district of Odisha during kharif and rabi season 2018-2019.

MATERIALS AND METHODS

Survey Site

Field study was carried out in three villages namely Kamalpur, Lalbagh and Tentulidhia of Jajpur district of Odisha. The blackgram fields were visited and observations were recorded during kharif and rabi season of 2018-2019.

RESULTS AND DISCUSSION

Field surveys were carried out in three villages namely Kamalpur, Lalbagh and Tentulidhia village of Jajpur district, Odisha. Pulse production and productivity are low, with substantial insect, fungal, bacteria, and viral attack being one of the main causes. The black gram is found to be susceptible to a number of pathogens. The following diseases were observed.

Powdery mildew Disease

These are powdery mildew, which is caused by *Erysiphe polygoni*. The disease generally noticed on all aerial parts of the plants. Powdery mildew causes small, irregular powdery spots on the top surface of the leaves, and in some cases the symptoms appear on both surfaces. During the flowering and pod development stages, the disease gets more severe. The leaves, petioles, stem, and even the pods are totally covered in white powdered patches. Powdery mildew is more severe in late-sown kharif crops, but it can appear at any time during the year in favorable conditions.Powdery mildew is one of the most significant constraints in the production of black gram, resulting in a potential yield reduction of about 40-90% (Reddy et al., 2005; Reddy et al., 2008)

Anthracnose Disease

Anthracnose disease is caused by *Colletrotrichum lindemuthianum*, sexual stage (*Glomerellal indemuthianum*).The fungus produces dark brown to black sunken lesions on the hypocotyl region and causes seedling death. The fungus is transmitted through seeds and causes primary infection. It can also be found in soil infected plant tissues. Dissemination is also aided by rain splashes.

Leaf spot Disease

Leaf spot diseases is caused by *Cerospona canescens* (Fig. 1). Small irregular patches with a grey centre and a brown edge appear on the leaves after infection by the fungus. The fungus can be found on damaged plant detritus as well as seeds. Airborne conidia are the second mode of transmission. The disease can be controlled by removing contaminated plant material.

Ascochyta leaf spot

Ascochyta phaseolorum is a fungus the causal organism of ascochyta leaf spot. A small irregular area with a grey to brown centre and a yellow border is one of the symptoms of this fungal infection.

Mung Bean Yellow Mosaic Disease

Mung bean yellow mosaic is caused by Mung Bean Yellow Mosaic Virus (MYMV). Initially small yellow patches or spots appear on green lamina of young leaves. Soon it develops into a characteristics bright yellow mosaic symptom.

Leaf Crinkle Disease

Leaf crinkle disease is caused by Urd bean Leaf Crinkle Virus (ULCV). Symptoms include crinkling and curling of the tips of leaflets and increase in leaf area. White fly, *Bemisia tabaci* is responsible which helps in the secondary spread. The virus is also sap transmissible. Presence of weed hosts like *Aristolochia bracteata* and *Digera arvensis* and kharif season crop and continuous cropping of other legumes serve as source of inoculum. The virus is seed- borne and primary infection occurs through infected seeds.

Leaf curl/Necrosis

Leaf curl disease is caused by Groundnut Bud Necrosis Virus (GBNV). Symptoms include upward cupping and curling of leaves with vein clearing.

CONCLUSION

Greater emphasis is now laid on increasing the productivity and thereby the total production of pulses in order to mitigate the protein hunger of growing population of our country. Black gram is a dry season pulse crop which requires low inputs and serves as an excellent source of seed protein. Hence, cultivation of high yielding input responsive varieties of black gram is being recommended. In addition to other management practices such as irrigation and plant protection, black gram responds markedly to plant population level and mineral nutrition especially, when applied in balanced amount and by appropriate methods. However, the response of blackgram to fertilizer application varies from place to place and variety to variety. Powdery mildew caused by *Erysiphe polygoni* DC is one of the major constraints in the production of black gram, which lead to a potential decrease in yield (40-90%). Powdery mildew, cercospora leaf spot, anthracnose, and black gram yellow mosaic virus (MYMV) are the main diseases of black gram among the biotic stressors. Powdery mildew is more severe in late-sown kharif crops, but it can occur throughout the year under favourable conditions.

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Fig 1. Cercospora leaf spot and leaf blight disease of black gram

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RESEARCH ARTICLE

Integration of Renewable Energy Sources with Control in Railway Microgrid

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ABSTRACT

The traffic rail increment infers an increment in the electric energy utilization. Hybridizing the railroad substations with half breed fuel sources dependent on sustainable power sources and capacity units associated with a dc transport might be an answer for give to the fractional freedom of energy makers in the area traffic in rail. A savvy control is strongly prescribed to try not to upset the traffic it nature of railroad lines. This work proposed an reversible, self-versatile, independent, and clever appropriated generator associated with the catenary of transport dispersed control by the multiagent framework. The results investigation has shown that the proposed control engineering can be an answer for face the issues identified with the traffic railroad issues.

Keywords: Railway microgrid, tracking of energy, renewable energy sources.

INTRODUCTION

This kind of fuel source remainder as different to energy derivatives, which are being utilize definitely more it recharged. Sustainable power regularly gives energy in four significant regions: power age, air and water warming/cooling, transportation, and country (off-grid) energy administrations. It comprises of hybridizing the substation by cross breed power age framework (HPGS) attached to a dc transport that it is straightforwardly associated with the centennial as portrayed. It offers all administrations brought by the half breed frameworks and doesn't need any progressions in the current engineering of the substation. Connecting environmentally friendly power sources (RES) and capacity is transport, associated with the centennial, is the half breed substation (HSS) concentrated in this paper. It's anything but a generator stockpiling units. Two RES from various nature are joined, to limit their intermittence and furthermore to keep away from their regulating that can happen if by some stroke of good luck of accessible. If their principle trademark, of rail route line in integrally. A capacity unit, for example, of

attached one. It gives the energy important to satisfy the pinnacle power need and devours the overabundance on account of the related converter. In reality the interconnection of the RES and the battery to a dc transport in the centennial can be seen as circulated generator (DG) that lessens the bought in power because of the RES age, gives the energy fundamental during speed increase stages to smother the bought in power surpassing and disposes of on account of the battery release and the age at the same time, and maintains a strategic distance from the voltage increment because of the deceleration cycle, Renewable energy is valuable energy that is gathered from inexhaustible assets which are normally recharged on a human timescale, including carbon unbiased sources like daylight, wind, downpour, tides, waves, and geothermal warmth. The term regularly includes biomass also, whose carbon nonpartisan status is under banter.

Two RES from various nature are joined, to limit their brokenness and furthermore to stay away from their oversizing that can happen if by some stroke of good luck one source is accessible. Following their fundamental trademark the RES supply the rail route line in reciprocally. A capacity unit, for example, the battery is attached to the RES. It gives the energy important to fulfill the pinnacle power need and burns-through the overabundance is on account of the related converter. In fact the interconnection of DC transport in the catenary can be seen as conveyed decreases the bought in power on account of the RES age, gives the energy vital during speed increase stages to stifle the bought in power surpassing and takes out the drop voltage because of release age at the same time, and keeps away from the voltage increment because of the deceleration interaction by burning-through the energy abundance, because of the cell. In general force of the future HSS surrendered (1). As indicated by (1) devour the energy required for flow just yet from a reasonable utilization of RES produced power PRES, $P_{Battery}$, and $P_{Substation}$ because of the Distributed Energy Management. The joining of the disseminated age, for example, RES in the rail line organization should meet a few specialized requirements [4-6]. The expected chance of coordinating RES in the rail line of concentrated in [7] [8], Is called attention to that to work on the proficiency and unwavering quality of the framework, hybridizing a fixed framework dependent on RES in the rush hour gridlock railroad must be accomplished by thinking about an astute methodology, in light of the fact that the greatest rail route traffic utilization is infrequently corresponded with most extreme RES creation. The HPGS comprises of a multi-source framework with decentralized fuel sources with various limits and distinctive age.

Rail-Way System Control by MAS

If control the rail line micro-grid is a similar rule of a force stream manage in a DC transport [14]. If current streaming uncovers various potential situations; a train or a few trains brake, or a speed up. In this way, the normal current addresses the result of the different potential situations that may happen. If administrator specialist' data to specialists intrigued data and is give the inventory or utilization of energy administration to meet the speed increase & decrese stages necessities. In reality, these specialists are specialist & res supervisor specialist'. On the opposite side, the 'battery specialist' gets from Simulink the sign demonstrating its condition of SOC. On the off chance that the proposition of the 'line supervisor specialist' is related in specialist' acknowledges the proposition and starts is required during the speed increase which isn't recuperated, that additionally take part in the inventory of energy during speed increase. The end devours the all out abundance of the because of deceleration and furthermore because of the RES age which isn't burned-through at deceleration time. On the off chance that the dissemination speed is non-consistent and a stock solicitation is recommended, the 'RES administrator specialist' will incorporate if transport the subsequent current which is the subsequent foothold and slowing down current of bi-directional burden as clarified previously. Subsequently, if observed by the battery is the distinction current, i.e., if speed increase is delivered if isn't shrouded it to take out the bought in power surpassing.

CONCLUSION

This work bargains the DEM of railroad of MAS micro-grid with Hybrid SS dependent of HPGS is convene the constraints of rail transportation frameworks as far as energy economy. HPGS comprises of a multi-source framework with decentralized fuel sources with various limits and an alternate age, thusly, sensible use and

combination of every component were regarded. Decreasing the bought in power, disposing of the voltage drop in the line because of the speed increase and prompting the bought in power surpassing and keeping away from the voltage ascend because of the deceleration by burning-through the absolute of the regenerative the energy doesn't recuperated by different trains of line, stay the primary issues that ought to be considering while at the same time hybridizing of substation is without altering the current engineering. Accordingly, this work reach the referenced limits and confines by planning reversible, dynamic, canny, self-versatile, and self-ruling DG associated with the catenary on account of the disseminated DC transport control of voltage by the MAS.

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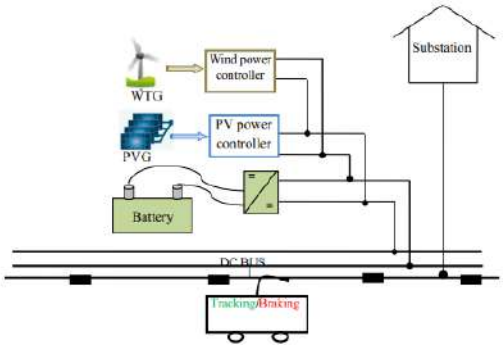

Fig 1. architecture of HSS

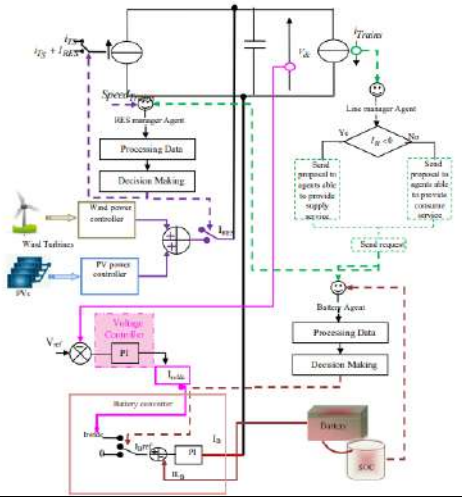

Figure 2. Circuit of Railway microgrid

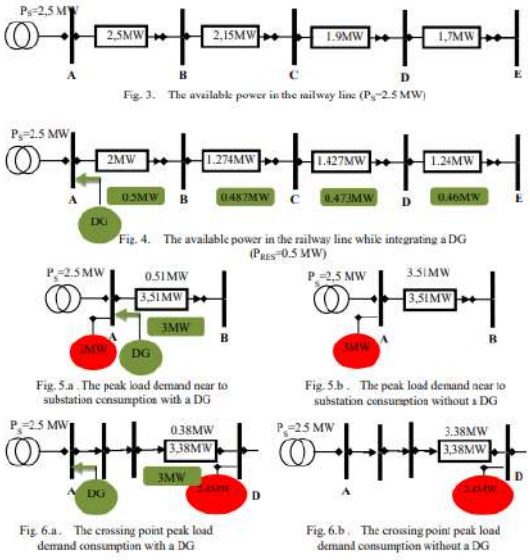

Fig. 3. The available power in the railway line (P_S=2.5 MW)

Fig. 4. The available power in the railway line while integrating a DG (P_{RES}=0.5 MW)

Fig. 5.a . The peak load demand near to substation consumption with a DG

Fig. 5.b . The peak load demand near to substation consumption without a DG

Fig. 6.a . The crossing point peak load demand consumption with a DG

Fig. 6.b . The crossing point peak load demand consumption without a DG

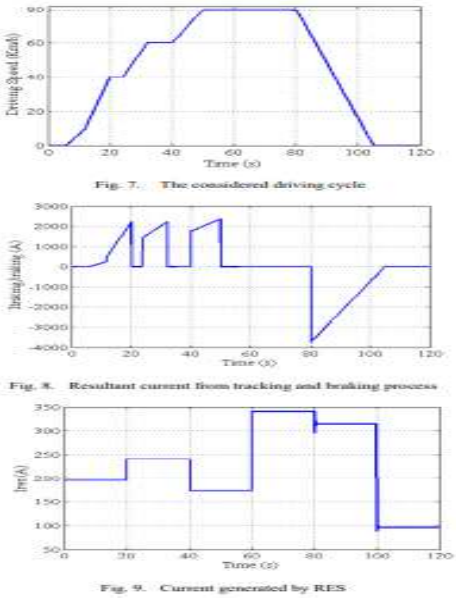

Fig. 7. The considered driving cycle

Fig. 8. Resultant current from tracking and braking process

Fig. 9. Current generated by RES

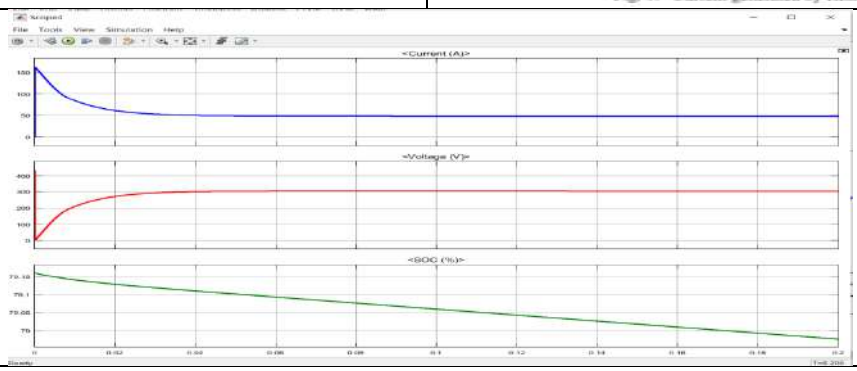

Fig 10. Battery (soc, current, voltage)

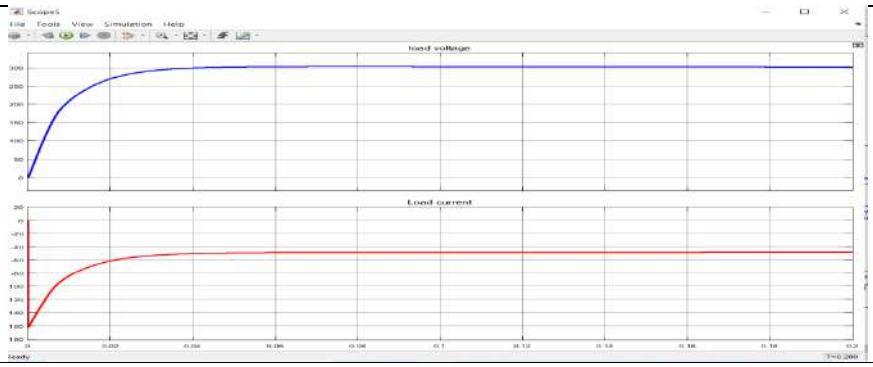

Fig 11. Load output voltage and current

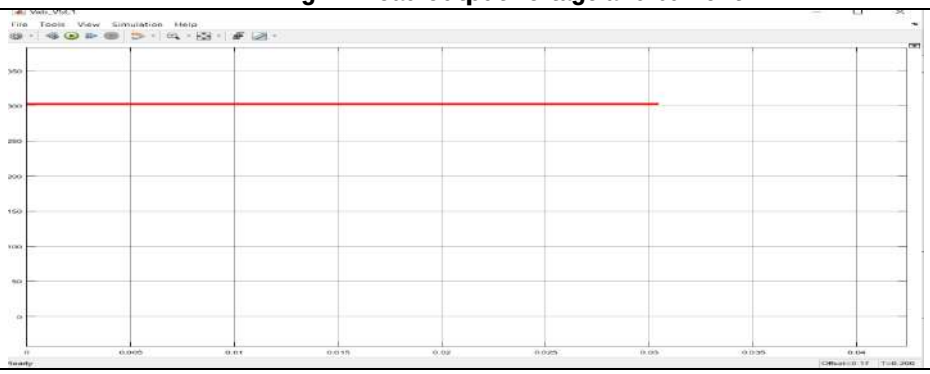

Fig 12. Solar output

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RESEARCH ARTICLE

Morphological and Meristic Characters of Fresh Water Fish *Puntius amphibious*

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ABSTRACT

Puntius amphibius was identified using morphometric measurements and meristic counts using a divider and measuring board with graduations in millimetres. For 17 morphometric and meristic characters, the rate of growth of different morphological body parts of fish in relation to total length was investigated.

Keywords: *Puntius amphibious,* meristic, measurements, characters, Rays

INTRODUCTION

Morphometric characters in fishes are important to study because they can be used to differentiate taxonomic units and detect differences between fish populations. Morphometrics can be used to quantify a trait of evolutionary significance and to infer something about their ontogeny, function, or evolutionary relationships by detecting changes in shape. The first step in studying species is taxonomic identification [1]. Many researchers used protein studies and DNA sequences to estimate the similarities and differences between Taxonomic categories. However, due to the costs, this is particularly difficult [2]. Fish morphology has been a reliable source of data for taxonomic and evolutionary studies. These are primarily classified as Morphometric and Meristic. This is the simplest and most direct method of identifying fish. Morphometric characters are those that can be measured, whereas Meristic counts are those that can be counted.

Morphometric and meristic characters are commonly used for fish identification, whereas morphometric characters are measurable characteristics of a fish. Meristic counts, on the other hand, are countable characters. Meristic counts include the number of fin rays, spines, vertebrae, and gill slits. Examining their body shape, proportion of body part to total length, pattern of arrangements of fins, position of mouth, coloration, and number of fin rays is the simplest way of distinguishing one species from another. They are used to assess intraspecific variation among species.

Each fish has a distinct body shape, which can be elongated, fusiform, cylindrical, dorsoventrally flattened, laterally compressed, or any combination of these. An organism's body shape is an adaptation to the environment in which it lives. For example, a fusiform shaped body is a feature of fish that live in the upper portion of the water column. Benthic bodies are dorsoventrally compressed. The fins are the most distinguishing feature of fish. A fish's mouth morphology reflects its feeding habits and can be inferior, sub terminal, terminal, or superior. Fins are classified into two types: median fins (dorsal, anal, adipose, and caudal) and paired fins (pectoral and pelvic). Dorsal fins are located on the dorsal side of fish and are used to protect the fish from rolling. On each side of the body, pectoral fins are located just behind the operculum. Pelvic fins can be found on the body's ventral side, just behind the pectoral fin. The caudal fin, also known as the tail, is located at the end of the caudal peduncle. Its primary function is propulsion. The caudal fin can be rounded, intended, straight, forked, or pointed. The caudal peduncle connects the anal and caudal fins. The soft fleshy structure located behind the caudal fin is known as the adipose fin. The function of this fin is not fully understood.

Fish fins are made up of spines, also known as hard rays and soft rays. Soft rays can be segmented or branched. Catfishes use their fins to defend themselves. Catfishes, for example, have soft fleshy barbels around their mouths. The maxillary barbels are the barbels on each side of the mouth. Mandibular or mental barbels are those that are located on the chin region. Barbels are typically sensory in nature and are used to search for food. Morphometric parameters of a fish species play a significant role in determining whether there is any disparity between the same species in different geographic regions. There are phenotypic differences in morphometric and meristic characteristics between fishes of the same species due to differences in sex, food availability, predator-prey interactions, physical parameters, and environmental conditions. Allometry refers to changes in body proportions that occur during the growth phases and result in significant changes in body form. Variations in morphometric characters explain the evolutionary adaptations of the species; for example, the mouth gap size of a species determines the species' feeding habit.

MATERIALS AND METHODS

From October 2019 to December 2019, fish specimens were collected on a monthly basis from Kausalyaganga, Daya River. The morphometric measurements and meristic counts were performed on 16 Puntius amphibious specimens ranging in size from 7.7cm to 11.7cm in total length (TL) and 20 to 45 g in weight. For various measurements, a divider, measuring board with millimetre graduations, string, and scale have been used. Seventeen morphometric and one meristic character were studied following the standard procedures described AppaRao (1966), as well as Dwivedi and Menezes (1974).

The following seventeen morphometric characters were obtained for each fish (Fig. 1).

