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RESEARCH ARTICLE

Artificial Neural Network Techniques for Automated Land Use/Land Cover Change Detection in Multispectral Satellite Time Series Imagery for the Long Term Urban Growth Predication of Salem City, Tamil Nadu

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ABSTRACT

Salem city is rapidly growing for the past three decades due to several factors such as income growth, population growth, the establishment of numerous education institutions in and around the urban areas, and migration of people from rural areas to the urban. This rapid urban growth usually occurs at the expense of agricultural lands, with the destruction of the natural landscape and public open spaces, leading to problems such as environmental degradation and depletion of natural resources due to improper urban planning and urban management activities. Urban planners, resource managers, economists, and urban ecologists require information on the magnitude, areal extent, and the spatial and temporal pattern of the urban expansion to ensure basic amenities. There is a need for assessing and monitoring urban expansion by using Artificial Neural Network techniques in multispectral satellite time series imagery that can provide solutions to address the problems of the urban expansion**.**

Keywords; Salem city, Urban growth,Urban planning, Urban management, Artificial Neural Network, Satellite imagery.

INTRODUCTION

Image classification remains one of the most difficult problems in machine learning, even today Kang, (2015). Vast efforts have been directed toward building neural network models and exploring their utility in classifying remotely sensed data, particularly its performance relative to conventional parametric classifiers Gao, (2009). Recently, deep

learning has become the new state-of-the-art solution for image processing and has been intensively used in several distinct tasks of different domains, including remote sensing Khelifa Djerriri et al., (2017). In the sub-field of data classification, neural-network methods have been found to be useful alternatives to statistical techniques such as those which involve regression analysis or probability density estimation Holmström et al., (1992). Artificial Intelligence approaches have been receiving favorable reviews for classification of hyperspectral data because the complexity of such data challenge the limitations of many conventional methods Manibhushanet al., (2011). So far ANNs have proved to be a viable alternative for automatically mapping land covers on the global scale because of the improved accuracy and their ability to provide additional information on uncertainty Gopal et al., (1999).

The feedforward, backpropagation algorithm is the most widely applied neural networks algorithms in image classification. The term "feedforward" indicates that the network has links that extend only one direction. Except during training, there are no backward links in a feedforward network; all links proceed from input nodes toward output nodes Erzs´ebetMer´enyi et.al., (2011) considered ANNs that can handle a large number of data sets and discriminate classes with subtle spectral variations. Therefore, they took hyperspectral AVIRIS data to delineate 23 lands covers using many classification methods such as the Maximum Likelihood, Mahalonobis Distance, Minimum Distance, Spectral Angle Mapper, and a hybrid ANN classifier and showed that the ANN outperformed the other methods and achieved more or less 90% accuracy of test data.Fabio Pacifi et al., (2009) used very high resolution panchromatic images of Quick Bird and World View-1 to accurately classify the land use of four urban including Las Vegas (USA), Rome (Italy), San Francisco (USA) using neural networks and made it possible to differentiate roads, highways, parking lots, residential houses, apartments, and towers with the kappa coefficient of 0.90 attributing to classification accuracy. An increase in the classification accuracy depends on the spatial resolution of the images. For instance, trees can be extracted from the fine resolution satellite images such as Quick Bird and Ikonos.

Venkatesh and Kumar Raja, (2002) took advantage of the multilayer Perception (MLP) to classify multispectral satellite data by training the neural network using both the ground truth and the output of a K-means clustering algorithm and distinguish vegetation, highways, rocky terrain, mountains, and water bodies with an accuracy of more than 99%. They compared the MLP outputs obtained from the two experiments with the results of the K-means algorithm for accuracy assessment. From the first experiment, they found that the maximum classification accuracy could be achieved with two or more hidden neurons. Finally, they compared their classification results of the second experiment with the results given by Bischof et al.,1992 and found that their results yielded 99.34% in terms of classification accuracy. Deilmaiet al., (2014) proposed a pre-trained Convolutional Neural Network (CNN) approach to extract urban areas from the 10 m spatial resolution Sentinel-2 images of Pooh Delta area in Italy and obtained results with a Kappa index more than 0.74,From the results, the authors concluded that the CNN outperformed the other classification methods.

Xuefei Hu and QihaoWeng, (2009) applied Self-Organizing Map (SOM) and Multilayer Perceptron (MLP) to the three ASTER images covering Marion County, Indiana, and the United States, which were obtained on April 5, 2004, June 16, 2001, and October 3, 2000. In this study, six impervious surface maps were generated, and an accuracy assessment was carried out for the results derived from SOM and MLP. The accuracy assessment result showed that the SOM produced a slightly better result of impervious surfaces than the MLP. Furthermore, the SOM provided more accurate results in the residential areas, indicating the effectiveness of SOM in the residential areas of mixed pixels when compared to the MLP. For the commercial area, both the SOM and MLP yielded results with very high RMSE values owing to the prevalence of shade, showing incapability of both algorithms in handling the shade problem well. The results corresponding to the rural area showed the lowest RMSE value because of the presence of less mixed pixels and shade. This research concluded that the SOM can be a promising alternative to the MLP neural network and also found the influence of different map sizes on the impervious surface estimation.