- Total length (TL): The distance from tip of the snout to the tip of longest ray of caudal fin.
- Fork length (FL): The distance from the tip of the snout to the end of the middle caudal fin rays.
- Standard length (SL): The distance from the tip of the snout to the end of hypural plate.
- Pre-pectoral length (PPL): The distance from the tip of the snout to the insertion of the pectoral fin.
- Pre-pelvic length (PPLL): The distance from the tip of the snout to the insertion of the pelvic fin.
- Pre-dorsal length (PDL): The distance from the tip of the snout to the anterior end of the first dorsal fin base.
- Pre-anal length (PAL): The distance from the tip of the snout to the insertion of the anal fin.
- Pectoral fin length (PL): Length of the longest fin ray of the pectoral fin.
- Dorsal fin length (LD): Length of the longest fin ray of thedorsal fin.
- Anal fin length (LA): Length of the longest fin ray of the anal fin.
- Caudal fin length (LC): Length of the longest fin ray of the caudal fin.
- Depth (D): The distance from the anterior end of first dorsal fin to the ventral surface of the fish at deepest part.

- Least width of caudal peduncle (LWCP): The minimum distance between the dorsal and ventral edges of the caudal peduncle.
- Pre-orbital (Snout length) (PRO): The distance from the tip of upper jaw to the front margin of the orbit.
- Post orbital length (POO): The distance from hind margin of orbit to the tip of opercular membrane.
- Eye diameter (ED): Distance from the anterior to the posterior rims of the eye in the longitudinal axis.
- Head length (HL): The distance from tip of the snout to the posterior point of opercular membrane.

Meristic characteristics are those that can be counted, such as vertebrae, fin rays, and scales. Only the Caudal fin ray was dealt with here for convenience. There are two types of fin rays: undivided rays or spines and divided rays or soft rays (also termed as rays). FAO, 2013; Jayaram, 1981).

Difference between spines and ray

Spines	**Rays**
Hard and pointed	Segmented
Unsegmented	Sometimes branched
Unbranched	Bilateral with Left and right halves
Solid	Flexible

Here we have considered only Caudal Fin Rays as the meristematic characters.

RESULTS

A total of 16 specimens ranging from 7.7 to 11.7 cm were used for the studies of morphometric and meristic characteristics. The important morphometric data in the present study is given in Tables1,2,3 and Figures. 1, 2, 3 & 4.

DISCUSSION

Morphometric analysis helps to understand the relation between the body parts. Proportion of each and every-body parts with its total length is used for morphometric analysis.

- From the above study I have found that fishes of the genus Pontious are found in South Asia and mainland Southeast Asia, with a single species. Thegreast richness in India.
- The maximum size for an adult of this genus is 25cm but most species reach 7 to 15 cm and some species do not surpass 5cm. In appearence they may resemble miniature car & are sometimes brightly coloured and patterned.

Meristic characteristics are those that can be counted, such as vertebrae, fin rays, and scales. Only the Caudal fin ray was dealt with here for convenience. There are two types of fin rays: undivided rays or spines and divided rays or soft rays (also termed as rays). FAO, 2013; Jayaram, 1981).

CONCLUSION

Above all, the experiment yielded a nice result demonstrating *Puntius amphibius* dominance in Bhubaneswar (Kausalyaganga, near Uttara, Dayariver, at the back of Dhauli) from October 2019 to December 2019. (Winter season). The specimens were collected and tested on a regular basis from the Daya River. According to the observations, these fish species grow significantly up to a few centimetres in length, which is their maximum length. These fish species are dominant in the Daya River and cannot grow larger than that, but some fish from this species can grow up to 25 centimetres, which is larger than the measurements recorded in table-1.

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Table1.Measurable Characters of Fish *(Pontius amphibious)* in cm

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
TL	11.3	11.7	11.1	11.5	8.5	10.3	8.7	8.5	7.8	7.7	8.8	8.5	8.5	9	9.2	8.8
FL	9	9.2	9.5	9.8	7.6	9.6	7.5	7.5	6.9	6.7	7.4	7.3	7.3	7.7	7.8	7.5
SL	8.5	8.9	8.5	9	7	8.2	6.9	7	6.1	5.6	6.8	6.6	6.6	7	7	6.9
PPL	2	2	1.8	1.9	1.6	2.1	1.4	1.7	1.6	1.4	1.5	1.5	1.4	1.7	1.7	1.6
PPLL	4.6	4.7	4.1	4.4	3.4	4.1	2.4	3.5	3	2.8	3	3.1	3.3	3.5	3.6	3.5
PDL	4	3.9	3.8	4.2	3	3.6	3.2	3.3	3	2.9	3	3	2.9	3.5	3.4	3.2
PAL	7.1	7.2	6.5	6.7	5.3	5.8	5.1	5	3.2	4.6	5.5	5	5	5.4	5.5	5.2
PL	1.7	1.7	1.6	1.6	1.2	1.4	1.1	1	1.1	1.2	1.2	1	1.1	1.1	1.1	1.2
DL	2.2	2.2	2	2	1.6	1.9	1.5	1.4	1.3	1.3	1.5	1.5	1.5	1.6	1.6	1.6
LA	1.5	1.5	1.5	1.4	1.1	1.3	1.1	1.2	0.7	1	1.1	1	1	1.1	1.1	1.2
LC	2.7	3	2.8	2.7	2.1	0.9	1.9	1.8	1.6	1.5	2	1.7	1.7	1.9	2	2.1
D	2.2	2	2.1	2	1.8	2.4	1.9	1.6	1.5	1.6	1.7	1.6	1.7	1.6	1.7	1.6
LWCP	1	1	0.9	1	0.8	0.9	0.7	0.8	0.7	0.7	0.8	0.6	0.7	0.8	0.8	0.8
POO	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.5	0.6	0.3	0.6	0.6	0.5	0.6	0.6	0.8
PRO	0.5	0.5	0.5	0.4	0.3	0.6	0.4	0.5	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.5
ED	0.7	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.6
HL	2	1.9	1.8	1.8	1.5	1.9	1.6	1.2	1.4	1	1.5	1.5	1.4	1.5	1.6	1.6

Table 2.Relationship between total length & depth

Sample No.	TL in Cm	D in Cm
1	11.3	2.2
2	11.7	2.0
3	11.1	2.1
4	11.5	2.0
5	8.5	1.8
6	10.3	2.4
7	8.7	1.9
8	8.5	1.6
9	7.8	1.5
10	7.7	1.6
11	8.8	1.7
12	8.5	1.6
13	8.5	1.7
14	9.0	1.6
15	9.2	1.7
16	8.8	1.6
Mean	**9.3687**	**1.8125**
Standard Deviation	**1.343**	**0.260**

Table 3. Shows the maximum and minimum value

	Maximum	Minimum
TL in cm	11.7	7.7
D in cm	2.4	1.5

Figure 1.

Figure 2.

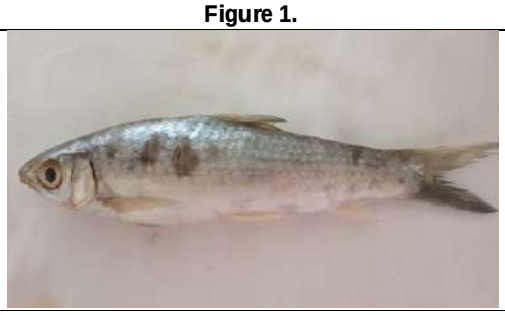
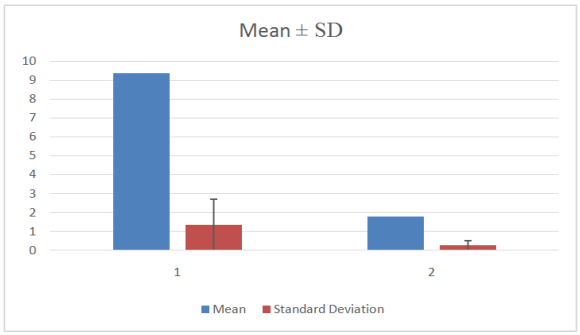


Figure 3.

Figure 4.



Graph 1. Relationship between total length & depth

Graph 2. Shows the maximum and minimum value

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RESEARCH ARTICLE

Latitudinal Study of Space Weather Influence on Ionosphere

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ABSTRACT

The ionospheric variability is mainly depending upon space weather conditions. It is disrupted when there are geomagnetic storms. The Satellite navigation and communication systems are affected as well. The results presented here describe the responses of six intense geomagnetic storms (-100 nT > Dst_{min} > -250 nT) at low, mid, and high-latitudes that develop during the solar cycle 24. The dual-frequency GPS Vertical Total Electron Content (VTEC) data in the Receiver Independent Exchange Format (RINEX) has been retrieved from the International GNSS Service (IGS) database Scripps Orbits and Permanent Array Center (SOPAC). We also use the solar Dst index and IMF Bz component. We find enhancement in VTEC values at all three latitudes (low-latitude, mid-latitude, and high-latitude). The change in VTEC values was observed maximum at low-latitude, followed by mid and high latitudes as compared to previous or quiet days.

Keywords: Ionosphere, Space weather, Total Electron Content (TEC), Geomagnetic storms.

INTRODUCTION

Basically, space weather can be defined as the dynamic and highly variable nature of the geophysical environment, as a result of solar flares that are borne in by solar wind plasma. A number of factors affect the solar wind, such as its speed and density, the interplanetary magnetic field, solar flares, and coronal mass ejections (CME). Space weather research involves the study of the variation of physical processes of space due to the sun and impacts on geo-space, interplanetary space, on the earth's surface. Research involves observing data, analyzing data from ground-based and satellite-based instruments, and interpreting theoretical and empirical models. The GPS drove Total Electron Content is a helpful parameter to study a better accepting of the behavior and dynamics of the ionosphere under different geospatial conditions.

When storms occur, the ionospheric electron density significantly changes (Buonsanto, 1999) during the storm's main phase, a positive increase in electron density may be noticed due to the thermo-spheric wind directed toward the equator ward (Lin et al., 2005). At the same time, depression in the electron density in the geomagnetic storm's recovery phase shows may be due to the neutral composition ratio (O/N2), (Fagundes et al., 2016). These factors contribute significantly to the development of equatorial ionization anomalies (EIAs) during storms. The electron density of the ionospheric changes during geomagnetic storms can be easily understood by analyzing total electron content. Previously many researchers have studied ionospheric variation with the use of Total electron content (TEC). (Fuller-Rowell et al., 1997; Balan et al., 2010; Sharma et al., 2017; Sori et al., 2019). Bhattacharya et al., (2008) have study Ionospheric TEC for the geomagnetic quiet and disturbed period at equatorial anomaly region and find higher values TEC during disturbed periods in winter then quite periods, while TEC during equinox season has higher values during the quiet period. Balan et al., (2010) reported the positive storm at low and mid-latitude during the storm time, and Neutral winds from equatorward slow down Plasma diffusion downward as well as recombination processes. Sori et al., (2019), analysis of the ionospheric TEC variation data and solar wind, electron density, geomagnetic indices, IMF, and thermospheric neutral wind data for the geomagnetic storm that occurred between 7 and 8 November 2004 . They reported that the TEC enhancement where observed during the initial as well as in main phases of the storm over North America and Europe region of mid-latitude and rapid longitudinal expansion within one h, and then enhancement was also observed over Japan. This implies that TECs enhanced in Japan were associated with a magnetic conjugacy in the southern hemisphere, which is a characteristic of storm-enhanced density (SED). In the present study, we investigate the six geomantic storms of solar cycle 24 with the Dst value less than -100 nT. The GPS total electron contents (TEC) data of three different latitudes (low, mid, and high) during the geomagnetic storms are taken from the SOPAC database and then processed. For geomagnetic storms, we use the Dst index and the Bz component of the IMF. This work is divided into different sections, in section 2. Methodology and data availability are explained. In section 3, results and discussions from graphs are presented. Finally, in section 4 the major finding of our work is outlined.

Data and Methods Used

We use the following data set
Total Electron Content (TEC)
Disturb storm time (Dst) Index
IMF Bz

Total Electron Content (TEC)

The GPS-TEC data is downloaded from International GPS Service (IGS) http://sopac.ucsd.edu/dataArchive/ and IGS provides free data access. The downloaded data is in RINEX form, converted using GOPI Seemala software, A application tool for the analysis of GPS observation's TEC (Seemala and Valladares, 2011).We use three IGS stations for ionospheric TEC data, situated at different low, mid, and high latitudes. The detail of geomagnetic coordinates, name and station code is shown in Table 1. And the selected six intense geomagnetic events are shown in Table 2.

Disturb storm time (Dst) Index

Disturb storm time (Dst) is the H (horizontal) component of the magnetic field surrounding the earth during a geomagnetic storm event. This is associated with the ring current. It is measured by several ground-based stations in nanotesla (nT), Dst index explains the geomagnetic storm that when Dst = 0, no storm or quite condition is detected, moderate (Dst value between −50 nT to −100 nT), intense (Dst value −100 nT to −250 nT), and super-storm (Dst value < −250 nT) (Gonzalez et al., 1989; Burke et al., 2009). For our work, we obtain Dst index hourly values from the OMNI Web interface data centre (website www.omniweb.gsfc.nasa.gov) of the National Geographical data centre.

Interplanetary Magnetic Field (IMF) Bz

We obtained interplanetary magnetic field's Bz component as the north-south component values, in nano-Tesla unit (nT) from the OMNI website. Furthermore, the geocentric solar magnetosphere coordinates (GSM) for the IMF Bz. (Tsurutani et al., 1990) used interplanetary magnetic fields, such as the Bz, to describe geomagnetic storms.

RESULTS AND DISCUSSIONS

Event 1: 09 March 2012

In March 2012, two geomagnetic storms were recorded, one on 07th March of DST -88nT and one on 09th March of DST -145nT. We consider only DST < -100nT, so taking only the second geomantic storm for our study. Figure 1 displays hourly values of, (a) Disturb Storm Time (DST), (b) interplanetary magnetic field (IMF Bz), and (c) total electron content (TEC) from top to bottom for three latitudes (low, mid, and high) for 07-11 March 2012 against universal time. The geomagnetic storm's main phase began on 09th March around 0100 UT. DST gradually decreased to the minimum peak of -145nT at 0800 UT on 09th March, and then after the recovery phase started. While IMF Bz turns southward on 08th March about 1800 UT due to sudden storm commencement (SSC), reaches the positive peak of 16.5 nT at 2000 UT on 08th March, and remains southward for almost 08 h. Then, around 0200 UT of 09th March, Bz starts turning north and gradually drops to -16.5 nT at 0500 UT on 09th March 2012. Bz remains in a northward direction for another 14 hours. A southward orientation or negative value of Bz is a significant factor in the relationships between storms and solar flayers and interplanetary plasma parameters (Kane, 2005). The response of Dst index and the IMF Bz are simultaneous. On storm day (09th March), the TEC values increased at all latitudes, and on 8th March, the TEC value reached 63.22, 29.87, and 15.66 TECU at low, mid, and high latitudes. On 9th March TEC values reached 71.29, 43.70, and 17.34 TECU, while the change from 08th March to 09th March was 8.06, 13.83, and 1.67 TECU, respectively at low, mid, and high latitudes. (Adebesin et al., 2013), Studied the same storm and observed the increase in electron density during the storm's main phase at low and mid-latitudes. In the daytime, the eastward electric field can be found lower due to the upward drift.

Event 2: 19 February 2014

Figure 2 shows the geomagnetic storm that hit 19th February 2014. Hourly plots of the DST index, IMF BZ component and GPS-derived TEC for three latitudes (low, mid, and high) are shown for 17 to 21st February. Around 1300 UTC on 18th February, a sudden storm began (SSC) leading to the first phase of the geomagnetic storm. The initial phase remains about 01 h. Then DST gradually decreased to a minimum peak of -119nT at 0800 UT on 19th February, the main phase extended for almost 19 h, and then after recovery phase started. Due to sudden storm onset (SSC), IMF Bz turned southward on 18th February at about 0200 UT, reached 3.6 nT at 1100UT, and continued southward for about 12 hours. Then Bz turns northward on 1400 UT of 18th February and reaches a negative peak of -12.9 nT at 0500 UT on 19th February 2014. Bz remains northward about 20 h, then turns southward orientation on 1000 UT on 19th February. From 1400 UT of 18th March to 0800 UT of 19th March, an increase in VTEC values was seen at all three stations, with a change in TECU of 10.17, 12.61, and 9.65 at low, mid, and high latitudes. (Atulkar, 2014), examined the same storm for the effect of fof2. The study concluded that thermodynamic wind contributed significantly to increased fof2 values in low, mid, and high latitudes.

Event 3: 20 December 2015

From 18 to 22nd December 2015, graphs 3 show the hourly DST index, the IMF Bz component, and the GPS-derived TEC. On the 19th December 2015 at 1500 UT, the storm's initial phase began with a sudden storm commencement (SSC). It remains about 13 h till 0500 UT on 20th December. At 2200 UT on the 20th December, DST gradually decreased to a minimum height of -155nT during the main phase. The main phase extended for almost 18 h followed by the recovery phase. While IMF Bz turns southward only for 02 h, and on 19th December between 1600-1700 UT reaches a positive peak of 11.4 nT. Then Bz turns northward for 02 h December and again turn southward during the storm main phase IMF Bz negative peak of -18.7 nT at 2200 UT on 20th December 2015. VTEC enhancements were observed at all latitude values. Changes in VTEC values of storm day 20th December compared to the previous day, 19th December, are 0.31, 7.67, and 1.03. (Parwani et al., 2019), presented the variation of VTEC of the same event at low mid and high latitudes and found the high variation of VTEC at mid-latitude compared to low and mid-latitude.

Event 4: 13 October 2016

The graph 4 shows the DST index, IMF component Bz, and GPS-derived TEC values for the period between 11 to 15 October 2016. Initial geomagnetic activity began on the 12th of October, around 2200 UT, with an abrupt start to a

storm (SSC). The initial phase remains about 09 h and then on 13th October main phase begin around 0700 UT. During the main phase, DST decreased to a minimum peak of -104nT at 0800 UT on 17th October, the main phase extended for almost 17 h, and then recovery phase started. At the same time, IMF Bz on 12th October about 1700 UT turns southward due to sudden storm commencement (SSC) and remains southward for 05 h and at 0500 UT of 13th October. It turns southward for only one hour; A geomagnetic storm peaks of -20.8 nT at 1600 UT on 13th October 2016 during its main phase. Similarly, the enhancement in VTEC values was observed due to storms at all the GPS stations under consideration. The change in VTEC values of storm day 13th October compared with 12th October is 0.31, 4.39, and 2.28, respectively for low, mid, and high latitudes. (Wan et al., 2018), shows the enhancement in fof2 values at high and low latitudes and enhancement at high latitude seen earlier than low latitude.

Event 5: 08 September 2017

Fig. 5 illustrates the hourly plots of the DST index, BZ component of the IMF, and GPS-derived TEC of low, mid, and high latitudes for the 6-to-10th September period of 2017. Geomagnetic activity began on 07th September around 0000UT with a sudden storm commencement (SSC). The initial phase remains about 05 h. The main phase started when DST decreases to the peak of -142nT at 0100 UT on 08th September. After that, the main phase extended for almost 21 h and then after the recovery phase started. While IMF Bz on 07th September 2017 in southward between 1100-1900 UT and reaches a positive peak of 07.5 nT at 1700 UT on 07th September, and then Bz turn northward on 2000 UT of 07th September and gradually reaches a negative peak of -24.2 nT at 0000 UT on 08th September 2017. The storm enhanced the VTEC values at low and mid-latitudes. At the same time, a slight decrement in TEC was observed at high latitudes, and the change in TECU is at low, mid, and high are 3.99, 2.13, and -0.54 were observed. (Wen and Mei, 2020) also see the enhancement over mid and low latitude during the storm. The enhancement at mid-latitude is due to the neutral wind that uplift the ionosphere, and at the low-latitude, eastward prompt penetration electric field (PPEF) plays a prominent role.

Event 6: 26 August 2018

Fig. 6 highlights the hourly plots of the DST index, IMF Component Bz, and GPS-derived TEC for 24-28 August 2018. The first phase of the storm began on 25th August around 0300 UTC with the sudden storm commencement (SSC). The minimum DST peak was recorded at 0700UT on 26th August with -174 nT. The main phase lasted approximately 13 hours, and then the recovery phase began. As IMF Bz turns southward on 24th August, about 1600 UT, it reaches a positive peak of 8.2 nT at 0900 UT on 25th August and remains southward for almost 24 h. Bz then turns northward at 1600 UT of 25th August and gradually reaches -16.8 nT by 0500 UT of 26th August; Bz remains northward about 21h. The response of Dst and the IMF Bz are simultaneous. The storm enhanced the VTEC values at all the latitudes, and the change in TECU is 22.72, 9.20, and 2.22 at low, mid, and high latitudes. The upward vertical plasma drifts, neutral winds, and disturbed neutral composition plays a prominent role in enhancement of TEC values during the main phase.

CONCLUSIONS

In this work, we have studied the six intense geomagnetic storms during solar cycle 24 at low, mid, and high latitudes, using GPS-TEC values. The main conclusion of this paper is the Dst index and IMF Bz show synchronously with ionospheric TEC. The TEC values are enhanced during geomagnetic storm main phase at all the latitudes (Low, Mid, and High). And the change in TEC values at low latitudes is highest, moderate at mid-latitude, and lowest at high latitudes due to geomagnetic storms.

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Table 1. GPS Stations detail

Sr. No.	Latitude	Location	Latitude	Longitude
1	Low	Bangalore (iisc), India	13.02^0 N	77.57^0
2	Mid	Urumqi (urum), China	43.81^0 N	87.60^0
3	High	Norilsk (nril), Russian Federation	69.36^0 N	88.36^0

Table 2. Events date and maximum Dst values

Sr. No.	Event date	Minimum Dst value
1	09 March 2012	-145nT
2	19 February 2014	-119nT
3	20 December 2015	-155nT
4	13October 2016	-104nT
5	08 September 2017	-142nT
6	26 August 2018	-174nT

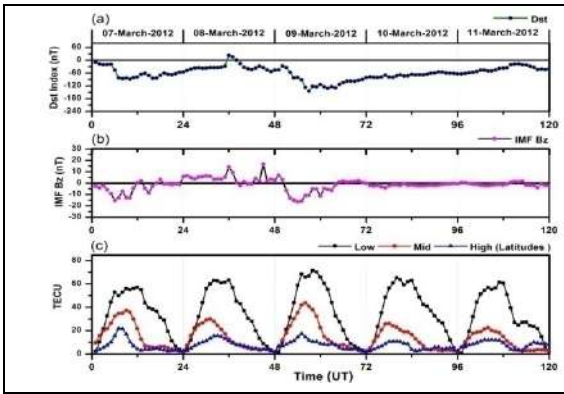

Figure 1. hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 07 – 11 March 2012.

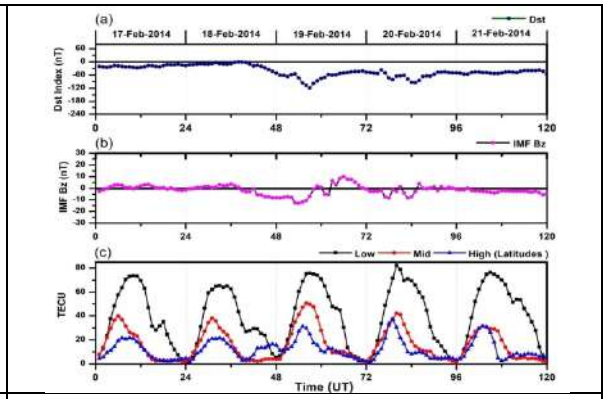

Figure 2. Hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 17 – 21 Februry 2014.

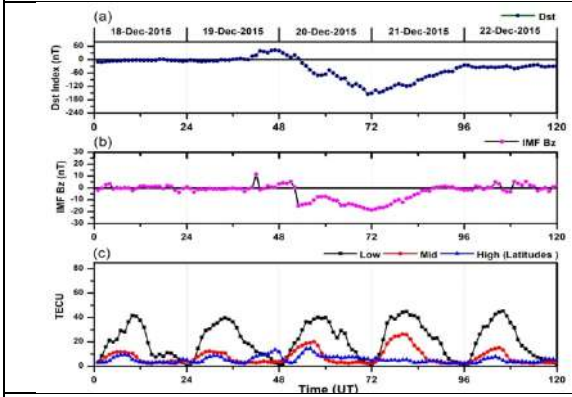

Figure 3. Hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 18 – 22 December 2015.

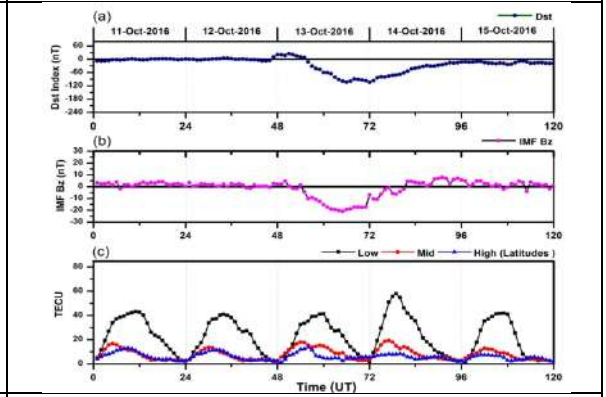

Figure 4. Hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 11 – 15 October 2016.

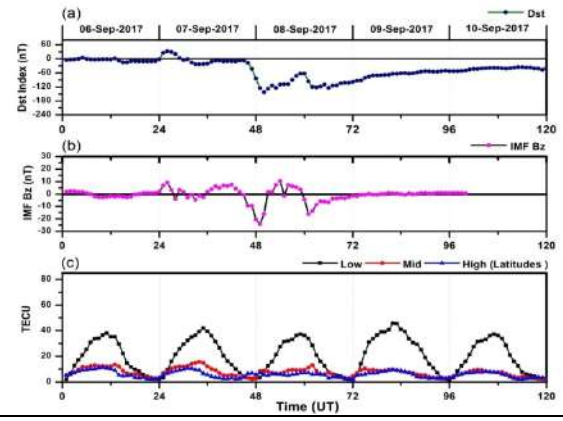

Figure 5. Hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 06 – 10 September 2017.

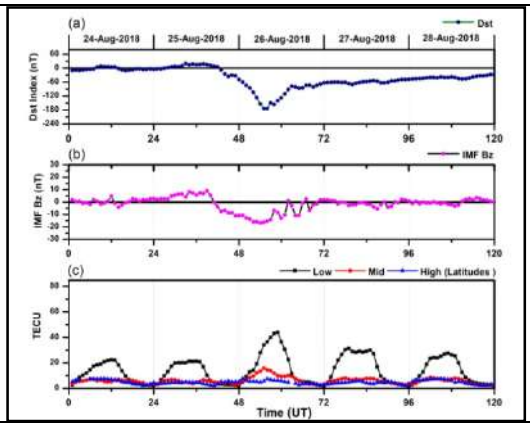

Figure 6. Hourly vaiation of (a) Dst index, (b) IMF Bz, and (c) TECU at low, mid, and high latitudes for 24 – 28 August 2018.

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RESEARCH ARTICLE

Statistical Analysis of Rainfall and Temperature of Paralakhemundi, Odisha, India

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ABSTRACT

Rainfall is a key input for different designing plan like hydraulic constructions, culverts, bridges, trenches, storm water sewer and road drainage system. The detailed statistical analysis of each region is vital to estimate the relevant input value for design and analysis of engineering structures and also for crop planning. A rain gauge station located at Paralakhemundi, Gajapati district, Odisha was selected for statistical analysis where agriculture is the prime occupation. The daily rainfall, maximum and minimum temperature data for a period of 30 years (1984 - 2013) was used for the selected site in this research. The mean, standard deviation, coefficient of variation and coefficient of skewness for these weather parameters were calculated in this study. This investigation will help farmers, water resources planner and engineers to survey the accessibility of water and make the capacity likewise.

Keywords: Rainfall, skewness, agriculture, analysis.

INTRODUCTION

Water is essential for any life cycle and there cannot be a viable alternative for it. Water is also utilized for transportation. Water is a source of power and serves many other useful purposes for domestic consumption, agriculture and industry (Cheung et al., 2008). The primary significant source of water in any space is rainfall and it considerably affects agriculture (Barnwal and Kotani, 2010). Crops get their water supply from normal sources and through irrigation. The crop production especially in rain-fed regions relies upon the rainfall pattern, which makes it very important to forecast the probability of rainfall occurrence from the past records of hydrological data using statistical analysis (Rajendran et al., 2016). Frequency or probability distribution helps to narrate the magnitude of

the extreme events like floods, droughts and severe storms with their number of occurrences such that their chances of occurrence with time can be forecasted easily. A high inconsistency in temperature and rainfall and a raise in the frequency of extreme climate events can have various severe negative effects on society, particularly in India where the population is mainly dependent on rain-fed agriculture (Pachauri and Reisinger, 2007). Economic losses, damages to water resources, decline in crop production and human deaths are among the direct and indirect impacts that must be brought up (Crétat et al., 2014). Some researches asserted that variations in rainfall patterns and temperature have adversely impact on the economic and social integrity of populations for some decades (Juana et al., 2013). During the last few decades, Orissa has been marked by unprecedented rainfall and temperature variability, differing from the past century. Therefore, this study aims to assess the most important changes in rainfall and temperature patterns over 30 years (1984-2013) for Paralakhemundi, Odisha, India.

MATERIALS AND METHODS

Study area

The rainfall and temperature data (1984-2013) used for the present study was obtained from one station in Paralakhemundi of Gajapati district, Odisha. The study site is located at 18.78°N latitude and 84.09°E longitude. The normal annual rainfall in the study area is around 1926 mm, 80% of which occurs during monsoon period (June - September). The climate of this site is extreme type. Summer season is intensely hot and winter is very cold. The whole year is divided into four seasons. The hot season starts from March to May followed by the South-West monsoon (June - September). The post monsoon season is constituted by October and November. The cold season is from December to February. The South-West monsoon is the principal source of rainfall in this district.

Statistical analysis

Annual rainfall and temperature data analysis

Annual rainfall and temperature data for 30 years (1984 - 2013) period were statistically analysed with the help of arithmetic mean, standard deviation, coefficient of variation and coefficient of skewness. The formulae used for estimation of those different parameters are tabulated below (Table 1).

Table 1: Formulae for analysis

Description	Formula
Arithmetic mean (X_{avg})	$\sum X_i / n$
Standard deviation ($\sum$)	$[\sum (X_i - X_{avg})^2 / (n - 1)]^{1/2}$
Co-efficient of variation (C_V)	$100 \times (\sigma / X_{avg})$
Co-efficient of skewness (C_S)	$(1 - \sigma^3) \times [(N / (N^2 - 3N + 2))] \times \sum (X_i - X_{avg})^3$

RESULTS AND DISCUSSIONS

Rainfall analysis

Table 2: Total annual rainfall and standard deviation for 30 years (1984 - 2013)

Year	Wet season (June - September)		Dry season (January - April)	
	Total rainfall	Standard deviation	Total rainfall	Standard deviation
1984	1501.826804	13.71840565	75.18768983	3.111311841
1985	2348.749214	16.03552681	153.5356793	5.258098981
1986	1478.423206	13.83652937	36.03000504	1.278300531
1987	939.8108403	9.102000665	106.6746977	3.45010376
1988	2155.095299	18.48955719	7.71274355	0.469523447
1989	2344.727858	21.15287976	186.6225222	3.179913186
1990	2349.238401	23.2202808	72.25054991	1.892072678
1991	2651.66105	22.77518735	27.31133209	0.752485075
1992	1318.740442	19.74769513	46.16235146	2.590546059
1993	1910.64699	16.23362612	162.5521928	6.257530745
1994	1727.810944	14.17838108	164.8842244	3.935221887
1995	1416.172971	10.17437357	61.81783009	1.517256015
1996	1590.554103	13.34163497	84.51664766	3.073634065
1997	1571.72728	11.2647822	319.7828636	4.708762269
1998	2228.974111	16.00253057	0.923537966	0.04786685
1999	1309.128269	10.79183692	115.4989288	3.112924139
2000	1607.019634	16.15579395	82.53654785	3.37844164
2001	2062.282382	59.16503664	29.02193011	1.116520321
2002	1714.06291	26.59184085	43.87665808	0.917417083
2003	2568.068828	14.2204864	3.641678439	127.1530163
2004	1426.565257	9.381487758	112.8106755	3.590968376
2005	1475.808759	13.63992309	68.92547129	3.143005755
2006	1906.159129	23.92710906	32.68947433	1.090893566
2007	2000.008586	13.64121743	231.7364522	11.49061998
2008	1934.167135	14.012361	0.041198738	0.003190992
2009	1832.674924	26.12345082	33.04309552	1.823977859
2010	1726.092871	10.20115139	61.16464923	1.661192957
2011	2155.948722	18.17348912	31.91356053	0.719334062
2012	2241.008267	20.0400855	96.85218325	5.921841462
2013			96.85218325	5.921841462

Table 3: Coefficient of variation and skewness of annual rainfall for 30 years (1984 - 2013)

Year	Wet season (June - September)		Dry season (January - April)	
	Co-efficient of Variation	Co-efficient of Skewness	Co-efficient of Variation	Co-efficient of Skewness
1984	111.4406283	-13.5502426	496.5672197	-0.000159248
1985	83.29259925	36.88166422	410.9610749	0.031016132
1986	114.1795243	-16.4746189	425.7453294	-0.003977409
1987	118.1561261	-147.8773671	391.3416809	0.000380513

1988	104.6694305	9.132016758	730.5158447	-0.005327774
1989	110.0618702	47.44577917	204.4713456	0.064463822
1990	120.5869211	53.56910654	314.2518939	-0.000175819
1991	104.7861248	225.8168467	333.3806415	-0.003753071
1992	182.6909018	-66.18913884	673.4178767	-0.004237027
1993	103.6561121	0.029490943	461.9462073	0.054503655
1994	100.1129491	-0.765446771	286.398913	0.037619432
1995	87.6498564	-18.9385854	296.982242	-0.000658657
1996	102.3341151	-5.70252618	436.4064335	-4.31085E-06
1997	87.43905167	-5.871032883	176.6984841	1.251010245
1998	87.58777054	15.64428709	461.2858145	-0.000684244
1999	100.5710544	-38.11097927	326.1188866	0.001175772
2000	122.6498308	-5.739653778	491.1920928	-1.57305E-05
2001	350.0070859	9.144766468	461.6592967	-0.005058778
2002	189.2698667	-1.908032539	250.9080107	-0.001756609
2003	67.55657488	100.6091017	12100	0.144756999
2004	80.23057486	-16.28020331	381.9817612	0.001102708
2005	112.7565213	-16.57138527	547.2007424	-0.000509854
2006	115.4832249	141.7486839	400.4568156	-0.004084144
2007	83.21106912	0.670867825	599.9768288	0.696202363
2008	88.38471153	0.09065385	929.4437515	-4.75279E-05
2009	173.9021448	-0.019813303	662.399632	-0.00669982
2010	72.10159379	-0.571522355	325.9123649	-0.000732503
2011	102.8394437	9.055644095	272.7349127	-0.002846284
2012	109.0977872	21.60554137	733.7170434	7.2995E-05
2013			96.85218325	5.921841462

Temperature analysis

Maximum temperature analysis

Table 4: Average and Standard deviation of maximum temperature for 30 years (1984 - 2013)

Wet Season (June - September)			Dry Season (January - April)		
Year	**Average**	**Standard Deviation**	**Year**	**Average**	**Standard Deviation**
1984	32.26	3.72	1984	34.87	6.54
1985	31.83	4.77	1985	36.86	6.15
1986	32.25	4.82	1986	34.02	5.77
1987	34.43	5.14	1987	34.26	5.43
1988	32.21	4.69	1988	35.16	4.69
1989	31.48	4.26	1989	37.11	5.18
1990	30.5	2.89	1990	34.4	4.68
1991	31.13	3.63	1991	35.65	5.34
1992	33.83	4.52	1992	35.65	6.12
1993	32.16	3.97	1993	35.72	4.24
1994	30.84	4.08	1994	35.87	5.09
1995	33.06	4.00	1995	33.56	6.52
1996	31.32	3.26	1996	35.25	5.38
1997	33.76	4.26	1997	35	5.89

1998	32.68	4.33	1998	33.45	4.81
1999	33.03	4.5	1999	39.62	4.54
2000	32.17	3.45	2000	35.11	5.64
2001	32.24	2.92	2001	36.93	3.98
2002	33.93	4.27	2002	35.28	5.71
2003	32.02	4.6	2003	36.29	4.68
2004	32.55	3.38	2004	35.13	6.59
2005	33.39	5.82	2005	35.91	4.8
2006	32.23	4.5	2006	36.5	4.88
2007	32.09	3.73	2007	35.95	4.82
2008	32.38	3.1	2008	34.39	5.21
2009	33.57	5.25	2009	38.03	4.7
2010	32.32	3.66	2010	37.15	6.08
2011	32.54	3.64	2011	35.11	5.12
2012	32.4	5.12	2012	37.81	5.9
2013	31.75	3.11	2013	36.76	5.34
2014			2014	36.21	5.71

Table 5: Co-efficient of variation and skewness of maximum temperature for 30 years (1984 - 2013)

Wet Season (June - September)			Dry Season (January - April)		
Year	**Co-efficient of Variation**	**Co-efficient of Skewness**	**Year**	**Co-efficient of Variation**	**Co-efficient of Skewness**
1984	11.53130812	-0.000481863	1984		
1985	14.98586239	-0.034853854	1985	16.68475312	0.292048603
1986	14.94573643	-0.000756168	1986	16.96061141	-1.15706512
1987	14.92884113	1.569103862	1987	15.84938704	-0.700376284
1988	14.56069544	-0.001428187	1988	13.33902162	-0.040409418
1989	13.53240152	-0.127909018	1989	13.95850175	0.458095046
1990	9.475409836	-0.749625306	1990	13.60465116	-0.451237587
1991	11.66077739	-0.283753866	1991	14.97896213	-0.000382761
1992	13.36092226	0.478832101	1992	17.16690042	-0.00043867
1993	12.34452736	-0.002349511	1993	11.87010078	-2.55065E-05
1994	13.22957198	-0.588100277	1994	14.19013103	0.00016452
1995	12.09921355	0.040472957	1995	19.42789035	-2.629020865
1996	10.40868455	-0.157469933	1996	15.26241135	-0.02882496
1997	12.61848341	0.387713842	1997	16.82857143	-0.101605353
1998	13.249694	0.003106145	1998	14.37967115	-2.243120798
1999	13.6239782	0.03949939	1999	11.45885916	9.585587353
2000	10.72427728	-0.00180792	2000	16.06379949	-0.061447692
2001	9.05707196	-0.000548467	2001	10.77714595	0.227973638
2002	12.58473327	0.554931207	2002	16.18480726	-0.025638191
2003	14.36602124	-0.010261659	2003	12.89611463	0.023801317
2004	10.38402458	0.000332206	2004	18.75889553	-0.065508353
2005	17.43036837	0.202345444	2005	13.36675021	0.000443214
2006	13.96214707	-0.001001724	2006	13.36986301	0.069185168
2007	11.62355874	-0.00460932	2007	13.40751043	0.000967094

2008	9.573810994	-3.65493E-06	2008	15.14975284	-0.513383227
2009	15.63896336	0.302949235	2009	12.35866421	2.002289774
2010	11.32425743	-0.000104671	2010	16.36608345	0.587462271
2011	11.18623233	0.000285647	2011	14.58273996	-0.055782302
2012	15.80246914	-3.0187E-07	2012	15.60433748	1.847416856
2013	9.795275591	-0.033449378	2013	14.52665941	0.189757833
2014			2014	15.76912455	

Minimum temperature analysis

Table 6: Average and Standard deviation of minimum temperature for 30 years (1984 - 2013)

Year	Wet Season (June - September)	Dry Season (January - April)	Wet Season (June - September)	Dry Season (January - April)
	Average	Average	Standard Deviation	Standard Deviation
1984	16.56	23.32	3.19	1.74
1985	16.02	23.46	3.7	1.45
1986	15.84	23.18	3.86	0.99
1987	15.05	24.07	3.78	1.44
1988	16.53	24.09	4.33	1.01
1989	13.66	23.6	4.91	0.97
1990	16.76	23.56	5.21	0.97
1991	17.04	23.65	3.44	0.98
1992	14.79	23.6	3.25	1.23
1993	14.64	23.78	4.48	1.16
1994	15.92	23.23	3.96	1.18
1995	17.78	24.24	3.64	1.39
1996	16.62	23.46	3.79	0.99
1997	16.05	24.09	4.14	1.21
1998	19.82	24.42	3.13	1.13
1999	16.47	24.01	2.67	1.46
2000	15.88	23.8	4.77	0.95
2001	14.9	24.09	6.54	0.97
2002	17.04	24.28	3.71	1.54
2003	16.19	24.12	5.22	1.12
2004	16.43	23.92	3.90	1.04
2005	17.24	24.1	4.66	1.44
2006	15.73	24.12	5.00	1.41
2007	15.37	24.34	4.34	1.13
2008	16.42	24.09	5.21	1.03
2009	14.31	24.56	3.72	1.49
2010	16.42	24.28	4.45	1.38
2011	16.23	23.86	3.60	1.3
2012	14.71	23.54	3.9	1.18
2013	14.62	23.37	3.56	1.01
2014	16.07		3.35	

Table 7: Co-efficient of variation and skewness of minimum temperature for 30 years (1984 - 2013)

Year	Wet Season (June - September)	Dry Season (January - April)	Wet Season (June - September)	Dry Season (January - April)
	Co-efficient of Variation	Co-efficient of Variation	Co-efficient of Skewness	Co-efficient of Skewness
1984	7.461406518		-0.011004546	
1985	6.180733163	23.09612984	-0.003829372	-5.42519E-07
1986	4.27092321	24.36868687	-0.01230408	-0.001075916
1987	5.982550893	25.11627907	0.000400519	-0.134454909
1988	4.192611042	26.19479734	0.000376167	0.019403899
1989	4.110169492	35.9443631	-0.000743562	-2.44469429
1990	4.117147708	31.08591885	-0.001118544	0.073470259
1991	4.143763214	20.18779343	-0.000410788	0.129336355
1992	5.211864407	21.97430696	-0.000942868	-0.233316247
1993	4.87804878	30.6010929	-3.61546E-05	-0.452338457
1994	5.079638399	24.87437186	-0.011719894	-0.000228351
1995	5.734323432	20.47244094	0.002523369	0.71712573
1996	4.219948849	22.80385078	-0.002614537	0.028055588
1997	5.02283105	25.79439252	0.000450655	4.39525E-07
1998	4.627354627	15.79212916	0.006816644	6.297588138
1999	6.080799667	16.21129326	0.000135357	0.008114677
2000	3.991596639	30.03778338	-1.44872E-05	-0.000669861
2001	4.02656704	43.89261745	0.000361269	-0.355795652
2002	6.342668863	21.77230047	0.003817144	0.139487755
2003	4.64344942	32.24212477	0.000616548	0.000710524
2004	4.347826087	23.73706634	3.6774E-06	0.00886958
2005	5.975103734	27.03016241	0.000614433	0.302123633
2006	5.845771144	31.78639542	0.000776189	-0.005309087
2007	4.642563681	28.23682498	0.004236564	-0.047560194
2008	4.275633043	31.72959805	0.000383616	0.010969778
2009	6.066775244	25.99580713	0.017833446	-0.709952182
2010	5.68369028	27.10109622	0.003420558	0.00936958
2011	5.448449288	22.18114603	-1.42133E-07	0.000978851
2012	5.012744265	26.51257648	-0.00163731	-0.337454237
2013	4.32178006	24.3502052	-0.004810434	-0.375121152
2014		20.84629745		

CONCLUSIONS

Rainfall and temperature are the most determinant climatic parameters in Odisha, as more than 60% of the agriculture is reliant on rain. The present study attempts to analyze the changes occurring in the temporal distribution of rainfall and temperature in one district of Paralakhemundi, India for a period from 1984 to 2013. Statistical evaluations were carried out using arithmetic mean, standard deviation, coefficient of variation and skewness. The overall analysis of the data for 30 years (1984 - 2013) in Paralakhemundi clearly indicated significant changes in rainfall and temperature patterns during the study period. This investigation will help farmers, water resources planners and engineers to survey the accessibility of water and make the capacity likewise.