Shifali and Gurpreet Kaur, (2016) implemented back propagation feed forward neural network to increase the accuracy of an image following the use of median filter and the Scale Invariant Feature Transformation (SIFT)

algorithms for image segmentation and feature extraction respectively. Then they compared classification results of the neural network with a hybrid algorithm known as Artificial Bee Colony and Fuzzy C-Means(ABC-FCM) and found that the results of ANN were better than that of ABC-FCM, giving 99.91% accuracy. Amin Tayyebi et al.,(2010) chose ANNs, geographical information systems, and remote sensing to create Urban Growth Boundary (UGB) models of Tehran, Iran. They trained ANNs to identify slope, aspect, roads, elevation, green spaces, service stations, and built-up area. The ANNs results were used to build UGB models that could foresee urban growth boundaries with an urban area with an accuracy of 80–84% until the year of 2012.

Profile of the Salem City

Salem city is one of the rapidly growing city in Tamil Nadu in the recent years owing to anthropogenic influences. The city is bounded by the Godumalai hill to the east, the Kanjamalai hill to the west, the Nagaramalai hill to the north, and the Jarugumalai hill to the south. It falls within the Geo-coordinates ranging between 11°35' 50" N to 11°42'10" N and 78°5'0" E to 78° 14'30" E. The city comprises of 60 wards constituting fourzones named as Suramangalam, Hasthampatty, Ammapet and Kondalampatty Zones with an aerial extent of 338.38 Km^2. It experiences temperature ranging from 20° C to 37.9°C and the temperature is usually very high during summer. It receives 363.5mm rainfall as an average annual rainfall. From the 2001 census report, the total population of the Salem city is about 30, 16,346 of which 12, 79,846 are employed and the rest are unemployed. Railway junction is situated at the centerof the city. The city is well connected by road network and has well established connections with the adjacent cities including Bangalore, Chennai, Trichy and Coimbatore through the rail and highway with a road networks of NH47, and NH38.

Artificial Neural Networks

The artificial intelligence (neural networks) is a model that simulates the function of a human brain in decision making process. A neural network consists of features like synapses, neurons, and axons of the brain. Synapses (lines connecting neurons) are meant for input weights, neurons for processing the input data, and the axons for the resultant output. The most widely used ANNs contain three/more layers including an input layer, an output layer, and one or more hidden layers. It requires no a priori knowledge about the spectral characteristics of the study area. Several researchers have applied ANNs to derive information on LU/LC from Landsat imagery and have proven that this technique provides more reliable and accurate results than the other traditional statistically based classification methods. The back propagation learning algorithm has been successfully applied to complex image classification problems.The back propagation rule minimizes the error resulting from the difference between observed and calculated result by adjusting synapse weights according to the observed and calculated value of a processing element. The following steps in the box describe the back propagation algorithm.

Artificial neural networks were first proposed by Warren McCullock and Pitts (1943). They, members of a family of computational architectures were originally inspired by biological nervous systems in the 1940s. In the earlier days, a lot of research was carried out on the networks. As training of the neural networks required more computational power and a long time, the popularity of them started to decline and the research about them slowed down, leading to the negligence of artificial neural networks and development of other machine learning techniques. All the math behind them was not really fig out and there was not a lot of good solutions on how making them work until the 1970s, when the back propagation algorithm was discovered by Paul Webs to train neural networks based on the disparity between the expected and the calculated results. The neural networks were used to analyze multispectral satellite data in the late 1980s. They could not take off due to different computing theories until the advent of the idea of deep learning in 2000s. Back then, neural networks started gaining popularity when Google and Facebook adopted the networks to process a huge amount of data gathered from every day user to target suitable ads to customers.

Step 1:Assign all network inputs and outputs
Step 2:Initialize all weights with small random numbers, typically between -1 and 1
Step 3:For every node in the hidden layer of the network, calculate the sum of the weighted inputs
Step 4:Activate the following signed function (1.1) to transfer the input data into the hidden layer to the output layer.

$$o_i = f(net_i) = \frac{1}{1 + e^{-net_i}} \qquad (1.1)$$

Where

o_i = the output transferred to the corresponding neuron in the output layer

$$net_i = \sum_j \omega_{ij} o_j + bias_i \qquad (1.2)$$

Where

ω_{ij} = the weight associated with the j^{th} input, Oj = the value of the j^{th}input

Step 5:
Calculate the RMS error by using the following equation(1.3):

$$\varepsilon = \frac{1}{k}\sum_{i=1}^{k}(t_i - o_i)^2 \qquad (1.3)$$

Where
i = index of the output nodes of the network,
K = the number of information classes to be mapped
Step 6:If the RMS error exceeds the desirable limit, update each node's weight in the hidden layer
Step 7:Repeat the same procedure from step-1 to step-6, but with the updated weights
Step 8:Calculate the RMS error
Step 9:If the error is within the permissible limit, end the ANNs training and classify the input Landsat image. If not, repeat the same procedure described above until RMS error becomes allowable.

Artificial neural networks based image classification is one of the most advanced classification methods. A feed-forward multi-layer perception has been confide and trained with back-propagation learning algorithm.The back propagation rule is used to minimize the error resulting from the difference between observed and calculated result by adjusting synapse weights according to the amount of the error. Neural networks are useful for solving image classification problems. In addition to the many advantages of neural networks, one of some drawbacks is taking a long time during training when they are run on older computers. Another drawback is determining the number of hidden layers, right number of nodes in hidden layers, and learning rate.

METHODOLOGY

The first four principal component images were fed as inputs into the MLP (fig 1) module in IDIRISI Selva,and then training site files containing pixels of seven LU/LCs were given as inputs for the networks, training and testing procedures. The training parameters were assigned automatically by the module. The number of nodes selected during the training and testing processes ranged from 50 to 50, after successful completion of the training and testing processes, the principal component images were subjected to classification processes.

Topology

The Multilayer Perception (MLP) neural networks consist of an input layer, one or more hidden layers, and an output layer. The number of neurons in the input and output layers is determined by input bands and number of LU/LC classes respectively, while there is no rule to determine the number of neurons in the hidden layers. A large

number of neurons in the hidden layers can make the neural network generalize more complex data patterns. Generally, smaller networks tend to have higher generalization capabilities and require less training time. Artificial neural networks were trained using various numbers of hidden layer nodes to achieve maximum accuracy of training and testing. The input and output layer nodes are equivalent to the number of input bands and the number of land use and land cover classes respectively. Hence, the number of nodes of these two layers remained unchanged during the training and testing processes. But the number of hidden layer nodes was changed in order to train the network as accurate as possible. The layer information and the overall accuracy of the network have been provided in the table 1. The structures of the networks that trained to classify the principal component images of 1990,1995,2000,2005,2010, and 2015 have been drawn and shown in fig. 2.All the network structures in this study used automatic training and dynamic learning rate with the momentum factor of 0.5.

ANNs Training Data

The raster images fig. 3 of training sites used in ANNs training and testing and MLP module snapshots fig. 4 showing details about training parameters, RMS errors of training and testing, iterations, number of layers and nodes used, and overall accuracy of the network training.Training sites for all the year, including 1990, 1995, 2000, 2005, 2010, and 2015 were created with the aid of false color composite images of original bands and the principal components and google earth images for input into an MLP module to train and test the artificial neural networks as the first step of the ANNs classification process. The number of pixels assigned to each training site has beenshown in table. 2and in column graphs fig. 5 for the six time periods.

During the preparation of the training data set for the year 1990, road network was assigned 2542 training pixels. Urban land was provided 3111 pixels. The land cover, vegetation was shown to the network using 1973 pixels. Water bodies were included in the training data set using 479 pixels. Fallow land, mines, and barren land were given 755, 6647, and 7911 pixels.During the training process of the ANNs for classifying the Landsat image of 1995, 388 pixels were added to the training data set under the category of road, 1381 pixels to urban class, 1727 pixels to vegetation class, 528 pixels to water bodies, 1402 pixels to fallow land, 4097 pixelsto mines, and 4146 pixels to barren land. Each training site was allocated the number of pixels ranging between 3531 and 859 for classifying the Landsat image of the year 2000. 976 pixels were set aside to the training site, road, 2816 pixels to urban, 2986 pixels to vegetation, 859 pixels to water bodies, 1450 pixels to fallow land, 3531 pixels to mines, and 2496 pixels to barren land. From the Table.2, it can be seen that the number of pixels included in each training site of 2005 varies from 1885 to 667. Mines show the highest number of training pixels (1885), followed by the water bodies (1859). Barren land consists of the lowest number of training pixels (667). The Road network has been given 888 training pixels and urban has been assigned 1606 training pixels. Vegetation and fallow land reserve 1175 and 787 training pixels respectively.