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REVIEW ARTICLE

A Review on Yellow Fever Mosquito *Aedes aegypti*

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ABSTRACT

Mosquitoes are disease-carrying vectors that are the primary cause of human filariasis, malaria, and a variety of viral diseases such as dengue, Japanese encephalitis, Zika, and West Nile virus. Yellow fever is a viral hemorrhagic vector-borne disease that is spread by the bite of an infected *Aedes aegypti* mosquito and can be fatal to humans. The 'yellow' refers to the jaundice that some patients with severe disease experience. Numerous factors are to blame for the disease's emergence. Yellow fever has three transmission cycles: Sylvatic, Intermediate, and Urban. Enzyme linked immune-sorbent assay and PCR can be used to diagnose this disease.

Keywords: *Aedes aegypti*, fever, human, PCR, mosquito

INTRODUCTION

Mosquito is a common name for biting insects and blood sucking fly pests that belong to the family-Culicidae, suborder-Nematocera, order-Diptera, class-Insecta, and phylum Athropoda and have been on the planet for millions of years. In our country, mosquitos were previously referred to as 'gnats.' The name 'gnat' was changed around 1900, when Ross discovered the mosquito life cycle in malaria and the importance of these insects to man became more widely known. Hust's paper was titled 'The Life Development and History of a Gnat' in 1890, and Giles authorised his book 'Handbook of Gnats' in 1900. Since 1901, Theobald has essentially used the term "mosquito" to refer to all English authors. When researching old literature, keep in mind that the reference in the index may be for gnats or culex (Aldrovando. U, 1602). Certain passages in Aristotle (384-322 B.C.) relating to empis, a name which, as previously stated, is generally accepted as referring to the mosquito, are among the earliest references in the literature to the mosquito. Thus, in Aristotle's Historia Anima Zium, it is stated that many animals live in water at first and then change their form, as is the case with empis (Book I, cap. I, as given in Aubert and Wimmer, p. 197, lines 8-12). In another passage (Peck, p. 47), this time in De Generatione Animalibus, empis is mentioned as one of the creatures that, coming from putrifying liquids, are neither produced by other animals nor copulate, implying that

they were thought to arise by spontaneous generation of life, a belief that the mosquito larva still held some two thousand years later. The structure of the proboscis was one of the features of the mosquito that piqued the interest of early naturalists, and there are numerous references to the number and nature of its components in the early literature. Most of these observations are now purely historical in nature, having been made under conditions very different from those of today's miscroscope." Swammerdam (1737-8) describes five stylets and the sheath without naming them. All of the stylets end similarly, with fusi form swollen ends and no further detail. Up to a century or more later, authors provided little more detail than this, and the naming of the parts was not always correct.

***Aedes aegypti* and Its Role on Diseases**

There is no doubt that the discovery that mosquitoes were disease vectors sparked and was responsible for the massive amount of research on these insects that began with the beginning of what has been referred to as the modern period of research on these insects. While this is especially true for the subfamily Anophelini due to their role as malaria vectors, it also applies to the Culicini, particularly the genera Culex and ASZes, both of which are important disease vectors in humans and animals. Furthermore, this is especially true for the single species *Aedes aegypti,* which is found almost everywhere in the tropical and subtropical zones, and of the four major human diseases transmitted by mosquitoes, namely malaria, yellow fever, dengue, and filariasis, *Aedes aegypti* is the usual vector species for two of them, and has been the subject of extensive research in the past that it could be known that it did not play an important role as vector of a third, filariasis.

Furthermore, its reputation as the yellow fever mosquito has given it an importance to the sanitarian, particularly in the New World, that no other single species of mosquito possesses. The discovery that insects and other arthropods were involved in disease transmission came relatively late in the game. Agramonte (1908) provides translated extracts from an article by Beauperthuy (1854), in which this author identifies the mosquito as the agent responsible for yellow fever. Beauperthuy held that yellow fever (and the intermittents, etc.) were caused by the direct injection of a poison by mosquitoes, much like snake-bite, though he did not believe this poison was caused by the mosquito itself, but by a virus derived from swamps. Regardless of how wrong he was, he was correct in his conclusion that mosquitoes were to blame for yellow fever, and his theory presumably also applied to malaria. Regarding the former disease, it is worth noting that he specifically mentions what is clearly *Aedes aegypti*, namely the 'zancudo bobo' with white-striped legs, which he says may be regarded as more or less the house-haunting kind, one of the earliest references to this species in the tropics. Though the discovery that *Aedes aegypti* transmitted yellow fever by its bite after an interval was solely the work of Reed, Carroll, Agramonte, and Lazear, who first transmitted the disease to volunteers by the bite of *Aedes aegypti* previously fed on cases of the disease, it is impossible to overlook the work of Finlay, who was led to investigate the habits and bionomics of the species from 1881 onwards by his belief that it was this mosquito. Following the work of the American Commission in Havana was that of the French Commission's observers, Marchoux, Salimbeni, and Simond, and, later, of the German observers Otto and Neumann in Brazil. Much of what we now know about disease transmission was established at this time, with subsequent advances primarily focused on increased knowledge of the virus's properties and findings regarding transmission by species other than *Aedes aegypti* in jungle yellow fever.

With the realisation that mosquitos were important disease vectors, there was a surge in interest in methods to prevent them from breeding or destroying them, as well as methods to protect against their bites. Howard, Dyar, and Knab contain references to a writer (Delboeuf) who speaks of destroying mosquitoes by pouring oil on water where they breed in 1847. (1912). Southey makes the same suggestion (1812). However, the first recorded experiments in this direction appear to have been those conducted by Howard (1893-4) who observed the effect of such treatment on a small selected pond. For many years, oil was the primary method for treating breeding sites, but it has now been largely replaced by more effective larvicides. It wasn't long after the role of *Aedes aegypti* in yellow fever transmission was discovered that large-scale control measures were implemented in seaports and towns throughout America's yellow fever zone, and later elsewhere. Recently, measures aimed specifically at the adult mosquito have been greatly developed in connection with air traffic.

Aedes aegypti has also been extensively used as a research animal in a variety of fields. It has been used extensively in the testing of insecticides and repellents, the testing of essential food requirements, genetic research, and other studies. *A. aegypti* has many outstanding advantages for such laboratory work; its hardiness, readiness to feed, and ease with which it can be reared, with the added benefit that eggs can be stored for months if necessary without losing vitality, make it almost uniquely useful for such a purpose.

Identifications

Fortunately, *Aedes aegypti* identification is usually simple because it has distinct thoracic markings that almost always serve to distinguish it. Furthermore, the marking of the mid-femur, namely a white line on the anterior surface extending from the base almost to the tip, is unique to this species of Stegomyia, as is the patch of white scales on each side of the clypeus in the female. The male's clypeus, on the other hand, is usually bare or has only a few scales. The shoulder marked are more coiled or flattened in the majority of species, and either yellow or a single median white line are present instead of two median parallel white lines. Edwards (1941) gives some useful figures which show the thoracic decoration of a number of species. A study of Roth and Willis's genus comportement. The human arm had all forms indifferent and two women exposed did not give a male answer. But they did like males, because after the females settle they remained in the cage back and forth. Normal men tried to match 5 gynandromorphs with ordinary wings of women and showed that they sounded the same as that of a woman. The proboscis has no pale banding in most species of this genus. The tibia are normally dark in Stegomyia. This tarsal band is used in synoptic tables to differentiate between species, with some of the sub-argenteus white on the base of the fourth hind tarsal segmente, but all the black in wood and sympsoni white in kivuensis. The banding extent of the third hind tarsal and other tarsal segments may also be of a difference. The wings are normally black, but there is a common small light spot in the base of the coastline in *Aedes aegypti* and many other species.

Genetic Characters

Male terminalia in *Aedes aegypti* are distinguished by the following characteristics: (a) ninth tergite with two prominent conical lateral lobes carrying a few short hairs, between which is a deeply hollowed out V-shaped embayment; (b) clasper style widened in the middle; (c) paraproct lateral processes nearly as long as terminal processes; and (d) phallosome lateral plates with fine teeth only In *Aedes aegypti* and related species, the basal lobe is a flattish plaque on the inner membranous aspect of the coxite.As in some species, *Aedes aegypti* lacks a projecting process that results in a separate claspette or harpago. Iyengar and Menon (1955) recently described three or four distinct hooked spines arising from the lobe of *Aedes aegypti* (Ross and Roberts, 1943).

Pupal Characters

The following characteristics distinguish *Aedes aegypti*: (a) triangular trumpets with no transverse folds (see Theodor, 1924); (b) paddles with a single hair about one-quarter the length of the paddle; (c) no accessory hair; and (d) chaeta A on abdominal segments II-VI spine-like. Ingram and Macfie (1917), Theodor (1924), Baisas (1938), and Edwards (1938) provide synoptic tables for pupal characters in a number of Stegomyia species (1941).

Larval Characters

The following larval characteristics distinguish *Aedes aegypti*: (a) smooth antennae, antenna1 hair simple; (b) comb teeth 8-12 in a single row with strong basal lateral denticles; (c) pecten teeth on the syphon 15-20, none widely spaced; and (d) pleural hairs of the meso- and metathorax with conspicuous thorn-like processes.

Varieties

1. The type form is *Aedes aegypti* (L.) s.str. A brown form with variable colour depth but always paler and browner (at least in the female) than the black African subspecies (no. 3). If there is any extension of pale scaling, it is limited to bleaching of the two dark areas on the back of the head, the presence of pale scaling on the first abdominal tergite, or both.

2. *Aedes aegypti* var. queenslandensis Theo. Any form displaying any of the following: bleaching of the mesonotum's dark scales from mid-brown to buff to almost white; encroachment of the pale basal bands on the abdominal tergites on the apices of the preceding segments, or extension of these bands in the mid-line to form pale basal triangles or a continuous median pale line on the abdomen; presence of scattered pale scales on the dark areas of the tergites or normally dark areas on the legs.
3. Walker's *Aedes aegypti* s.sp. formosus. Except for coastal districts and one or two inland trade-frequented areas, this is the only known form in Africa south of the Sahara. This form differs from the type form in that the dark areas of the thorax and abdomen are markedly black, and there is no bleaching or extension of pale scaling on any part of the body. Mattingly's name formosus is that given by Walker (1848, p. 4) to a specimen from Sierra Leone (type in the British Museum) that exhibits the characteristics described above, and is the first description of any form from the area given as the subspecies' distributional area. All of the characters in the storey are female in some way. Males are typically darker in all forms.

Mutants

Certain changes caused by gene action result in forms that differ in some ways from the normal species characteristics. Wigglesworth's chapter on growth includes a brief description of such gene action. The condition has been studied extensively in certain Lepidoptera and Drosophila in Diptera. There are two modes of action recognised: (1) action confined to the cells in which the genes occur, such as determining the nature of the wing ornamentation in the moth Ephestia; and (2) action in which the cells are caused to liberate chemical substances that act at a distance and determine the character of other tissues. The latter type of action is responsible for Ephestia's black-eye and red-eye forms, as well as Drosophila's vermilion-eye form. The white-eye mutant of *Culex molestus* described by Gilchrist and Haldane appears to be the only known instance in mosquitoes. The condition in this case is characterised by the imago's normally dark eyes being devoid of pigment, resulting in a dull white appearance.
The strain described by the authors was bred from four females and ten males with white eyes found in a cage of hundreds of *C. molestus*. The larva and pupa both have a lack of pigment in their eyes. White-eye mutants had normal phototaxis but did not respond to moving shadows like normal people. The authors attribute this to the fact that because the ommatidia were not separated by pigment from one another, visual acuity was lost.

The affected larvae and pupae, on the other hand, displayed a distinct shadow response. They also responded to changes in light intensity, which was thought to be the reason for the shadow reaction. It is not stated whether the ocelli were pigmented or not. It would be interesting to note the condition of these functional visual organs in the larva, and possibly even in the pupa, because they are the functional visual organs in the larva and possibly in the pupa.

CONCLUSION

In tropical areas around the world, *Aedes aegypti* is the primary vector of many arthropod-borne diseases, including dengue, chikungunya, yellow fever, and Zika viruses. Here, we combine available information on the mosquito's genetics, morphology, behaviour, and ecology with human history. And, because this mosquito is easy to rear in the laboratory, many studies have been conducted to test the relative competence of different populations of the mosquito to transmit many different strains of viruses for the potential implications on the spread and incidences of vector-borne diseases such as Dengue/Yellow fever.

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RESEARCH ARTICLE

Simulation of Thermodynamic Properties of Lithium Fluoride using Density Functional Perturbation Theory

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ABSTRACT

We have stated the first principles simulation results for Lithium fluoride using linear response theory and DFPT (density functional perturbation theory) using the CASTEP package from BIOVIA Material Studio. simulation. The results are predicting he phonon dispersion curve with the corresponding vibrational frequencies.

Keywords: Lithium Fluoride, Density Functional Theory.

INTRODUCTION

Lithium fluoride owing to its large band gap has enormous applications pertains to industries [1,2]. Lithium fluoride when reacts with hydrogen fluoride-HF , it forms a potent material for lithium ion battery [3] electrolyte. LiF has nearly 14.1 eV bandgap which is a primary reason behind its transparency to High frequency UV radiation which can deal with dedicated optics [4] for the vacuum. It can further be used as a diffracting crystal in X-ray spectrometry. Lithium fluoride is extensively used in OLED (organic light emitting diode) [5] to augment electron injection. Here, we have tried to realize the thermodynamic properties of LiF [6] using the first principles approximation technique [7] in the CASTEP package of BIOVIA Material Studio.

Simulation Details

We have used the CASTEP [8] module to execute a linear response mechanism to compute dispersion curve for phonons and DOS (density of phonon states) as well as forecast the thermodynamic behaviour. CASTEP is a hi-tech quantum theory-grounded package designed exactly for research in materials field. CASTEP involves the DFT (Density functional theory) plane-wave pseudopotential process, which lets us to achieve DFT calculations that discover various intriguing properties relevant to various materials. Here, we have used this technique to predict the phonon properties of lithium fluoride. We have imported the structure directly from the libraries available. For geometry optimization, the exchange-correlation functional was chosen as local density approximation with fine quality to calculate the phonon properties of various materials. The pseudopotentials were chosen as Nonconserving

potential [9]. Linear response method including interpolation was utilized to predict the phonon related properties, where the distance between the q-vector grid is 0.05 $1/A^0$. Phonon dispersion curves display how energy of the phonons that hinge on the q-vector in the brillillouin zone. These simulated datas are valuable for founding the legitimacy of a simulationtactic to establish the projecting power of first principles simulations. Figure 1.3 shows the frequencies for each of the q-points and each branchare specified in cm^{-1}, Similarly, the q-points locations are clearly revealed in the reciprocal space. The high symmetry points Γ, L and X are at reciprocal space positions (0 0 0), (0.5 0.5 0.5) and (0.5 0 0.5) respectively.

Figure 1.3 shows the density of phonon states for LiF is revealed. This graph clearly tells that two significant satellite peaks (one at7 THz and another 10 THz). These two peaks indicated a large number of densities of phonon states are available in Lithium Fluoride. Other small peaks are also achieved from O to 20 THz. The Mid frequency range is showing largely available phonon density of states.

Thermodynamic properties

We can determine various thermodynamic properties like entropy, Gibbs free energy, Helmholtz free energy, specific heat capacity of a material using CASTEP module. These simulated details can be associated with the already established experimental calculations which is used to forecast phase stability of diverse modifications in the structure or transitions in the crystal phase. The precise form of the curvature at very small temperatures is not precise. We have to choose a finer sampling to obtain the accurate predictive data. Figure 1.4 exposed LiF's highest normal mode of vibration i.e. The Debye temperature, i.e., the largest temperature we can accomplish because of a single normal vibration. The corresponding Debye temperature that is simulated about 660 K, which lies is in the experimental agreement 735 K. The dip revealed in figure 1.4 at nearly 80 K estimated by CASTEP results.

Figure 1.5 shows the disparity of enthalpy, free energy, and entropy vis-à-vis temperature. Here, we have detected the entropy growing exponentially but with unhurriedrapidity (designated by blue curve), whereas free energy (denoted by red curve) is exponentially declining which displays understandable results. The blue curve demonstrations the modification of enthalpy vis-à-vis the temperature that illustrates the sluggish change with respect to temperature. The difference between these three amounts is very unimportantat room temperature.

CONCLUSION

We have stated the computational forecast of LiF using the CASTEP module of BIOVIA Material studio. We have found out the phonon density of states (vibrational properties) of LiF using density functional perturbation theory. The thermodynamic properties predicted the parameters like Debye temperature and heat capacity with variation to temperature which can be compared with the experimental datas.

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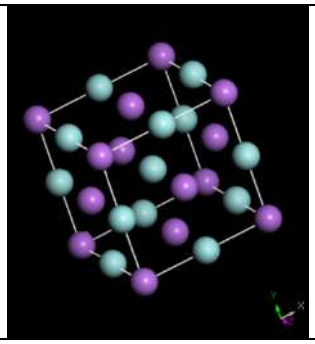

Fig. 1. The conventional unit cell for Lithium Fluoride.

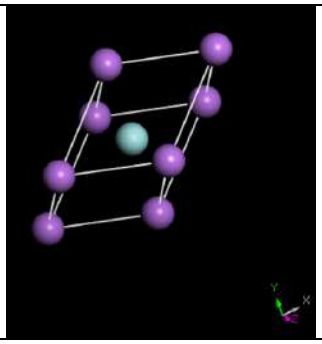

Fig. 2. The primitive unit cell for Lithium Fluoride

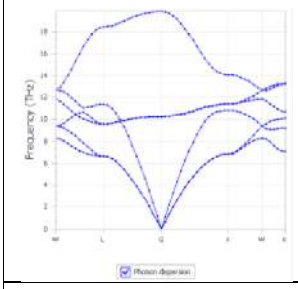

Fig .3 The phonon dispersion curve for Lithium Fluoride.

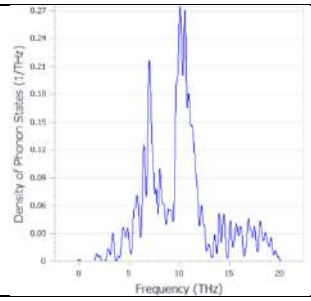

Fig. 4 The Density of Phonon States curve for Lithium Fluoride.

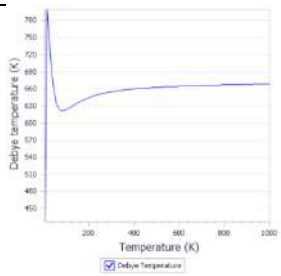

Fig.5 The Debye Temperature simulated for Lithium Fluoride

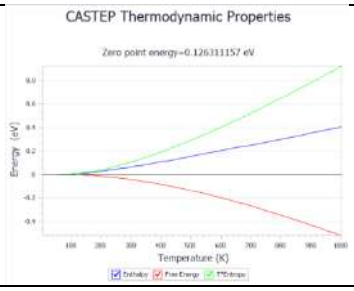

Fig.6 The Debye Temperature simulated for Lithium Fluoride.

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RESEARCH ARTICLE

Layer Wise Soil Moisture Depletion Rate During Potato Growing Period Under Different Irrigation Frequencies with Different Planting Dates

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ABSTRACT

A field study was carried out with two different potato (*Solanum tuberosum*) varieties (i.e. *Ashoka* (V_1) and *Jyoti* (V_2)) at "C" block farm of the Bidhan Chandra Krishi Viswavidyalaya at Kalyani during the period of November-March (2009-2010) to study the performance of different potato varieties under three different irrigation levels (I_1, I_2 and I_3) with 30 mm irrigation depth. The total experiment was accomplished under two treatments (D_1 and D_2) during the above period. The soil was sandy-loam with medium land situation. The plot size was 4.5 m × 3.7 m. The maximum yield recorded under D_1 was 50.56 t ha^{-1} and the maximum yield under D_2 was 63.65 t ha^{-1}. Among two cultivars, *Ashoka* variety produced the highest average yield (49.23 t ha^{-1}) and it declined by 6% under *Jyoti* variety. Soil moisture content in the soil profile varied widely in the two varieties. Among different treatments, the maximum soil moisture (3.50 mm d^{-1}) depletion rate was recorded under I_3 V_1 followed by I_2 V_2, I_1 V_2, I_1 V_1, I_3 V_2 and I_2 V_1 for D_1; whether for D_2, the maximum value of 3.07 mm d^{-1} was recorded under I_1 V_1 followed by I_1 V_2, I_2 V_2, I_2 V_1, I_3 V_1 and I_3 V_2. Thus, the soil moisture depletion rate varied under different treatments.