For training and testing the ANNs, 480 pixels were assigned to the road network, 815 pixels to urban, 1351 pixels to vegetation, 459 pixels to water bodies, 922 pixels to fallow land, 7115 pixels to mines, and 3419 pixels to barren land. A wide range of pixels was assigned to training sites of 2015, ranging from 1029 to 282. The vegetation, land cover has 1029 pixels as the maximum number of pixels, followed by urban having 872 pixels. Barren land shows 282 pixels as the least number of pixels because of its last occurrence in the study area. The training site, road has been assigned 745 pixels, water bodies 366, fallow land 628, and mines 530.

RESULTS AND DISCUSSION

Result and Discussion of Anns Classification

The finial output of the ANNs LU/LC are given in the fig 6 and table3 , it reveals that in the year *1990* the road network covers 26.69 km^2 (7.88%), urban land 68.13 km^2 (20.13%), vegetation 63.50 km^2 (18.76%), water bodies 0.0046 km^2 (0.00%), fallow land 22.66 km^2 (6.75%), mines 29.96 km^2 (8.85%), and barren land. In the year 1995 road covers

7.47 km^2 (2.21%), urban 6.58 km^2 (1.95%), vegetation 82.30 km^2 as the second largest land cover (24.32%), water bodies 2.79 km^2 (0.83%), fallow land 33.91 km^2 (0.83%), mines 22.94 (6.78%), and barren land 182.38 as the first largest land cover (53.90%).and 12.In the year 2000 theroad covers 2.92km^2 (0.86%), urban 65.04 km^2 (19.22%), vegetation 117.03 km^2 as the first largest land cover (34.59%), water bodies 2.42km^2 (0.72%), fallow land 64.32 km^2 (19.01%), mines 20.70 (6.12%), and barren land 65.95 as the second largest land cover (19.49%),7.25 km^2 (37.60%), occupying the largest portion of the study area.In the year 2005it is obvious that road covers 90 km^2 as the second largest land cover (26.60%), urban 31.06 km^2 (9.18%), vegetation 105.68 km^2 as the first largest land cover (31.23%), water bodies 5.08 km^2 (1.50%), fallow land 72.72 km^2 (21.49%), mines 16.39 (4.84%), and barren land 17.45 (5.16%).In the year 2010 indicates that road covers 5.22 km^2 (1.54%), urban 9.68 km^2 (2.86%), vegetation 75.78 km^2 (22.39%), water bodies 2.44 km^2 (0.72%), fallow land 25.36 km^2 (7.49%), mines 70.55 (21.28%), and barren land 149.36 (45.07%), covering the largest portion of the study area.In the year 2015, shows that road reserves 47 km^2 (13.89%), urban 52 km^2 (15.37%), vegetation 116.9 km^2 (34.55%) covering the largest portion of the study area, water bodies 9.52 km^2 (2.81%), fallow land 72.3 km^2 (21.37%), mines 10.22 (3.02%), and barren land 30.44 (9.00%).

CONCLUSION

LU/LC Change detection analysis has been performed after the classification evaluation procedure.The change detection analysis on ANNs classified LU/LC images between 1990 and 2015 showed a dramatic increase in the extent of road (20.31 km^2), an unusual decrease in vegetation land(11.5 km^2), an increase in urban land (48.77 km^2), water bodies (9.52km^2), and fallow land (49.44 km^2) as well as a decrease in mines (19.74 km^2) and barren land (96.81 km^2). LU/LC changes were detected successfully using Landsat images for the years of 1990,1995,2000,2005, and 2015. Particularly, the urban area has increased by 48.77 km^{2}during the years between 1990 and 2015. This increase in the urban area is due to construction of new buildings on vegetation and barren lands. As the result, the vegetation has decreased by 11.5 km^2 during the 25-year time period. This paper shows a scientific way to understand the urban growth and also provides a less expensive methodology for assessing urban land use change effectively in a short period of time. It is also useful for decision making process and helpful for planners and authorities to formulate a suitable plan for sustainable urban development in the study area.

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Table 1. Number of Neurons/Nodes Used in Classification and the Resultant Accuracy of ANNs

YEAR	Input Layer Nodes	Hidden Layer Nodes	Output Layer Nodes	Overall Accuracy in %
1990	4	50	7	84.90
1995	4	14	7	90.93
2000	4	6	7	80.03
2005	4	8	7	90.88
2010	4	5	7	91.01
2015	4	5	7	88.06

Table 2. Training Pixel Statistics of LU/LC Training Sites for the six Time Periods

Training Sites/year	1990	1995	2000	2005	2010	2015
Road	2542	388	976	888	480	745
Urban	3111	1381	2816	1606	815	872
Vegetation	1973	1727	2982	1175	1351	1029
Water bodies	479	518	859	1859	459	366
Fallow land	755	1402	1450	787	922	628
Mines	6647	4097	3531	1885	7115	530
Barren land	7911	4146	2496	667	3419	282

Table 3. ANNs Classification's Land Use/ Land Cover Status of the Salem Urban in the Years between 1990 and 2015