Keywords: Soil moisture, Irrigation, Potato, Yield.

INTRODUCTION

Agriculture supports human life and its mismanagement leads to acute shortage of food grain. The increase in population makes it very important for agriculture to flourish so as to feed the teeming millions (Islam et al., 1990). Along with this, scarcity of fresh water resources makes irrigated agriculture in an alarming condition during dry period (Sharma et al., 1992). The irrigation network in the country is not also well established. Hence, agricultural scientists need to meet the challenge and plan for improving the irrigation potential and to preserve for its best utilization at present and in future (Nasare et al., 2009). In designing new systems and rehabilitating old ones the need of user should be of supreme importance. The system must deliver the precise amount of water to the crop according to the needs and just sufficient to meet this demand (Kumar and Minhas, 1999). This will not only save the wastage but also will assure an increased yield (Nagaz et al., 2008). Scientific irrigation in proper time, amount and at a desired depth is essential for the successful production of potato (Kang et al, 2002).

Variable-rate irrigation (VRI) is an eminent practice to further increase water use efficiency by spatially matching irrigation rates to crop water demand. Field scale spatial variability of crop water status and soil is well established, and utilizing VRI to match spatial crop water demand during a growing period could reduce irrigation inputs while sustaining crop yield (Daccache et al., 2015). This was seen in potato production where the total irrigation utilization was not substantially higher, however water productivity enhanced under VRI managements. The VRI reduced total applied irrigation up to 26% compared to uniform irrigation in New Zealand. Despite documented reductions in irrigation requirements, widespread water savings from VRI systems have not been realized because decision support systems need further development (Evans and King, 2012). Keeping these in view, the present investigations on two different potato cultivars, 3 different irrigation levels were planned under furrow irrigation. This study was conducted to estimate layer-wise soil moisture depletion rate under different planting dates.

MATERIALS AND METHODS

The experiment was carried out at "C" block farm (lat - 22.5^0 N, long - 89^0 E and altitude 9.75 m above msl) at Kalyani during the period of November-March, 2009-10. The soil of the study site is sandy-loam with medium land situation.

Experimental design and treatments

The treatments were distributed in a split split plot design, where the date of sowing was considered as the main plot treatment, the irrigation levels as sub plot treatment and varieties as sub-sub plot.
The treatment combinations were as follows:

Main plot treatment (Dates of planting; D)
D_1 – 20th November
D_2 – 29th November

Sub plot treatment (Irrigation level; I)

		IW/CPE
I_1	=	1.40
I_2	=	1.20
I_3	=	1.00

Total plot size was 4.5 m × 3.7 m. In a particular plot, the spacing is 45 cm × 15 cm.

Methods and Observation

Soil moisture content:

Gravimetric soil moisture was measured before and after irrigation and also at the initial and harvest time of potato crop.

Yield

The crop was harvested on two phases. In the first phase, crops were harvested on 16th February. It was 88 dates after planting (DAP). In the other phase, crops were harvested on 3rd March. It was 95 DAP.

RESULTS AND DISCUSSION

Layer wise moisture depletion rate

It was revealed from the data irrespective of varieties and irrigation treatments soil moisture depletion rate was at the lower level during initial crop growth stage ((0-12 DAP) for D_1) and ((0-4 DAP) for D_2) followed by a sharp increase during (34-52 DAP) for D_1 I_1 V_1; (77-88 DAP) for D_1 I_1 V_2; (36-55 DAP) for D_1 I_2 V_1; (77-88 DAP) for D_1 I_2 V_2; (66-73 DAP) for D_1 I_3 V_1 and D_1 I_3 V_2; (34-51 DAP) for D_2 I_1 V_1; (57-64 DAP) for D_2 I_1 V_2; (59-71 DAP) for D_2 I_2 V_1 and D_2 I_2 V_2; (61-74 DAP) for D_2 I_3 V_1 and D_2 I_3 V_2, where these attained the highest value (1.00 to 2.96); (1.00 to 3.01); (1.00 to 2.72); (1.00 to 3.36); (1.00 to 3.55); (1.00 to 2.87) mm d^{-1}; for D_1 and (0.90 to 3.07); (0.90 to 3.06); (0.90 to 2.89); (0.90 to 2.99); (0.90 to 2.76); (0.90 to 2.33) mm d^{-1}; for D_2 respectively. Thereafter, in the most of the cases depletion rates decrease gradually till final harvest of the crop. Among different treatments, the maximum soil moisture (3.50 mm d^{-1}) depletion rate was recorded under I_3 V_1 followed by I_2 V_2, I_1 V_2, I_1 V_1, I_3 V_2 and I_2 V_1 for D_1; whether for D_2, the maximum value of 3.07 mm d^{-1} was recorded under I_1 V_1 followed by I_1 V_2, I_2 V_2, I_2 V_1, I_3 V_1 and I_3 V_2. Thus, the depletion rate varied under different treatments and those variations are shown by the following tables.

Yield

In the present study, it was observed that average potato yield was at the highest level (50.15 t ha^{-1}) under 2nd planting date (D_2: 29th November) irrespective of variety and irrigation level and it declined by 5 t ha^{-1} (on an average) when the crop was planted 9 days earlier (Table 13). Irrespective of date of planting and variety, the highest average yield (53.59 t ha^{-1}) was attained under I_1 treatment, which declined by 13% under I_2 treatment. The same was at its lowest peak (42.67 t ha^{-1}) under I_3 treatment. Among two cultivars, *Ashoka* variety produced the highest average yield (49.23 t ha^{-1}) and it declined by 6% under *Jyoti* variety.

CONCLUSIONS

The different crop varieties may require different amount of irrigation water for better water use efficiency and cultivar selection should be such that minimum water can produce maximum, making the slogan 'more crop per drop'. The annual requirement of potato in West Bengal is quite high. Potato is sown in the month of November and harvest to March. In this study, two different potato cultivars (*Ashoka* (V_1) and *Jyoti* (V_2)) were selected and the layer-wise soil moisture depletion pattern was investigated under two different planting dates (D_1 and D_2) and three different irrigation (I_1, I_2 and I_3) water regimes with different IW/CPE ratio. Among different treatments, the maximum soil moisture (3.50 mm d^{-1}) depletion rate was recorded under I_3 V_1 followed by I_2 V_2, I_1 V_2, I_1 V_1, I_3 V_2 and I_2 V_1 for D_1; whether for D_2, the maximum value of 3.07 mm d^{-1} was recorded under I_1 V_1 followed by I_1 V_2, I_2 V_2, I_2 V_1, I_3 V_1 and I_3 V_2. Thus, the depletion rate varied under different treatments. The results of investigation also indicated that the average potato yield was at the highest level (50.15 t ha^{-1}) under late planting irrespective of variety and irrigation level and it declined by about 10% when the crop was planted 9 days earlier. Among two cultivars, *Ashoka* variety produced the highest average yield (49.23 t ha^{-1}) and it declined by 6% under *Jyoti* variety.

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Table 1: Layer wise soil moisture depletion rate (mm/d) from D_1 I_1 V_1 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.52	0.46	0.31	0.32
10-20	-0.16	0.48	0.63	0.28	0.26
20-30	-1.05	0.37	0.73	0.28	0.31
30-40	-0.19	0.15	0.56	0.56	0.11
40-50	1.00	0.09	0.28	0.20	0.15
50-60	1.48	0.03	0.31	0.22	0.37
Total	**1.00**	**1.64**	**2.96**	**1.85**	**1.52**

Table 2: Layer wise soil moisture depletion rate (mm/d) from D_1 I_1 V_2 treatment combination during crop growing period:

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.35	0.46	0.26	1.01
10-20	-0.16	0.24	0.12	0.15	0.23
20-30	-1.05	0.56	0.35	0.09	0.53
30-40	-0.19	0.56	0.24	0.31	0.41
40-50	1.00	0.33	0.61	0.89	0.46
50-60	1.48	0.03	0.57	0.61	0.37
Total	**1.00**	**2.07**	**2.35**	**2.31**	**3.01**

Table 3: Layer wise soil moisture depletion rate (mm/d) from D_1 I_2 V_1 treatment combination during crop growing period:

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.04	0.91	0.74	0.69
10-20	-0.16	0.53	0.40	0.20	0.40
20-30	-1.05	0.92	0.20	0.11	0.30
30-40	-0.19	0.87	0.08	0.30	0.36
40-50	1.00	0.53	0.32	0.75	0.12
50-60	1.48	-0.48	0.81	0.09	0.27
Total	**1.00**	**2.39**	**2.72**	**2.18**	**2.15**

Table 4: Layer wise soil moisture depletion rate (mm/d) from D_1 I_2 V_2 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.43	0.83	0.77	0.55
10-20	-0.16	0.30	0.67	0.58	0.48
20-30	-1.05	0.37	0.51	0.54	0.27
30-40	-0.19	0.03	0.48	0.55	0.41
40-50	1.00	0.02	0.41	0.28	0.85
50-60	1.48	0.02	0.39	-0.04	0.79
Total	**1.00**	**1.16**	**3.29**	**2.69**	**3.36**

Table 5: Layer wise soil moisture depletion rate (mm/d) from D_1 I_3 V_1 treatment combination during crop growing period:

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.44	0.63	1.34	0.66
10-20	-0.16	0.40	0.38	0.58	0.17
20-30	-1.05	0.40	0.27	0.69	0.22
30-40	-0.19	0.30	0.23	0.08	0.49
40-50	1.00	0.22	0.48	0.28	0.54
50-60	1.48	0.24	0.45	0.57	0.60
Total	**1.00**	**1.99**	**2.43**	**3.55**	**2.68**

Table 6: Layer wise soil moisture depletion rate (mm/d) from D_1 I_3 V_2 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-12	14-32	34-52	54-69	71-88
0-10	-0.06	0.69	0.59	1.28	0.63
10-20	-0.16	0.24	0.32	0.35	0.55
20-30	-1.05	0.59	0.37	0.70	0.51
30-40	-0.19	0.08	0.27	0.10	0.23
40-50	1.00	0.08	0.14	0.14	0.09
50-60	1.48	0.15	0.21	0.30	0.12
Total	**1.00**	**1.82**	**1.91**	**2.87**	**2.13**

D_2: Total depletion rate:

Table 7: Layer wise soil moisture depletion rate (mm/d) from D_2 I_1 V_1 treatment combination during crop growing period:

Soil depth (cm)	Days after planting					
	0-4	5-32	34-51	57-64	68-79	81-95
0-10	0.27	0.37	1.05	1.20	0.73	0.55
10-20	0.21	0.27	0.64	0.59	0.47	0.36
20-30	0.15	0.22	0.54	0.58	0.96	0.72
30-40	0.12	0.25	0.29	-0.03	0.08	0.06
40-50	0.08	0.31	0.23	0.20	0.08	0.06
50-60	0.08	0.28	0.32	0.47	0.21	0.16
Total	**0.90**	**1.70**	**3.07**	**3.01**	**2.54**	**1.90**

Table 8: Layer wise soil moisture depletion rate (mm/d) from D_2 I_1 V_2 treatment combination during crop growing period

Soil depth (cm)	Days after planting					
	0-4	5-32	34-51	57-64	68-79	81-95
0-10	0.27	0.30	0.65	0.67	0.56	0.36
10-20	0.21	0.23	0.51	0.91	0.36	0.53
20-30	0.15	0.28	0.39	0.10	0.43	0.48
30-40	0.12	0.18	0.61	0.75	0.40	0.45
40-50	0.08	0.20	0.38	0.47	0.35	0.44
50-60	0.08	0.25	0.14	0.16	0.36	0.50
Total	**0.90**	**1.44**	**2.68**	**3.06**	**2.46**	**2.76**

Table 9: Layer wise soil moisture depletion rate (mm/d) from D_2 I_2 V_1 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-4	5-32	34-57	59-71	74-95
0-10	0.27	0.27	0.34	0.59	0.36
10-20	0.21	0.24	0.45	0.59	0.29
20-30	0.15	0.22	0.44	0.58	0.27
30-40	0.12	0.16	0.47	0.45	0.26
40-50	0.08	0.17	0.36	0.28	0.33
50-60	0.08	0.18	0.40	0.40	0.32
Total	**0.90**	**1.24**	**2.46**	**2.89**	**1.84**

Table 10: Layer wise soil moisture depletion rate (mm/d) from D_2 I_2 V_2 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-4	5-32	34-57	59-71	74-95
0-10	0.27	0.14	0.56	0.61	0.28
10-20	0.21	0.31	0.45	0.63	0.27
20-30	0.15	0.39	0.34	0.67	0.33
30-40	0.12	0.38	0.35	0.44	0.41
40-50	0.08	0.21	0.44	0.37	0.36
50-60	0.08	0.16	0.47	0.27	0.37
Total	**0.90**	**1.60**	**2.61**	**2.99**	**2.02**

Table 11: Layer wise soil moisture depletion rate (mm/d) from D_2 I_3 V_1 treatment combination during crop growing period:

Soil depth (cm)	Days after planting				
	0-4	5-32	34-57	59-71	74-95
0-10	0.27	0.30	0.43	0.48	0.18
10-20	0.21	0.30	0.46	0.50	0.14
20-30	0.15	0.39	0.38	0.49	0.54
30-40	0.12	0.39	0.36	0.46	0.57
40-50	0.08	0.31	0.42	0.39	0.60
50-60	0.08	0.30	0.46	0.44	0.65
Total	**0.90**	**1.98**	**2.51**	**2.76**	**2.68**

Table 12: Layer wise soil moisture depletion rate (mm/d) from D_2 I_3 V_2 treatment combination during crop growing period

Soil depth (cm)	Days after planting				
	0-4	5-32	34-57	59-71	74-95
0-10	0.27	0.16	0.41	0.38	0.13
10-20	0.21	0.16	0.40	0.25	0.03
20-30	0.15	0.25	0.33	0.38	0.49
30-40	0.12	0.25	0.31	0.20	0.47
40-50	0.08	0.19	0.36	0.52	0.42
50-60	0.08	0.18	0.34	0.60	0.43
Total	**0.90**	**1.18**	**2.16**	**2.33**	**1.97**

Table 13: Yield of Potato crop

Treatment	Yield (t ha^{-1})
D_1 I_1 V_1	45.17
D_1 I_1 V_2	50.28
D_1 I_2 V_1	44.27
D_1 I_2 V_2	48.97
D_1 I_3 V_1	50.56
D_1 I_3 V_2	31.98
Average	**45.21**
D_2 I_1 V_1	63.65
D_2 I_1 V_2	55.26
D_2 I_2 V_1	41.52
D_2 I_2 V_2	52.34
D_2 I_3 V_1	50.24
D_2 I_3 V_2	37.90
Average	**50.15**

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RESEARCH ARTICLE

Marketing Mix in Management Education: Relevance to Management Students in Bengaluru

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ABSTRACT

The Purpose of this research paper is to present a adoption of marketing mix by management education institutions to impact on enrolment and satisfaction of MBA students. Hence, students and parents attitude, perceptions, opinions & expectations towards marketing initiatives of management education institutions Bengaluru. The MBA education market is increasing demand, institutions aggressive efforts to attract prospective students, and satisfy the current pursuing students and alumni's. The 4Ps of traditional marketing mix tools (Product, Price, Promotion and Place) are considered in this quantitative approach for the survey. The primary data has analysis Correlation, Chi- square and One way ANOVA statistical tools are used, secondary information's has gathered from research articles. The sample size is 250 drawn in MBA institutions Bengaluru. In the research findings the many institutions are covering traditional 4Ps of marketing mix, however, it need to change as different elements : Programme, Premium(Course fee), Prospectus marketing (targeted customers) and last Prominence (Infrastructure facilities) to face the strong competition in educational market. This article highlighted that the traditional 4 Ps of marketing mix for management education may not useful for MBA programme enrolment, every institution need to look towards quality education as a main motto. the finally the concluded the paper with the message for students enrol in the best institution where satisfy your needs, MEIs need to understand the expectations of stakeholders act accordingly to get successes in education market in Bengaluru.

Key Words: Marketing, Mix, Management, Education, MBA, 4PS, Institution, MEIs, Students, Product, Price, Place, Promotion, Bengaluru

INTRODUCTION

While MBA Graduates are increase in numbers every year, Similarly the Management education institutions also increasing in Bengaluru in the form of Government, Private & Deemed to be Universities, also as B- Schools, and Private institutions affiliated to Government Universities. The more number of traditional Management Education institutions are created highly Competitive market in Bengaluru, that is benefited to MBA students to get varieties of choices while selecting, pursuing and best placements. More Management Education institutions are differentiating themselves with special combination of 4Ps. The Institutions are realised the importance of Marketing Mix, to attract the end users (students), As per the common calculation where competition increases, the problems also increases. due to tough market many institutions are have been declined the enrolment of MBA students in Bengaluru.

For Students, MBA education is highly impacting decision on their life. But, for the students the more number of Management Institutions availability makes more complexity for selecting a best institution. When students get more varieties of options they may take longer time for decision making process. Where students gathered the more information with evaluation may done critically be decision takers. some point of time the institution reputation, publicity and opinion leaders may impact on decisions. Even many institutions are facing financial issues and Competition making them to become idle in the market.

Marketing of management education is not new concept, many of the researcher are enlighten on the role of marketing and its importance while selection of best institutions by decisions makers (Students or parents). When MEIs are offers high importance to satisfy the student need, teaching method which fulfils their expectations. 100% genuine information on the facilities provided by institution to decision makers wields the results. the management programs prices need to match with the value which is offered by institutions. The programs are need to filled with tools of Marketing Mix elements like Product, Price, Place & Promotion are effected not only on program, which may effect on improvement in enrolment of students and satisfaction and influence aspirants.

The Marketing Mix (4Ps)

The Marketing Mix elements are controllable by any Management Education Institutions in order to build a response from the market which they targeted. The marketing Mix tools can create a wide range of demand with offers by any MEIs. Any product based industries mainly concentrate on 4Ps, but Management Education Institutions falls in service sectors it need to focus on 7Ps of Marketing Mix. But as a education sector mainly focus on traditional 4Ps approach to satisfy the MBA students expectations. those Ps are named as Product, Price, Promotion and place.

The Product: The product can be sold for buyers with tangible features, which carries the bundle of benefits to satisfy the consumer. Hence, service sector the product is a concept which can be sold for the purchaser. But in case of Management Education Institutions the MBA aspirants/ Admitted students are raw materials, during the course time they will comes under processing stage, Once the degree awarded alumni's are treated as finished products. In another argument, The students are the customer who are paying for benefits are buying a MBA degrees as products. the designing curriculum of MBA programme need to meets the students need, corporate expectations and market demands.

The Price: The Price element is Service Marketing Mix which highly impacting on programme what being charged for MBA degree/ Course tuition fees that charges for students to enrol for MEIs. The price element is not only affects on the institutional profit which derived from students enrolment for the course. the Price may also affect on the quality perception of the MBA students. The students attitude towards the quality, decided on the basis of the cost which is charged by MEIs. Many institutions are generating revenue with offering with best structured course curriculum in order to create high profit. majority of the MEIs are involved for research activities to generate revenue for universities. Hence, the Pricing becoming more critical element to run the MEIs.

The Promotion: The Promotion tool which is conveying the marketing information of MEIs which is offering to their targeted customer: which may be a elements of Advertising, Direct Marketing, Personal Selling, Publicity, Public Relations, Internet and Interactive marketing efforts need to communicate. In targeted market, some of the Commerce/Management prospective students are aspirants are so important to target them with promotion tools to attract them to make enrol them for MBA programme. In recent days, the Education fairs, TV Ads, Direct mails, Social/ Digital media promotions, online counselling Applications/ Videos are few promotional tools which is contributed for MEIs to inform, persuade, remind prospective MBA students to select best institutions.

The Place: Where the MBA students meets and consume the benefits, is offered by the Management Education Institutions. The Place meets the exceeds the expectations of MBA Students. Hence, alternative modes of actions are significantly grown in the market as online platforms. But, no longer meeting the students their service providers to get an course inputs in physical classroom. Accessing of programme supported study materials/ learning facilities are available virtually through electronic/ social media. Classroom teaching on black board, practical lab based education facilities are declining and emerging the distance learning opportunities have developed like, though e-mail, Webinars, Video and Tele conferencing, Online classes, YouTube, etc.