LU/LC Class	LU/LC-1990	LU/LC-1995	LU/LC-2000	LU/LC-2005	LU/LC-2010	LU/LC-2015	Change from 1990 to 2015 in km²
Road	26.69	7.47	2.92	90	5.22	47	▲20.31
Urban	68.13	6.58	65.04	31.06	9.68	116.9	▲48.77
Vegetation	63.50	82.30	117.03	105.68	75.78	52	▼11.5
Water Bodies	0.004	2.79	2.42	5.08	2.44	9.52	▲9.52
Fallow Land	22.86	33.91	64.32	72.72	25.36	72.3	▲49.44
Mines	29.96	22.94	20.70	16.39	70.55	10.22	▼19.74
Barren Land	127.25	182.38	65.95	17.45	149.36	30.44	▼96.81
Total	338.38	338.38	338.38	338.38	338.38	338.38	

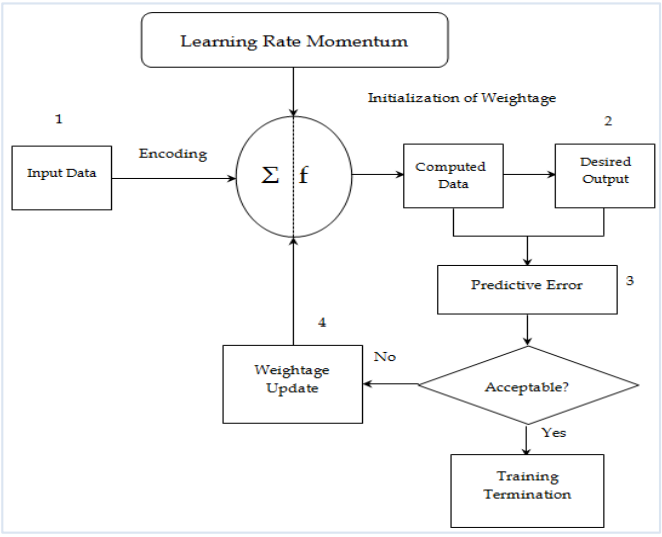
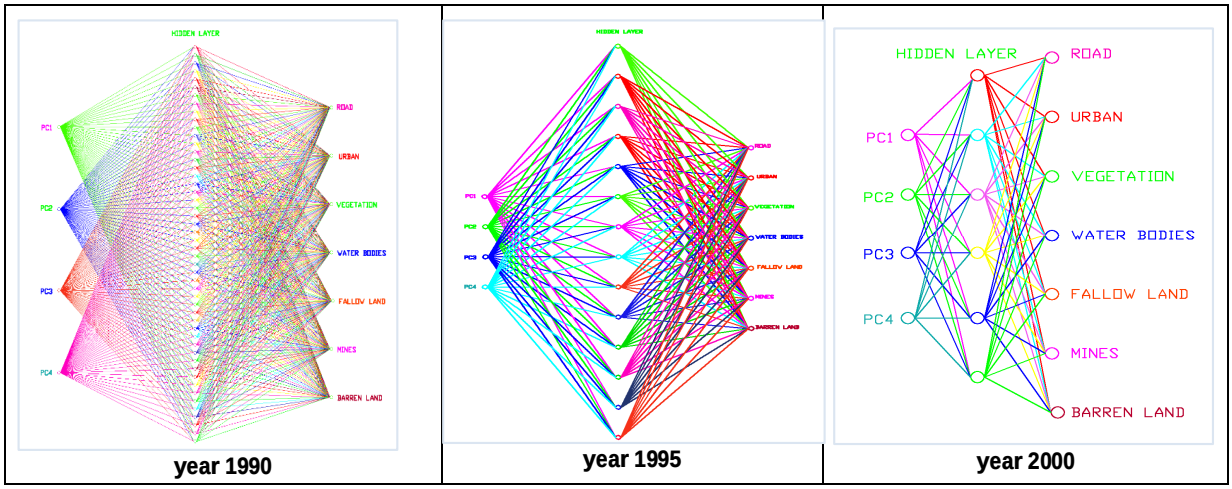
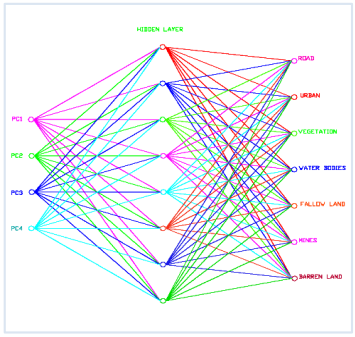