REVIEW OF LITERATURE

Irshad Ahmad, Abu Bashar, Anurag Chandra (Dec, 2013), Paper had title as marketing of higher educational services : An Empirical study of students perspective. The new enormous extension of schooling through private arrangement has presented new skylines for advertisers simultaneously with the increment in number of instructive foundations ceaselessly the opposition is likewise expanding with same speed, it is astounding that more consideration has not been paid to showcasing issues that have been stimulated because of expanding contest. A portion of the consuming issues, for example, are instructive organizations truly " Consumer Oriented"? Do they pick the most suitable market fragment? would advanced education advertiser are rehearsing the most proper systems to draw in and enlist understudies ? also, the intricacy of the choice interaction of the " Buyers" must be tended to at was level. Here discovered the overall issues looking by the instructive advertisers and endeavours would be utilized to comprehend the understudies assumptions and factor that draw in then towards a specific organizations.. E. Thangaswamy, (Feb 2014), In his examination article - marketing of higher education services in India: a critical study, The fruitful advertising of the significant instructive assistance in home nation and Abroad by, giving Quality schooling to the Prospective clients turns out to be, fundamental as they will go about as makers in the days to come improvement of Information and Communication Technology in training has likewise been Constantly working with the smooth stream and Exchange of Educational Production between the Educational Institution and the trying dearness. In this paper is an endeavor to assess the status and pattern of Higher Education System in India and Suggest fitting measures to work on the nature of training and Research to achieve its guest objectives by fulfilling the hopeful mass over the long haul. Le quang Trucand Hoa Van Tran (July 2017) the research article named as marketing mix in higher education -perspective from students, This study examines the level of importance and responsiveness of seven P's of Marketing Mix in higher education institutions in Vietnam. Hence, several policies for Universities in Vietnam to improve their education marketing are suggested, including (1) treating students as customers (2) Investing in human resources (3) Changing the perception of education services (4) Establishing a marketing division & (5) Providing funds for marketing activities in Universities

Research Objectives

1. To Understand current situations regarding Marketing Mix in Management Education.
2. To Determine the Traditional Marketing Mix Elements considered by MBA students in Bengaluru.
3. To Analyse the Management Educational Institutions Frame works towards Marketing Mix to attract the MBA students in Bengaluru.

RESEARCH METHODOLOGY

For Research a highly structured close ended questionnaires with 5 points Likert Scales was developed to measure the MBA Students different attitude towards marketing mix covered mainly 4Ps in MEIs in Bengaluru. In the questionnaire demographic and Students Motivational factors also measured. As a part of primary data collection the questionnaires was shared to around 760 population has considered, Newly enrolled, pursuing and Alumnis of MBA programme has considered has sample, The response rate was achieved 40% (304), in the achieved response just 33% are responded completion questionnaire response that was in satisfactory level to consider for the research. Hence, considered 250 responses has sample size for the study. The primary data has collected through the Random sampling technique. Hence, The study has limited for Bengaluru, Considered Some Government, Private & Deemed to be University, B- Schools, Private Affiliated Colleges as research locality. The Statistical Tools are used to measure (Questionnaires) a Primary data through SPSS software. Also reviewed previous research articles as part of secondary information.

Data Analysis & Interpretation

Correlations

Interpretation-

From the above table, It was observed that the Correlation value is 0.333. So it falls between
0.01 to 0.50. hence it is a positive correlation linking to the service providers quality and teaching methodology and the best experiences leads to positive word of mouth & positive impression on institutions.

Chi- Square test

Gender * Institution Cross tabulation

Interpretation

From The Above table, Its can be interpreted as asymptotic significance Two sided value is 0.306, Which is more value of 0.05. So, Null hypothesis can be agreed that if there is no relationship between gender and Institution category increases the relationship productivity.

One way ANOVA

Post Hoc Tests Multiple Comparisons

Homogeneous Subsets Fee

Interpretation

Hence, Significance variable was more than 0.05 that is 0.596, So, Null hypothesis is acceptance and There is no significance between Fee charging for MBA Degree and their institution category.

Findings

The Findings of this research indeed 4Ps marketing mix may not best way to approach the marketing of the MBA programme in Bengaluru. The MEIs are not designed the MBA programme as equal worthy to fee paid by student, technological element, Improper adaptation, lack of Implementation, extra curriculum, course quality, brand image, Placement & job packages, Course fee, Extra benefits, special offers, lack of advertisements, methodology in teaching, Students experience in classroom, tough competition and lack of digital marketing leading to negative impacts on management institutions in Bengaluru.

Recommendations & Suggestions

The MBA Programme / Product elements of the made up of curriculum related aspects through marketing mix most important element to improve the brand image and quality. The best teaching methodology and staff quality also second most important price element in the marketing mix. The institutions need to make pricing strategies on the basis of facility provided and stakeholder satisfaction level. for the promotional element, institutions need to standardised the mass media advertisement, like digital/ social media, hard copy promotional materials, direct mail or telephonic approach, best way to reach the aspirants through face to face. the place element needs to best infrastructure, classroom environment, digital study resources is very essential to satisfy the students and parents community.

CONCLUSION

The management education institution in Bengaluru has witnessed many significant challenges and changes in the competitive market. presently many challenges are mainly derived from recent competition in market, Global education opportunities, Financial conditions of the students or parents, education sector new demands and also many expectations of from the stakeholders. The MBA institutions have more scope for implementation of marketing strategies, strong brand image and reputation, transparent system strategies are need to apply the branding and marketing the Management institution in Bengaluru

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Table 1. Data Analysis & Interpretation

Correlations

		Q1. The service providers/ faculties quality and teaching methodology may create positive word of mouth by Alumni's to institutional Promotions for MBA aspirants.	**Q2.** The current pursuing students best experience in their Institutions, creates the positive impression on education market
Q1	Pearson Correlation	1	.333**
	Sig. (2-tailed)		.000
	N	250	250
Q2	Pearson Correlation	.333**	1
	Sig. (2-tailed)	.000	
	N	250	250

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2. *Chi- Square test*
Gender * Institution Cross tabulation

			Institution						Total
			Private affiliated	Government Institution	B-School	Private University	Deemed to be University	Government University	
Gender	Male	Count	34	26	12	20	16	21	129
		Expected Count	29.9	29.9	10.3	17.0	20.6	21.2	129.0
	Female	Count	24	32	8	13	24	20	121
		Expected Count	28.1	28.1	9.7	16.0	19.4	19.8	121.0
Total		Count	58	58	20	33	40	41	250
		Expected Count	58.0	58.0	20.0	33.0	40.0	41.0	250.0

Table 3. Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.004[a]	5	.306
Likelihood Ratio	6.035	5	.303
Linear-by-Linear Association	.576	1	.448
N of Valid Cases	250		

a. 0 cells (0.0%) have expected count less than 5. The inimum expected count is 9.68.

Table 4. Symmetric Measures

		Value	Approx. Sig.
Nominal by Nominal	Phi	.155	.306
	Cramer's V	.155	.306
N of Valid Cases		250	

a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis

Table 5. ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.530	5	2.106	.738	.596
Within Groups	696.194	244	2.853		
Total	706.724	249			

Table 5. Post Hoc Tests, Multiple Comparisons, Dependent Variable: Fee
Tukey HSD

(I) Institution	(J) Institution	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Private affiliated	Government Institution	.34483	.31367	.881	-.5562	1.2458
	B- School	.15345	.43801	.999	-1.1048	1.4116
	Private University	.54284	.36832	.681	-.5151	1.6008
	Deemed to be University	.07845	.34717	1.000	-.9188	1.0757
	Government University	.48150	.34465	.729	-.5085	1.4715
Government Institution	Private affiliated	-.34483	.31367	.881	-1.2458	.5562
	B- School	-.19138	.43801	.998	-1.4496	1.0668
	Private University	.19801	.36832	.995	-.8600	1.2560
	Deemed to be University	-.26638	.34717	.973	-1.2636	.7309
	Government University	.13667	.34465	.999	-.8533	1.1267
B- School	Private affiliated	-.15345	.43801	.999	-1.4116	1.1048
	Government Institution	.19138	.43801	.998	-1.0668	1.4496
	Private University	.38939	.47867	.965	-.9856	1.7644
	Deemed to be University	-.07500	.46259	1.000	-1.4038	1.2538
	Government University	.32805	.46071	.980	-.9953	1.6514
Private University	Private affiliated	-.54284	.36832	.681	-1.6008	.5151
	Government Institution	-.19801	.36832	.995	-1.2560	.8600
	B- School	-.38939	.47867	.965	-1.7644	.9856
	Deemed to be University	-.46439	.39723	.851	-1.6054	.6767
	Government University	-.06135	.39504	1.000	-1.1961	1.0734
Deemed to be University	Private affiliated	-.07845	.34717	1.000	-1.0757	.9188
	Government Institution	.26638	.34717	.973	-.7309	1.2636
	B- School	.07500	.46259	1.000	-1.2538	1.4038
	Private University	.46439	.39723	.851	-.6767	1.6054
	Government University	.40305	.37540	.891	-.6753	1.4814
Government University	Private affiliated	-.48150	.34465	.729	-1.4715	.5085
	Government Institution	-.13667	.34465	.999	-1.1267	.8533
	B- School	-.32805	.46071	.980	-1.6514	.9953
	Private University	.06135	.39504	1.000	-1.0734	1.1961
	Deemed to be University	-.40305	.37540	.891	-1.4814	.6753

Table 6. Homogeneous Subsets Fee

Tukey HSD

Institution	N	Subset for alpha = 0.05
		1
Private University	33	3.0606
Government University	41	3.1220
Government Institution	58	3.2586
B- School	20	3.4500
Deemed to be University	40	3.5250
Private affiliated	58	3.6034
Sig.		.743

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 36.546.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

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RESEARCH ARTICLE

Core Level Spectra Calculation of Indium Phosphide using First Principles Method

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ABSTRACT

We have testified the core level spectra of indium phosphide using first principles calculation using CASTEP module in BIOVIA Material studio. A super cell of 32 atoms of InP^3 is being well-thought-out for the core level spectra simulation. For In 1s, At nearly 12 eV the satellite peak is noticed and at 25 eV the peak broadening occurs. But for P 1s, four different peaks are being noticed. The variation in these peaks is due to the spin orbit interaction.

Keywords: InP, Core Level Spectra, Density Functional Theory.

INTRODUCTION

Density Functional theory [1] method deliversa robust and reliable foundation to observe the core-level spectra of various materials [2]. Recently Bethe Salpter equation [3] has been initiated very noteworthy in these simulations. Here, peak broadening is a vital point in explaining these kind of simulation owing to the electron-hole lifetime spectra. Still experimental results have shown to be successful in the forecasting the simulated data. Here, we have used systematic remarks of core-level effects. Quantitatively the strength of the core hole can be forecast in several materials which can support us in the explanation of simulation of core-level spectra. The noteworthy experimental results with an explanation for the spectrum can be accurately determined using Density Functional theory with ground state energy calculation [4]. But sometimes the core hole is essential to be involved with the simulation process.

Simulation Details

We have used the CASTEP [5] module to compute the spectroscopic properties of countless materials which is because of the electron transitions from the core level in the conduction band. We can also envisage the properties because of the electronic transition from the valance band to a conduction level. Both types of transitions are chosen as XABS spectroscopy and x-ray emission as well.

The core level spectroscopic method based on quantum mechanical simulation can provide a detailed data for a localized electronic structure. The experiments which are reliant on the angles which offer the different state that which involves orbitals. Finally, the entire symmetric states can be illustrated in which the straight importance is from the chemical bonding. Here we have used a conventional unit cell of indium phosphide which contains 8 atoms showing the FCC structure of the crystal lattice. For easier calculations, we have used the primitive lattice which completely uses the full symmetry. Here we have used on the fly ultra-soft pseudo potential [6] for the calculation of the core level spectroscopy. We have established the range of energy to 30eV which indicates that it allows the energy levels up to 30ev above the Fermi level.

Here, we have used a super cell of 32 atoms to simulate the contribution from the core hole . The super cell has an advantage of decreasing the artificial exchanges between the periodic imageries of the atoms that comprise the core holes. We have created 2 different core holes both using Indium and Phosphorus imposing the proper symmetry. The 1s spectrum corresponding to the core level spectrum [6] of a core electron in the 1s orbitals. The essential energy detected through the formation of the core hole is replicated by an absorption spectrum. Similarly, an emission spectrum will replicate the x ray photon energy producedin the relaxation of core hole returning to ground energy level.

RESULT AND DISCUSSION

Fig. 1.3 Core level spectra of Indium (Red curve representing the spectrum with consideration of core hole effects and the black curve representing the spectrum without consideration of core hall effect). This analysis of core level spectroscopic properties of In P [8] with and deprived of considerations of core hole effects is providing us two spectra. Fig 1.3 indicates the variation between intensity and the energy (eV) for InP [9]. At nearly 12 eV the satellite peak is observed and at 25 eV the peak broadening happens. In fig 1.3 both the curves have a similar representation.

Similarly, Fig 1.4 has shown core-level spectra for phosphorus 1s. We have obtained four peaks in phosphorus 1s. In the green curve the core-hole effect has been considered where at 5 eV a satellite peak has been obtained. At 14 eV we obtain the second small peak and the third peak happens at 26 eVwhere as the 4^{th} peak occurs at 32 eV. It also designated the separation at four different binding energy because of the spin-orbit coupling. At 8eV, an additional increment in the intensity shows the discrepancies in the curve which represents the consideration without a core level spectrum.

CONCLUSION

We have successfully simulated the core level spectra in InP using the CASTEP module in the BIOVIA material studio. We have achieved a difference in the intensity spectrum in the core level in phosphorus 1s.Four different peaks are obtained in each of the curves in the presence of the spin-orbit interaction.

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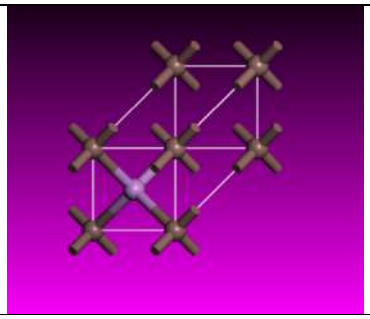

Fig 1.1 Primitive lattice of the crystal structure of InP (Brown dots are showing Indium atoms, and Magenta dots represents Phosphorous atoms)

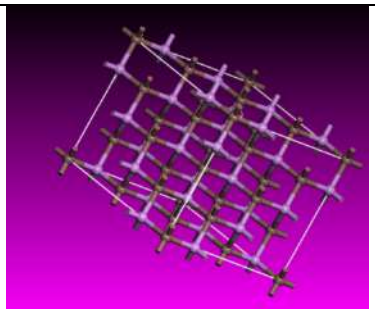

Fig. 1.2A super cell of 32 atoms of InP (Brown dots are showing Indium atoms, and magenta dots represents Phosphorous atoms)

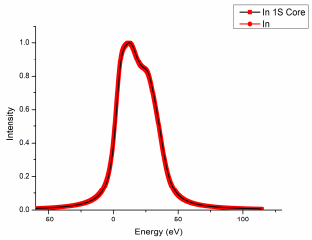

Fig. 1.3 Core level spectra of Indium (Red curve representing the spectrum with consideration of core hole effects and the black curve representing the spectrum without consideration of core hall effect)

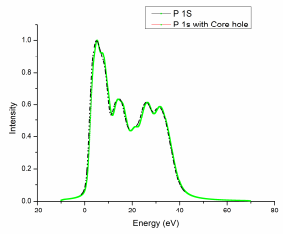

Fig 1.4 Core level spectra of phosphorus [10] (Black dotted curve representing the spectrum without consideration of core hole effects and the green curve representing the spectrum without consideration of core hall effect)

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RESEARCH ARTICLE

Review on the Synthesis, Structural and Electrical Properties of Perovskite Materials

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ABSTRACT

The perovskite is observed to be the single most versatile ceramic, which attracts the attention of researchers because of its huge number of applications. Perovskite materials show an enormous variety of structural modifications and variants. The perovskite-type oxides perform both physical and biochemical properties. Based on Perovskite-phase metal oxides, a specific range of characteristics in electrical conductivity, dielectric constant, dielectric loss, structural, and magnetic properties, etc. became applicable for different utilization. Perovskite materials showed energetic crucial characteristics for photovoltaic solar cells. The functionality of perovskite materials changes with the preparation methods by fabricating stoichiometric ratios of A and B cations. For energy storage device, perovskite having a high dielectric constant have to be synthesized by doping other elements like iron (Fe), Strontium (Sr), Manganese (Mn), Cobalt (Co), etc.

Keywords: Synthesis technique; Structure; Dielectric; Electrical,

INTRODUCTION

The mineral composite $CaTiO_3$ (Calcium Titanate) was first discovered by Gustav Rose in the year 1839 in the Ural Mountains of Russia which was later termed as Perovskite on behalf of the famous Russian Mineralogist Count Lev Alekseevich (1792-1856). The structure of the crystal was first identified by Victor Gold Schmidt in 1926 and published in 1945 by Helen Dick Megaw from X-Ray Diffraction data[1]. Perovskite is a reddish-brown, blackor pale-yellowmineral. Its generic structural form is ABX_3 where A & B are two positively charged ions (cations) of distinct sizes and X is a negatively charged ions (anion) which may be halides, sulfides, and nitrides, etc. but Oxygen is often used in Perovskite due to its huge applications[2]. As the catalyst, the A-site cations are responsible for thermal stability where B-site cations are responsible for catalytic activity[3]. Perovskite structures have different forms such as ABO_3-Perovskite oxides (Ex: -$CaTiO_3$, $PbTiO_3$, $PbSnO_3$), A_2BO_4- Layered Perovskite (Ex: - Sr_2RuO_4, K_2NiF_4), $A_2A'B_2B'O_9$-Triple Perovskite (Ex: - $Ba_2KNaTe_2O_9$, $Sr_3Sb_2CoO_9$) and $A_2BB'O_6$- Double Perovskite (Ex: -Sr_2FeMoO_6,

La_2ZnTiO_6) etc.[4],[5]Several investigations were focused on the study of Perovskite oxides i.e., ABO_3, their properties and applications during the last few years which are given in Table 1. These materials also have wide applications in the field of Physics, Chemistry, Material science, Geophysics, and Environment, etc.

Here in this study, we were only focused on the Perovskite oxides and Perovskite like oxides which have huge applications in various fields such as Volatile Memories, Tunable microwave devices displays, Superconducting digital circuits, Piezoelectric devices, Humidity Sensors, and Multilayer Ceramic Capacitors, etc. due to their different properties like insulator-metal transition, characteristics of thermal, electric and ionic conduction, dielectric, Pyroelectric, Piezoelectric, deviation of solid-state phenomena, metallic and superconductivity, etc.

SYNTHESIS OF PEROVSKITE

Various preparation methods of the perovskite oxides result in different physical or chemical properties of these materials which were discussed below.

SOLID-STATE REACTION TECHNIQUE

In this technique, the raw materials i.e. two or more nonvolatile solids react in presence of heat to form the required product which is also in the solid form[11]. Also, chemical ingredients like carbonates, nitrates, oxides can be added with the raw materials in the stoichiometric ratios at high temperatures to produce the final product of the desired composition. Then the samples are ball-milled successfully in presence of isopropanol or acetone. Then the collected product undergoes processes like calcinations, grinding, sintering and electroding to obtain an appropriate sample. As solid do not react with solid at room temperature, this method takes place at very high temperature [12],[13]. Several authors have been successfully demonstrated the structure and properties of perovskite oxides using a solid-state reaction process. Synthesis of $BaTiO_3$ materials was demonstrated using the solid-state reaction between $BaCO_3$ and TiO_2as a function of temperature (400^0C-1200^0 C)[14],[15]. Analyzing XRD data, $BaTiO_3$ particles were found to be the structure of tetragonal with high dielectric constant[16], high insulation temperature[17], and piezoelectric properties[18],and with the increase in calcination temperature, the size of samples also increased. Synthesis of Bismuth Ferrite ($BiFeO_3$) has been also developed successfully using this technique[7]. [19] the Crystallite size of Bismuth ferrite has fluctuated from 13 to 70nm. [20]We noticed that increase of dielectric constant and decreases of dielectric loss can be obtained with rising temperature and decreasing frequency which can be applied in the energy storage device, electronic device, etc. Also, the structure of the sample was in good match with rhombohedral at a high sintering temperature (800^0C-900^0C)[21],[22]. But from ferroelectric studies, saturation polarization in the P-E loop could not be achieved. Also, the Synthesis of $BaSnO_3$ powder was demonstrated using solid-state reaction technique by Jibi John[23] and U. Sujana Kumari [24]. Successfully the results were in good match with the previous experimental data. The samples were found to be in a cubic phase. The high reflectance of the sample in the 400-700nm range was observed. The bandgap energy was found to be 3.1eV.

A Cabrera Ramirez reported the synthesis of $LaYbO_3$ materials by applying this technique[25]. The orthorhombic structure of the materials was confirmed. The surface morphology was qualitatively analyzed as a function of the sintering process. The characterization of Lead Titanate ($PbTiO_3$) was reported in various journals[26],[27]. From XRD data, samples are confirmed to be in tetragonal crystal systems and it exhibits conduction because of the boundary effect in bulk and grain at high temperature [28]. Also, it was observed that the crystallite size of the sample can be enhanced by raising the temperature of the annealing process [29]. The structural, electrical, and dielectric properties of Perovskite materials can be greatly influenced by the doping of rare earth metals or transition metals in Perovskite materials which can be also synthesized triumphantly. Synthesis of Barium Strontium Titanate by doping Iron (Fe) taking x=0-0.3 ($Ba_{0.7}Sr_{0.3}Fe_xTi_{1-x}O_3$) was developed by A. Kaur[9] and Z. Guo [30]. The samples having x=0,0.1 were found to be a tetragonal and cubic phase whereas the samples containing x>0.1 were observed to be in the pure cubic phase. The samples with x=0,0.1 have confirmed its paramagnetic behavior where the sample for x>0.1 was weak Ferro- and ferri- magnetic at about 2K.