Fig.1. Flowchartof MLP-Back propagation network before the classification



year 1990 year 1995 year 2000



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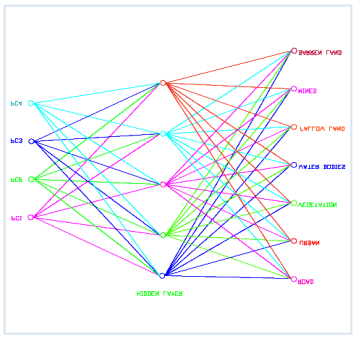


years 2010 and 2015

Fig. 2. The structures of the ANNs used to classify Landsat images from the year 1990 to 2015

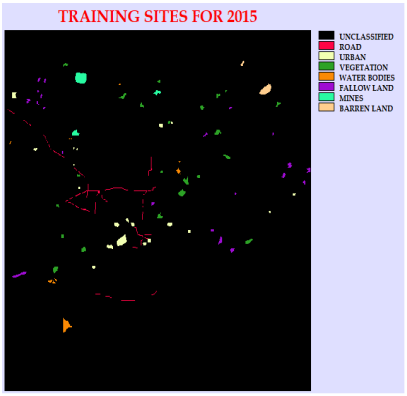


Fig.3 Training sites of LU/LC in the year 2015

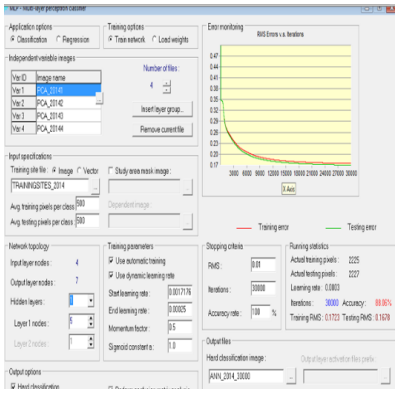
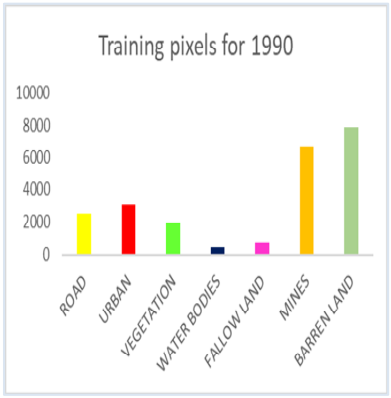
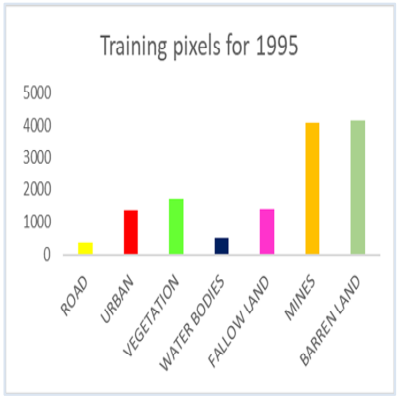
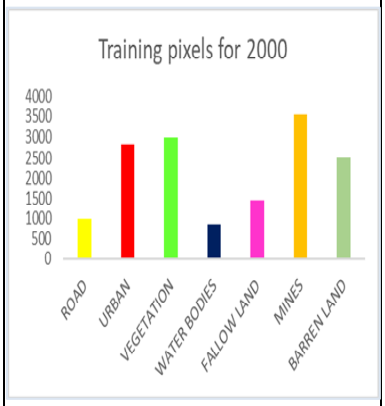


Fig. 4. A snapshot showing, training parameters and the accuracy during the image classification in the year 2015







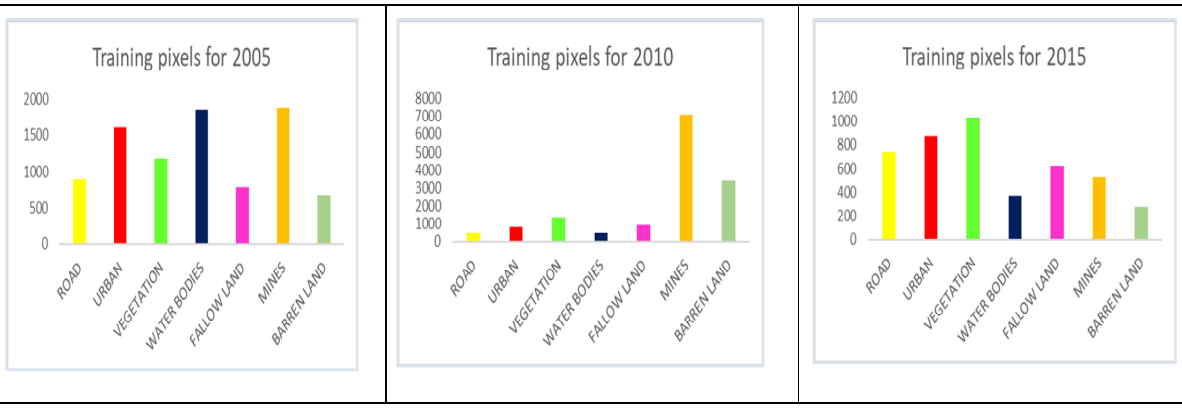
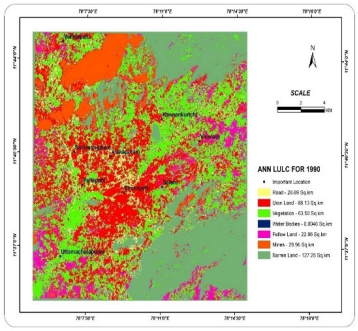


Fig.5 Column graph of training pixels of LU/LC in the year 1990 to 2015



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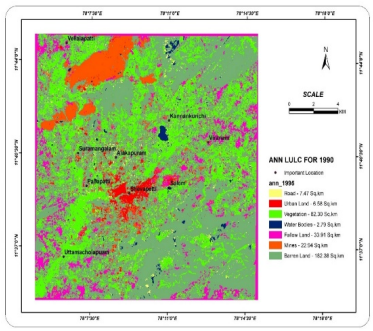

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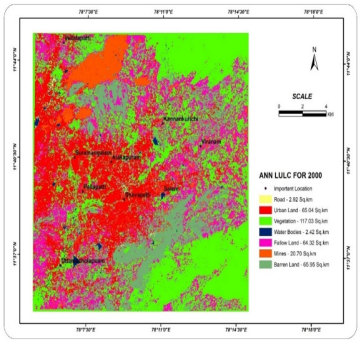

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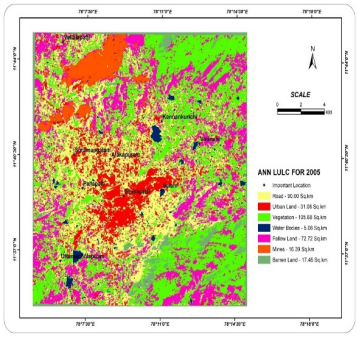

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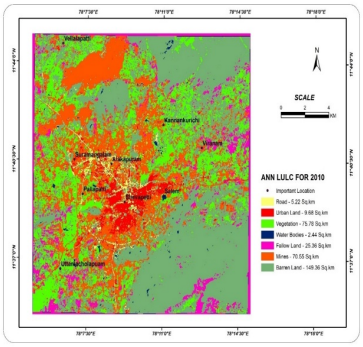

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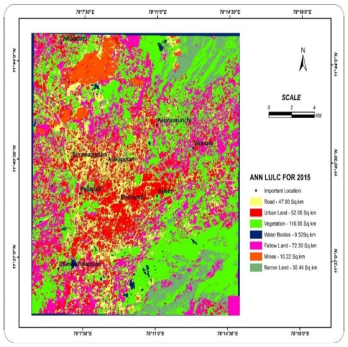

year 2015

Fig 6. LU/LC changes in the ANNs classifications from the year 1990 to 2015

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RESEARCH ARTICLE

Environmental Threat Analysis of Aquatic Toxicity in Underground Water, Dindigul District

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ABSTRACT

The paper presents the results of studying ground water quality plays a great role, where enormous population is dependent on ground water for utilization and other house hold uses. It possesses direct impacts on the health of the consumers including humans. The quality of ground water is mainly directed by the two factors, one is geogenic, while the other is man-made activity factor. The recent trend of excessive extraction of ground water for agricultural, industrial and domestic utilities is liable for the degradation of the quality of ground water. In light of these facts, ground water samples were collected at in and around kullanampatty- subrampattarai and malapatti pirivu, bharathipuram dindigul district and was assessed in terms of physico chemical parameters such as turbidity, pH, hardness, electrical conductivity, alkalinity, total dissolved solids, chloride, fluoride, nitrate, sulphate, iron and, arsenic content. Based on the results obtained iron and arsenic content in some locations were found surpassing the permissible limit. The results obtained through the research evoke the focus for a concentrated effort in a healthful approach for improving the ground water quality.

Keywords: Ground water, Physico chemical parameters, Iron, Arsenic health issues.

INTRODUCTION

Recently the available amount of water to agriculture is decreasing worldwide because the rapid population growth and the greater incidence of drought caused by climate change and different human activities [1]. Fresh water has

been steadily decreasing in arid and semiarid regions across the world, as the scarcity of fresh water has forced a greater reliance on saline and brackish water for agricultural irrigation. The two main sources of drinking water are surface water and underground [2]. The increased metals in the receiving water are lethal to the living communities in the aquatic ecosystem and also cause health problem inhuman. The aquatic ecosystems are tarnished by the increased nutrients and metals, water quality is busted and water availability is decreased [3]. Hence the current work is an attempt made to study water quality of ground water of several sites in Dindigul district with a spotlight to heavy metals.