Lead-FreeNanopowdersBa$Zr_{0.2}Ti_{0.8}O_3$(BZT)[31],[32]compounds were synthesized using this technique to obtain the microstructure of crystalline, surface morphology, and dielectric characteristics successfully. The tetragonal structure of the sample was identified[33]. It was confirmed that the dielectric constant depends on the temperature[34] and was maximum at curie temperature (T_c). These materials could be useful in the ferroelectric system.

1)

2) SOL-GEL PREPARATION

The Sol-gel technique is an outdated technique that was developed in 1940 in the field of chemistry[35]. In this process, Sol is transformed into a gel by following several steps. Here Sol stands for colloidal particles in a solvent of Silicate solution whereas gel is a 3D porous network structure of Silica[36]. The first step is the formation of sol by the mixture of molecular precursors that are either metal salts or metal organics. Condensation reactions are carried out in the sol stage, forming colloids or clusters which lead to the formation of a gel when they agglomerate and cross-link each other. After the gel stage, there is a heat treatment stage when drying and calcination steps take place[37].

The preparation of Perovskite $CaTiO_3$through sol-gel methods provides information about the structural, electrical, and dielectric properties of the materials[38],[39]. The luminous properties of the samples are gradually increasing with a rise in sintering temperature[40]. The spherical size of particles was varied from 26-31nm and the bandwidth was found to be 3-3.5eV[41].

Applying this method, the synthesis[42] of $PbTiO_3$was demonstrated at a very low temperature in the range 650-700^{0}C and concluded that $PbTiO_3$ exhibits the tetragonal structure[43],[26]with high dielectric constant and electrical conductivity[44]. Saturation polarization in the P-E loop was achieved from ferroelectric studies[45]. Perovskite $SrTiO_3$was synthesized in various papers via the sol-gel process[46].X-Ray Diffraction technique confirmed its structure to be pure cubic phase[47]at 400^{0}C temperature and the size of the particles were varied from 20-30nm[48].

Several authors demonstrated the synthesis of $LaCoO_3$ through this method[49]. The rhombohedral structure was obtained and good catalytic nature was confirmed. The electrical, optical, and thermoelectric properties[50],[51] of samples were investigated. Bismuth Ferrite was also synthesized by N. Shara Sowmya using the sol-gel method which paper was published in 2015[52]. From the X-ray Diffraction pattern, he found a rhombohedral structure at 60^{0}c with 90% density. He also observed that the samples $BiFeO_3$ have high permittivity and multiferroicity (coupling between magnetic and polarization orders).

3)

4) CO-PRECIPITATION METHOD

This method involves the induction of the super saturation state of certain species soluble in the solvent. The supersaturating conditions necessary to the precipitation of the species are usually induced by the result of a chemical reaction[53]. Precipitation agent is the combination of soluble metal cations solution and another solution which is known as co-precipitation solution. Inorganic or organic substances are generally considered as agents of co-precipitation [54]. Parameters like temperature, mixing rate, pH, and concentration are adjusted to obtain the desired products and their physical properties e.g., product size, morphology and particle size distribution, etc. After precipitation, the precipitate can be removed by centrifugation and filtration or decantation and then dissolved in acids or in an organic solvent, such as isobutyl methyl ketone to be measured[54],[55].

The synthesis of $LaCoO_3$ was determined by Z. Junwu and S. Xiaojie by operating this process [56]. The size of about 25nm of the particle was examined at an approximately low calcination temperature of 600^0. The Raman Properties of the samples was examined at different calcining temperature. La-based perovskite ($LaMO_3$, M= Al, Co, Fe, and Gd) were synthesized by W. Haron, A. Wisitsoraut [57]. $LaAlO_3$ showed important properties like semiconductivity having a band gap of 2.6eV and high surface area. Among these four perovskites, $LaAlO_3$ can be applied in gas sensing material and heavy metal ion adsorbent. The formation of crystal $NdFeO_3$ was developed by M. Khorasani-

Motlagh[58] by applying this method. The orthorhombic structure of the crystal was confirmed and the size of the particles was spherical at about 69nm. The ferroelectric nature of the crystal was investigated from the P-E loop.

Lead iron niobate Pb ($Fe_{0.5}Nb_{0.5}$) O_3 known as PFN was synthesized taking raw materials as Niobium Oxide (Nb_2O_5), lead nitrate, nitric acid (HNO_3), oxalic acid, and aqueous ammonia ($NH_3.H_2O$) by applying this technique. From XRD it was confirmed that the appropriate samples were obtained by calcinations at 800^{0}C. The PFN samples were amorphous, pyrochlore in nature. The grain size was identified to be very small i.e., nearly 1:1 ordered clusters. Synthesis of $BiFeO_3$ has been studied by N.A. Lamonova by using the co-precipitation method[59]. He described that with increasing temperature, sintering time should be decreased to keep the size of formed $BiFeO_3$ crystals.

DIELECTRIC PROPERTIES

Dielectric constant (K) considers as the polarity of a medium which is determined by the ratio ofthe capacitance of the substance in the medium(C) to the capacitance of the substance in free space (C_0)[60]$\mathbf{K} = \frac{\mathbf{C}}{\mathbf{C_0}} = \frac{\varepsilon}{\varepsilon_0}$, where ε &ε_0 are the permittivity of the medium and free space respectively. Capacitance of the substance in free space (C_0)can be calculated using the relation$\mathbf{C_0} = \frac{\varepsilon_0 \mathbf{A}}{\mathbf{d}}$, whereA = Area of pallets, d = pallet's thickness, and ε_0= permittivity of free space. The dielectric loss is the inherent dissipation of electromagnetic energy by dielectric material which depends on the frequency and dielectric material. It can be specified in the form of either loss angle δ or corresponding tangent loss tan δ. The dielectric loss value is defined as the ratio of theimaginary (ε') part to the real part (ε'') of dielectric constant i.e.$\mathbf{\tan \delta} = \frac{\varepsilon'}{\varepsilon''}$. High dielectric constants materials have very low value of tangent loss or dissipation factor.

Many researchers investigated the dielectric properties of various perovskite materials and concluded that doping of different metals in perovskite materials affects their dielectric properties which are discussed below. The characteristics of leadzirconate ($PbZrO_3$) were developed by doping Strontium (Sr)[61].and came to the conclusion that dielectric constant decreases with an increase in doping amount of Strontium (Sr) but attained maximum value at the transition temperature. The properties like ferroelectric, piezoelectric[62],and energy efficiency of lead zirconate were enhanced due to the doping of Strontium. The different properties of $BaTiO_3$ samples doping Iron (Fe) was characterized and noticed that after doping the samples were found in the mixture of tetragonal and hexagonal phases[63]. Dielectric constant decreases with an increase in doping concentrations. The dopant Fe enhanced the ferroelectric and ferromagnetic state of Barium Titanate for which it can be used for magneto electric applications[64],[65].

CONCLUSION

As discussed above, to obtain proper perovskite-type oxide materials, a solid-state technique at high temperatures can be used successfully. The functionality of perovskite materials changes with the preparation methods by fabricating stoichiometric ratios of A and B cations. For energy storage device, perovskite having a high dielectric constant have to be synthesized by doping other elements like iron (Fe), Strontium (Sr), Manganese (Mn), Cobalt (Co), etc.

Conflicts of interest

There are no conflicts to declare.

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Table 1 Different types of Perovskite Materials, their Properties, and Applications

Perovskites materials	Properties	Applications
$BaTiO_3$	Ferroelectricity, Piezoelectricity, Pyroelectricity, High dielectric constant, Insulating	Multi-layer ceramic capacitors (MLCCs), Positive Temperature coefficient resistor (PTCR), Embedded capacitance[3]
$SrTiO_3$	Nonlinear dielectric, High dielectric constant, Pyroelectric, Insulating	Tunable Microwave Devices, Pyro detector
$Pb(Zr, Ti)O_3$	Ferroelectricity, Piezoelectricity,Electro-optic, Pyroelectric[6]	Ferroelectric memories (FERAMs), Piezoelectric transducers and actuators, Optoelectronics devices
$BaSnO_3$	Semiconducting, Catalytic	Humidity sensors, thermally stable capacitors, Gas sensors,
$BiFeO_3$	Coupling of Magnetic & electric properties, High curie temperature	Magnetic Field detectors, Volatile Memories[7].
$LaMnO_3$	Magnetic, Catalytic, Insulating	Magnetic Field Sensors, Electronic devices
$LaCoO_3$	Electrical Conductivity, Catalytic[8]	Thermistor, Actuators
$YBa_2Cu_3O_7$	Superconductivity,	MRI, Superconducting digital circuits
$CaTiO_3$	Ferroelectric, Optical, Ion conductivity	Solar cells, Optoelectronics
$BaSrTiO_3$	High dielectric constant[9]	Dynamic RAM(DRAM)
$(Mg, Ca) SiO_3$	Thermal Stability, Phase stability, Elasticity	Ceramics, Thermoplastics
$LiNbO_3$	Piezoelectric, Electro-optic	Surface Acoustic wave device, Optical Modulator
$BaHfO_3$	High dielectric constant, Low dielectric loss	Electrical insulator, DRAM Capacitors[10]

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RESEARCH ARTICLE

Rapid Diagnostics for Asymptomatic SARS-CoV-2 Infections

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ABSTRACT

In the current novel coronavirus pandemic, the success of quarantine efforts depends on the rapid screening of potential asymptomatic as well as symptomatic infections. Unlike the symptomatic cases, the asymptomatic carriers in a population are too difficult to screen. There are considerable evidence of persistent fecal shedding of coronaviruses (including SARS-CoV-2) and avian influenza, consequently, rapid evaluation of community sewage samples could help in the detection of asymptomatic carriers. Furthermore, rapid diagnostics are also urgently needed to confirm the suspected cases of SARS-CoV-2 for effective clinical surveillance. To enable *in situ* rapid screening, the design process and prototype for a point-of-care (POC) paper-based microfluidic LAMP device is demonstrated. The present analytical device includes a pH indicator-based colorimetric assay for easy visualization of amplification by naked eyes or using simple optics such as a smartphone camera. The device is simple, inexpensive, portable, and sensitive enough to provide an opportunity to facilitate the rapid *in situ* detection of potential SARS-CoV-2 asymptomatic as well as symptomatic infections.

Keywords: SARS-CoV-2, Asymptomatic carriers, Reverse transcription-LAMP, Microfluidics

INTRODUCTION

The family Coronaviridae constitutes four genera, *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and *Gammacoronavirus,* and several subgenera and species. Human coronaviruses (HCoVs) comprise HCoV-229E and HCoV-NL63 belong to *Alphacoronavirus* while HCoV-OC43 and HCoV-HKU1 belong to *Betacoronavirus* [1]. In 1960, HCoV was first isolated in the cell culture from a patient with infection in the upper respiratory tract. In Guangdong province of southern China in 2002, a *Betacoronavirus* (of bat origin; subgenus *Sarbecovirus*) appeared in humans from civets caused severe acute respiratory syndrome (SARS) has been designated as SARS-CoV [2,3]. A SARS-like disease in humans known as the Middle East respiratory syndrome (designated as MERS-CoV) was also identified in Saudi Arabia in the year 2012 caused by a *Betacoronavirus* (of camel origin; subgenus *Merbecovirus*) [4–6].

The current outbreak of COVID-19 to emerge in humans appeared in Wuhan City, Hubei Province, China in December 2019 [7,8] which involves 165,158,285 confirmed cases of COVID-19, including 4,713,543 deaths as of 24 September 2021 [9]. The outbreak is caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [10]. The transmission of the disease occurs through respiratory droplets and may also be spread through contact with contaminated fomites [11]. According to many clinical reports, some carriers of SARS-CoV-2 are asymptomatic (show no symptoms of infection). To decrease the possible risk of disease transmission, it is highly crucial to detect these asymptomatic carriers at the early stage. However, it is too difficult and labor-intensive to test the whole population in a short duration of time. Similar to SARS coronavirus (2002-2004 outbreak), diarrhea has been reported in a significant proportion of the SARS-CoV-2 infections with high RNA copies in the stool samples [12-19].

A viable SARS-CoV-2 has been also detected in the feces and urine of infected people [20,21]. Because every individual contributes his or her microbiota in the form of feces, urine, and other biological fluids (like mouth wash) in the drainage system, therefore the waste water-based epidemiological studies could provide a solution to this problem [22-24]. Accordingly, the analysis of community wastewater (sewage) could trace SARS-CoV-2 to determine whether there are potential asymptomatic carriers in certain local areas or not. Although real-time quantitative PCR (RT-qPCR) methods, due to their high sensitivity and specificity, are used as the gold standard for the detection of infectious agents nevertheless rapid, portable, cost-efficient point-of-care devices are also urgently needed. To facilitate *in situ* detection of the asymptomatic carriers in a population in certain local areas and for the rapid diagnosis of symptomatic infections of SARS-CoV-2, the design and prototype for a paper-based microfluidic device is demonstrated (Fig.1).

The proposed device utilizes reverse transcription loop-mediated isothermal amplification (RT-LAMP) and includes a pH indicator-based colorimetric assay for easy visualization of amplification by naked eyes or smartphone camera. The amplification efficiency of the LAMP assay is known to be very high therefore with the prospective device, the unpurified RNA from many clinical samples (nasopharyngeal (nasal) swab, oropharyngeal (throat) swab, oral secretion (saliva), sputum, stool, blood, serum, plasma, and urine) can be used directly [25-30]. The miniaturization and easy operation of the device that couples sensitive LAMP assay could provide the alternate way of *in situ* rapid diagnostics [31-34]. The current prototype strategy could also serve as an early warning system for the rapid screening of infections to implement effective quarantine measures.

Sampling strategy in an *epidemiological* survey

The severe disease outbreaks caused by the enveloped viruses include Ebola, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), avian influenza H5N1, and SARS-CoV-2. Direct person-to-person contact or contact with contaminated objects (fomites) is considered to be the major route of transmission of these diseases [35,36]. The survival of enveloped viruses in water is low; however, there are substantial evidence of persistent fecal shedding of coronaviruses (including SARS-CoV-2) and avian influenzas that ultimately, after drainage, flow through the sewage system of the city as biosolid-residuals [37-48]. The samples (100-250ml) from these sewage systems can be collected from many areas of the city under investigation. Then these samples are pasteurized at 60^{o}C for 90 minutes to inactivate the viruses. The samples are then filtered using a 60-ml syringe (0.45μm followed by 0.2μm pore sized membrane) to remove bacterial cells and other solids. Since the outer membrane proteins and a lipid bilayer membrane make the enveloped viruses highly sensitive in an aqueous environment [49-51], two different approaches, polyethyleneglycol (PEG) precipitation [52,53] or ultrafiltration [54,55] or both can be optimized for their separation and concentration. Briefly, 4g of polyethylene glycol 8000 (8%w/v) and 0.9g NaCl (0.3M) are added to 50mL filtrate. It is followed by centrifugation at 12,000X g for 30 minutes or until a pellet is visible. This can be followed by concentrating the filtrate with 10kDa centrifugal filters to a final volume of 100 μL. To detect symptomatic SARS-CoV-2 infections, a wide range of clinical samples from suspected patients such as nasopharyngeal (nasal) swab, oropharyngeal (throat) swab, oral secretion (saliva), sputum, stool, blood, serum, plasma, urine) can be collected by following standard precautions as described by Center for Disease Control (CDC; **https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf**).

The design process of the prototype

It dates back to the early 18th century when the first analytical litmus test was performed [58]. Thereafter many analytical systems were introduced that employed 'paraffin' in paper patterning to create hydrophobic channels [59,60]. The conventional microfluidic devices were too expensive and complex to operate which caused a marked transition in the field of paper-based microfluidics. Consequently, the improved limit of detection (LoD) including detection capabilities in diagnosing various diseases, drug testing, and in many other analyses was registered [61-64]. The paper-based microfluidic devices can perform easy lateral flow tests (due to capillary action of the paper) with a small amount of analyte as well as complex analysis that requires multiple technical steps [65-70]. Multiple paper types can be tested for optimizing the paper to be used with the microfluidic device. The most common paper types are cellulose chromatography papers (Whatman) of grade 1 (pore size of 10 μm), grade 4 (pore size of 20-25μm), and nitrocellulose paper (pore size of 0.3μm). As a prototype for the present method, the current design of the paper-based microfluidic device consists of a central channel that directs the sample from the sample loading area to the separate test zone area (Fig. 1A). To control the volume of the sample, the design and size of the paper channel can be standardized. The long paper channel, that filters particulates from the lysed sample, is placed at the center of the microscope slide (75mm by 25mm). Thereafter, to make a hydrophobic barrier on the lower glass slide, a hydrophobic Parafilm® film can be shaped in such a way that it covers the outer area of the paper channel. Another microscope slide is placed on it which contains two specific holes to introduce the lysed sample and LAMP mixture into the sample loading area and test zone respectively. In this way, the paper channel can be sandwiched between two microscope slides. To complete fabrication, the device is heated using a hot plate at 95°C for 5 min until parafilm gets transparent (for patterning of the hydrophobic barrier a wax printer can also be used). The features of the device are large enough to be visible by eye but are small enough to limit the volume of fluid needed to run the assay. The portable and lightweight device will have defined areas of the hydrophilic matrix (cellulose or nitrocellulose paper) separated by the hydrophobic barrier (parafilm) which provides the spatial control of test fluid (Fig. 1A).

LAMP primer design and specificity

The consensus sequences of different SARS-CoV-2 strains can be worked out to identify areas of sequence conservation. To improve specificity, areas of divergence of COVID-19 with other coronaviruses can be identified using the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database [71] and nBLAST (Basic Local Alignment Search Tool at NCBI) (**https://www.ncbi.nlm.nih.gov/nuccore**) [72]. An area of the sequence that has high homology with other SARS-CoV-2 strains but divergent for Bat SARS-like coronaviruses can be targeted for LAMP primer design. The LAMP primers can be designed using LAMP Designer 1.15 (Premier Biosoft), and blasted using Primer-BLAST (NCBI) against the genomes of interest. Primer selection can be prioritized as described in "A Guide to LAMP primer designing" (https://primerexplorer.jp/e/v4_manual/). Percent mismatch can be calculated using the following equation:

$$\% \text{ Mismatch} = \frac{\text{Total number of nucleotides mismatched between each primer and sequence}}{\text{Total number of nucleotides in all primers}}$$

The LAMP primers can be selected to not have four guanines in a row to prevent the formation of tetraplex structures which might lead to interfering in the RT-LAMP reaction. Few sets of primers from the literature that have been used successfully in LAMP-based assays can also be employed (Table 1) which selectively target Nsp3 (target region ORF1a), RdRp (target region ORF1b), spike and nucleocapsid regions (Fig. 2). The PCR-standard (ssDNA positive control) can be synthesized commercially using the SARS-CoV-2 complete genome. Alternatively, the RNA from clinical samples can be extracted for subsequent cDNA synthesis [56]. DNA plasmids containing the target sequence (following *in vitro* transcription) can be used as positive controls [83]. For SARS-CoV-2, Center for Disease Control (CDC) provides a positive control sequence called nCoVPC [57].

Sample loading on the microfluidic device

The lysis buffer (100μL) that consists 40% saturated phenol (pH 4.3), 0.8M guanidine thiocyanate, 0.4M ammonium thiocyanate, 0.1M sodium acetate (pH 5.0), 5% glycerol is added directly to nasopharyngeal (nasal) swab

or oropharyngeal (throat) swab or ~100µL (w/v or v/v) of oral secretion (saliva) or sputum or stool or blood or serum or plasma or urine or sewage water (pre-filtered) sample in 1.5ml tube. The tube is then inverted 4-5 times rapidly and incubated for 10 minutes at room temperature. After incubation, 50µL of sterile MilliQ water is added. This is followed by 2-3 times gentle inversion. Thereafter, 50µL of solution from this step is pipetted to the sample loading area of the paper microfluidic device (Fig. 1B). The solution will spontaneously flow along the main paper channel via capillary action, and biomolecules (cell debris, proteins, DNA) can be filtered out (due to large size and increased retention time) at the beginning of the channel while RNA (due to small size it is pushed forward) goes through with the liquid towards the test zone of the device. The dimensions of the loading area and test zone can be optimized to sufficiently accommodate 50µL volume of the sample and its movement.

LAMP assay and its mechanism

The loop-mediated isothermal amplification (LAMP) assay is widely used to detect viral-specific genes [77-80]. The method also detects SARS coronavirus [81], influenza viruses [82], and MERS-CoV [83] with RNA genomes. The method was first described by Notomi et al. [84] and was further optimized by Nagamine et al. by utilizing highly specific loop primers for amplification [85]. The explicit amplification and high sensitivity of the LAMP assay (Fig. 3) are achieved using six specific primers which anneal specifically to the target sequence [86,87]. *Bst* polymerase further increases its specificity as it has been proven to be very sensitive even in the presence of PCR inhibitors in the unpurified samples [88]. In the current prototype strategy, the reverse transcription-based LAMP assay is performed in a total volume of 25µL of 1x isothermic amplification buffer, dNTPs (1·4mM), $MgSO_4$ (8mM), FIP and BIP (1·6µM), F3 and B3 (0·2µM), FL and BL (0·4µM) primers, 0·32U/µL *Bst* polymerase 2·0 or 3.0, 1U/µL Antarctic Thermo labile UDG (uracil-DNA glycosylase reduces crossover contamination), and 0·6U/µL reverse transcriptase (Warm Start) and phenol red as a pH indicator dye in sterile ddH_20. For visualization, instead of phenol red, SYBR Green (2µL) dye can also be added to the reaction at a 1:10 dilution [89-91]. Nuclease-free water is taken for no target control (NTC). Thus prepared, 25µL of the LAMP reaction mixture is pipetted to the test zone, followed by sealing to prevent evaporation. The device is then placed on a hot plate at 65°-68°C for 30-40 minutes (temperature and time requirements can be standardized for the assay).

Finally, the reaction is inactivated at 80°C for 10 minutes. The visual inspection of color change from orange to yellow is used to identify positive amplification. During and after amplification, the images of the paper microfluidic device are captured using a smartphone camera (Fig.1B). The intensities of the colour change from the control experiment are recorded. For the gel electrophoresis confirmation, the amplicons are eluted out from the paper by submerging it in 50 µL Tris-HCl and EDTA (TE) buffer. Amplification is confirmed using 3% w/v agarose gel and 1XTris-acetate-EDTA (TAE) buffer. The mechanism of the LAMP reaction is depicted in Fig. 3. Briefly, in step 1, the inner primer FIP contains two target sequences specific to two different regions in the template DNA, hybridizes to the target DNA, and starts synthesis of the complementary strand. The outer primer F3 starts strand displacement, which is elongated by FIP primer and subsequently ssDNA is released. It serves as a template for the backward primer. In step 2, the BIP primer starts strand synthesis of the ssDNA released in step 1. The elongated strand is displaced subsequently by the B3 primer. Step 3 involves complementary sequences, F1 to F1c and B1 to B1C form a stem-loop structure (dumbbell-shaped) which is the starting point for exponential amplification. Subsequently in step 4, the self-priming and elongation of F1 (3'end) induce displacement of B1c (5' end). Here unfolding of the hairpin structure and back folding of the newly synthesized strand will occur. Because of the repeated cycle of self-priming, it forms long amplicons with various sizes (consisting of alternately inverted repeats). Furthermore, the strand synthesis and its displacement are initiated by inner and outer primers (F3 and B3) that hybridize to the loop structures. Step 5; loop primers (Loop F and Loop B) that anneal to the loops in the dumbbell-shaped stem-loop structure further accelerate loop-mediated isothermal amplification (Fig.3B).

Real-time quantitative PCR validation

To validate the efficacy of the prototype paper-based microfluidic reverse transcription-based LAMP (RT-LAMP) assay for the detection of asymptomatic and symptomatic SARS-CoV-2 infection, real-time quantitative PCR (RT-qPCR) assays can be performed. For this purpose, commercially synthesized ssDNA (positive control) fragment of

the target gene can be used. In nanogram quantities, the control DNA is used in the SYBR Select master mix with 10M forward and reverse primers in a total reaction volume of 20 μL. The guidelines and specific primer sets are available at CDC, USA (**https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html**).

CONCLUSION

The prototype strategy for a rapid, simple, inexpensive, easy-to-fabricate, paper-based microfluidic device that can test *in situ* a wide variety of clinical and environmental samples is demonstrated. The present reverse transcription-based LAMP diagnostic device couples colorimetric assay (pH indicator-based) to assist in the detection of amplification which can be visualized by naked eyes or using simple optics such as a smartphone camera. Compared with the quantitative real-time PCR (RT-qPCR) assay, the present strategy does not allow quantification of SARS-CoV-2 RNA [56]. However, the sensitivity of the LAMP assays is found to be equivalent to the conventional PCR assays [84,85,92,93]. To perform these assays the requirement of sophisticated laboratories including the expensive instrument is a big determinant in the adoption of new diagnostic platforms [94,95]. Unlike these RT-qPCR assays, the present microfluidic device uses minimum or no sophisticated equipment. Furthermore, the present device utilizes economical Whatman or nitrocellulose paper-based platform for RNA filtration and its subsequent amplification by LAMP [96,97]. An added advantage of using paper is its strong negative polarity which makes the filtration of RNA and its amplification (strong '-' charges) from other cell debris and protein contaminants (weak '-' charges) quite uncomplicated, besides all the reaction steps can be performed on a single paper microfluidic device [98-101]. Based on the detection of SAS-CoV-2 in the sewage water [18], in an ongoing pandemic, the present device may serve as a particularly convenient platform to identify suspects, screen, and conduct virus surveillance. A similar kind of strategy has been also used previously during the 2002-2004 SARS outbreak in Israel, Egypt, and Sweden as an early warning system [102-107]. A diagnostic laboratory (Biosafety level-III) receives a lot of samples for diagnostic purposes, per-sample-test cost of which is usually very high, and the analysis also takes 24-48 hours to provide results. Conversely, the present strategy would provide a very low per-sample-test cost and rapid diagnosis. It is also expected that the rapid detection of SARS-CoV-2 asymptomatic and symptomatic infections in the population using the prospective paper-based microfluidic device would assist government agencies to take necessary steps to control the exponential spread of the current pandemic.

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Table 1: Primer sets for LAMP assay

LAMP primer	Primer Sequence (5' to 3')	Reference
S-F3	CTGACAAAGTTTTCAGATCCTCAG	73
S-B3	AGTACCAAAAATCCAGCCTCTT	
S-FIP	TCCCAGAGACATGTATAGCATGGAATCAACTCAGGACTTGTTCTTACC	
S-BIP	TGGTACTAAGAGGTTTGATAACCCTGTTAGACTTCTCAGTGGAAGCA	
S-LF	CCAAGTAACATTGGAAAAGAAA	
S-LB	GTCCTACCATTTAATGATGGTGTTT	
N-F3	GCCAAAAGGCTTCTACGCA	73
N-B3	TTGCTCTCAAGCTGGTTCAA	
N-FIP	TCCCCTACTGCTGCCTGGAGGCAGTCAAGCCTCTTCTCG	
N-BIP	TCTCCTGCTAGAATGGCTGGCATCTGTCAAGCAGCAGCAAAG	
N-LF	TGTTGCGACTACGTGATGAGGA	
N-LB	ATGGCGGTGATGCTGCTCT	
RDRP-F3	TGCTTCAGTCAGCTGATG	74
RDRP-B3	TTAAATTGTCATCTTCGTCCTT	
RDRP-FIP	TCAGTACTAGTGCCTGTGCCCACAATCGTTTTTAAACGGGT	
RDRP-BIP	TCGTATACAGGGCTTTTGACATCTATCTTGGAAGCGACAACAA	
RDRP-LF	CTGCACTTACACCGCAA	
RDRP-LB	GTAGCTGGTTTTGCTAAATTCC	

ORF1a-A-F3	CTGCACCTCATGGTCATGTT	75
ORF1a-A-B3	AGCTCGTCGCCTAAGTCAA	
ORF1a-A-FIP	GAGGGACAAGGACACCAAGTGTATGGTTGAGCTGGTAGCAGA	
ORF1a-A-BIP	CCAGTGGCTTACCGCAAGGTTTTAGATCGGCGCCGTAAC	
ORF1a-A-LF	CCGTACTGAATGCCTTCGAGT	
ORF1a-A-LB	TTCGTAAGAACGGTAATAAAGGAGC	
NSP3-F3	TCCAGATGAGGATGAAGAAGA	76
NSP3-B3	AGTCTGAACAACTGGTGTAAG	
NSP3-FIP	AGAGCAGCAGAAGTGGCACAGGTGATTGTGAAGAAGAAGAG	
NSP3-BIP	TCAACCTGAAGAAGAGCAAGAACTGATTGTCCTCACTGCC	
NSP3-LF	CTCATATTGAGTTGATGGCTCA	
NSP3-LB	ACAAACTGTTGGTCAACAAGAC	

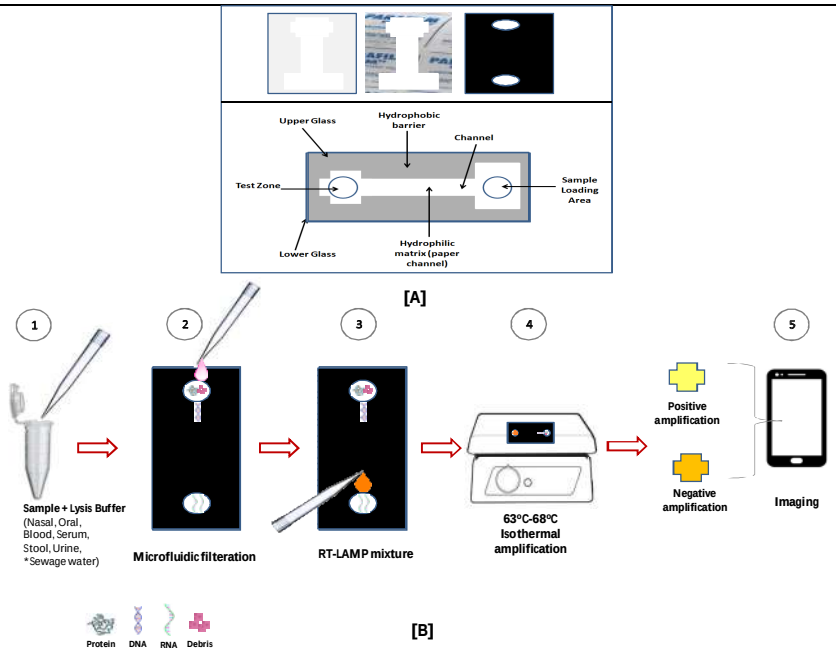

Fig. 1. (A) The prototype strategy for paper-based microfluidic LAMP assay. The device has consisted of a central paper channel (defined area of a hydrophilic matrix) that directs the sample from the sample loading area to the separate test zone area. (B) Step 1; the lysed sample is pipetted to the loading area of the device that spontaneously flows through the channel via capillary action. Step 2; biomolecules and contaminants like proteins, cell debris, and DNA in the sample are filtered during the process (due to large size and increased retention time), while target RNA, due to its small size will flow further (pushed forward). Step 3; LAMP reaction mixture is pipetted to the test zone of the microfluidic device. Step 4; to achieve amplification, the device is placed on a hot plate at 65°-68°C for 30-40 minutes. Step 5; visual inspection of color change from orange to yellow is used to identify positive amplification. During and after amplification, as shown in step 5, the images of the paper microfluidic device can be captured using simple optics like a smartphone camera. The intensities of the colour change from the control experiment are recorded. Nuclease-free water is taken for no target control.

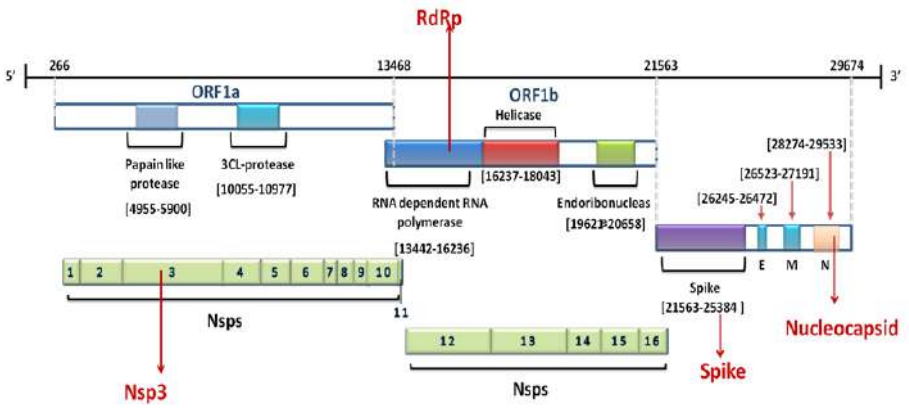

Fig. 2. Genome organization of SARS-CoV-2 and selection of primers. LAMP primer position of Nsp3 (target region ORF1a), RdRp (target region ORF1b), spike and nucleocapsid regions have been shown. The *orf1ab* gene encodes 16 non-structural proteins (NSPs) (shown in green). The other part of the SARS CoV-2 genome encodes spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins.

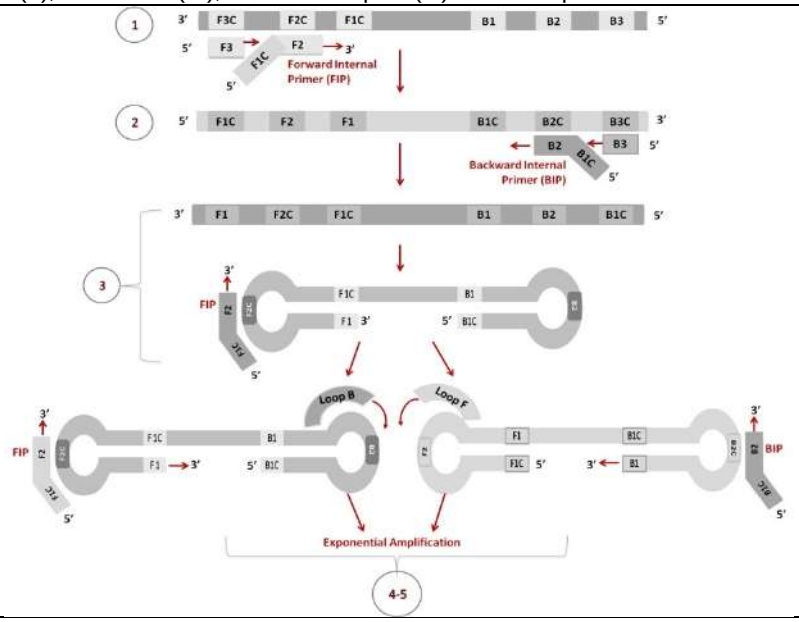

Fig. 3. Loop-mediated isothermal amplification (LAMP) mechanism.

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RESEARCH ARTICLE

Attitude Regarding Ill Effect of Mass Media among School Student

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ABSTRACT

A descriptive research design with cross sectional survey approach was used to assess the attitude of adolescent regarding ill effect of mass media among selected school in Veerapandi, Salem. Data were collected from 50 adolescent and data obtained was analyzed by using descriptive and inferential statistics. Highest percentage (84%) belonged to age group of 15-16 years. Majority (64%) of the people are males. Highest percentage (76%) of the people belongs to Hindus. Majority (76%) of them were from rural area. Most (50%) of the people were from nuclear family Highest percentage (50%) of them were from daily wager. Majority (64%) of the peoples are middle class family. Overall analysis attitude of adolescent regarding ill effect of mass media shows that high percentage (60%) of adolescent affecting by mass media so that adolescent attitude change ,(30%) of adolescent moderately affected due to mass media,(10%) of adolescent are slightly affected by mass media. Hence it is interpreted that Majority (60%) of the adolescents affecting by mass media and (10%) of them changing behavior due to mass media.

Keywords: Adolescents, Mass Media and Ill-effect.

INTRODUCTION

Adolescents are usually dealing with pre-pubertal and pubertal changes, which make them more aware of their own bodies and feelings, this is linked with a natural curiosity and television is a common media mode and research indicates approximately 83% of programming contains sex, cigarette smoking, alcohol consumption, crime, which leads to many discussions with their peers, can promote the belief among them that early smoking, alcohol, sex makes a person "look like an adult". In the 21st century technology has expanded the availability of information through various routes such as television, music, movies, internet and magazines, these routes avail the adolescents to endless learning venues about any issues that might be of interest to them. The role of mass-media in affecting knowledge, attitude and behavior towards health care may be thought of in terms of the following discussion. The mass media acts either as a "change agent "or as a "reinforcing agent "that is, media may function in such a way as

to change knowledge, attitude and behavior or to confirm existing behavioral pattern. In these respects the role of mass media in affecting knowledge, attitude and behavior towards other products and services.

Need for study

Mass media violence with a variety of physical, mental, social health children and adolescents including aggressive behavior, desensitization, to violence, fear, depression, nightmares, and sleep disturbance more than 3500 research studies examined. Children are influenced by media they learn by observing, initiating and making behaviors their own. Aggressive attitude and behaviors are learned by imitating observed models. Children younger than 8 years cannot discriminate between fantasy and reality. They are unequally Vulnerable to learning and adopting as reality circumstances, attitudes, and behaviors portrayed by entertainment media. Mass media initiation of sexual intercourse by younger adolescents is associated with risky sexual behaviours and increased risk of multiple sex partners, unwanted pregnancy, sexually transmitted infection. In the United States approximately 47% of high school students have had sexual intercourse, of them 7.4% report having sex before the age of 13 and 14% have had more than 4 sexual partners. Adolescents exposure to sexual content in the mass media, in India there are reports of messaging of sexual content through mobiles among school going adolescents.

Statement of the Problem

A descriptive study to assess the attitude of adolescent behavior regarding ill-effects of mass media among students in selected high schools

Objectives of the Study

To assess the attitude on ill-effects of mass media on adolescents behavior.

Research Design and Approach

A descriptive study design with cross-sectional survey approach was used to conduct the present study.

Setting of the Study

The study was conducted in a Government higher secondary school Veerapandi village, Salem. This place was approximately 2.5 kilometers from VMACON, Salem.

Population

The population was those who are adolescent (12-18 years) in government higher secondary school, Veerapandi, Salem.

Sampling Technique

Purposive sampling technique was used for the present study.

Sample size

The sample size consists of 50 adolescents who were studying in government higher secondary school, Veerapandi, Salem.

Development of Tool

The tool consists of items regarding the demographic data and adolescent attitude regarding ill effect of mass media among students in selected school in Veerapandi, Salem by used closed ended questionnaire.

RESULT AND DISCUSSION

Area wise analysis of adolescent regarding ill effect of mass media according to their behaviour problem

Overall analysis attitude of adolescent regarding ill effect of mass media shows that high percentage (60%) of adolescent affecting by mass media so that adolescent attitude change, (30%) of adolescent moderately affected due to mass media, (10%) of adolescent are slightly affected by mass media. Item wise analysis of problems of adolescent regarding ill effect of mass media shows that highest percentage (70%) of adolescent correctly responded for the item Are you develop any abnormal behaviour by reading fake news,(60%) of adolescent correctly responded for the item Is mass media affecting your health,(68%)of adolescent correctly responded for the item you think spend more times for mass media,(66%) of adolescent correctly responded for the item is mass media causing any emotional disturbances, (50%) of adolescent correctly responded for the item is mass media affecting your academic performance. (54%) of adolescent correctly responded for the item are you live without phone, (56%) of adolescent correctly responded for the item is internet is misguiding you in a wrong direction,(54%) of adolescent correctly responded for the item are you learning any bad habits like smoking, alcoholism, very lowest percentage (40%) of adolescent correctly responded for the item is mass media inducing you to do anti social activity , however (56%) of adolescent correctly responded for the item is mass media provoking any sexual behaviour.

CONCLUSION

A descriptive research design with cross sectional survey approach was used to, assess the attitude of adolescent regarding ill effect of mass media among selected school in Veerapandi, Salem. 50 adolescent were selected by purposive sampling technique and data was collected by using Questionnaire method and the result reveals the highest percentage (60%) of the adolescents affecting by mass media and lowest percentage (10%) of them changing behavior due to mass media.

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Table 1.Item wise analysis of adolescent regarding ill effect of mass media according to their behaviour problem.

Item	No of responders	percentage
Are you develop any abnormal behaviour by reading fake news	35	70%
Is mass media affecting your health	30	60%
You think spend more times for mass media	34	68%
Is mass media causing any emotional disturbances	33	66%
Is mass media affecting your academic performance	25	50%
Are you live without phone	27	54%
Is internet is misguiding you in a wrong direction	28	56%
Are you learning any bad habits like smoking, alcoholism etc…	27	54%
Is mass media inducing you to do anti social activities	20	40%
Is mass media provoking any sexual behaviour	28	56%

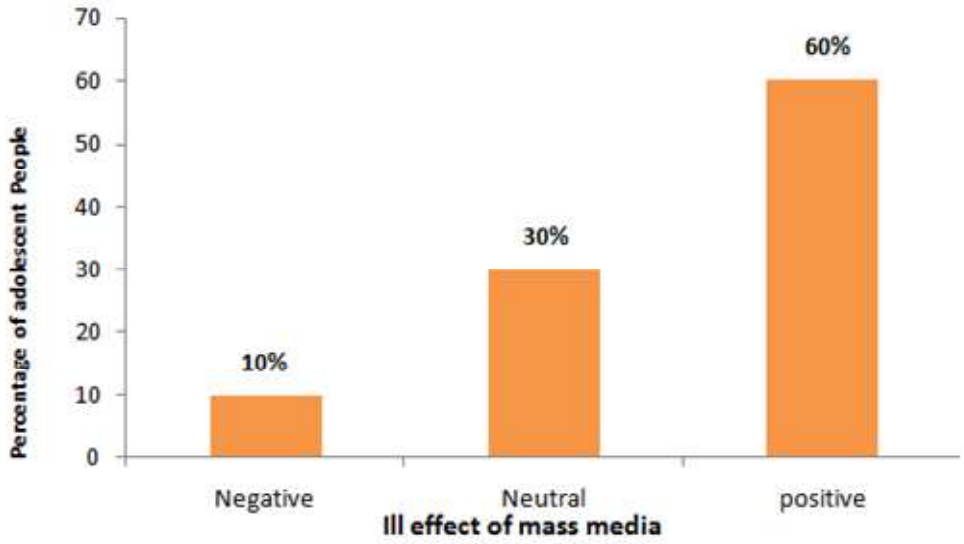

Fig.1. Bar diagram showing overall percentage wise distribution of adolescent attitude regarding ill effect of mass media.