MATERIALS AND METHODOLGY

Several samples of ground water were collected from different locations in dindigul district. The sampling locations are S1-kullanampatty, S2-subrampattarai, S3-malapatti pirivu, and S4- bharathipuram dindigul district. The physico-chemical characteristics were determined using the standard analytical methods [4]. The pH and electrical conductivity were measured by their meters. Total hardness is estimated by EDTA titrimetric methods. Total alkalinity, carbonate and bicarbonate were estimated by standard $AgNO_3$ titration. Nitrate content in ground water was determined electrochemically using EDT direct ion selective electrode methods. Iron was measured using 0.25% orthophenanthroline based on spectroscopic principle. TDS is estimated using conductivity meter and arsenic by field test kit.

RESULT AND DISCUSSION

In general, the quality of ground water of dindigul district is available for various purposes. In some block high concentration of some constituents are reported. The quality of ground water varies from place to place, with the depth of water table and from season to season thus hydrogeochemical investigation of ground water has been carried out to assess the suitability of water for various uses. High concentration of iron and arsenic has been reported from some areas of dindigul district. The results of various physico-chemical parameters like turbidity, pH, electrical conductivity, total dissolved solids, total hardness, alkalinity, fluorides, chlorides, iron, sulphates, nitrates, and arsenic are reported in a Table1. The comparision of various physicochemical parameters with Bureau Indian Standard (BIS) and World Health Organisation (WHO) is presented in Figure 1 and Figure 2. The turbidity of water samples ranged between 0.96-41.24 NTU which is above permissible limit prescribed by WHO and lower than BIS value, the highest value was recorded in and malapattipirivu, dindigul district.

The range of pH of the water samples ranged between 6.00-8.00. The dissolved solids of water samples are ranging from 160-450 mg/L which is under the WHO and BIS permissible limits. The conductivity of water ranges from 0.2-0.72 mS/cm which is under prescribed limit. Alkalinity of the water sample ranged from 160-300 mg/l which is also under BIS and WHO permissible limit. The range of fluoride of the water sample ranged from 0.10-1.50mg/L which is within permissible limit and highest value recorded in malapatti pirivu dindigul district. The chloride of water sample from 15-195mg/L which is also recorded with in prescribed limit governed by BIS and WHO. Total hardness ranged from 75-370 mg/L which is below permissible limit of BIS and WHO.

Iron content of the water sample ranged from 0.2- 1.80 mg/L , in which the highest value was found in subrampattarai, which is 55% higher than the WHO permissible limit and 25 % higher than the BIS standard. The presence of iron makes water unsafe for drinking and other purpose because water containing iron has pale yellow color, unpleasant taste and risk of possible health hazards such as causes conjunctivitis, choroditis, retinitis and siderosis and in some case may also lead to lung cancer development. The sulphate of water sample ranged between 40-58.4 mg/L which is under desirable limit prescribed by BIS and WHO Standard. Nitrates of the water sample ranges between 0.222-10.2 mg/L which is below permissible limit. Arsenic concentration in the water sample ranges between 0.01-0.10 mg/L which is high in all different sites exceeding the permissible limit governed by BIS values.

The highest value was recorded in malapattipirivu, dindigul district which may be due to excessive abstraction of ground water which caused changes in chemical environment leading to appearance in ground water .It is because of accelerated ground water level depletion in the last few years to the extent of 8-10 meter, the complexity of arsenic problem aggravated. Arsenic is a highly toxic metalloid in the environment which is posing worldwide public health problem. Due to its toxicity to living things it is tending towards a global concern with the ever-increasing contamination of water, soil and crops in many regions of the world. These analyses are supported by the presence of arsenic consumption in the underground.

CONCLUSION

The quality of the water in various spots of dindigul district was tested with the help of several phyico chemical parameters. On the basis of findings, it was concluded that most of the parameters such as turbidity, pH, electrical conductivity, total dissolved solids, total hardness, alkalinity, fluorides, chlorides, sulphates, and nitrates are under the prescribed limit of BIS and WHO standard. The iron content and arsenic content in all the locations was found to be slightly higher than the permissible limit of BIS and WHO standard. Among, the four location the maximum value was found in malapatti pirivu. This would make the water unsafe for drinking.

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Table 1. Physico chemical parameters

S.No	Blocks		Turbidity NTU	pH	TDS mg/L	EC mS/cm	Alkalinity mg/L	Chloride mg/L	Fluoride mg/L	Hardness mg/L	Iron mg/L	Nitrate mg/L	Sulphate mg/L	Arsenic mg/L
1	S1	Range	0.96-15.10	6.00-8.00	160-400	0.20-6.3	160-270	15-100	0.10-0.92	120-375	0.20-1.80	0.222-1.02	40-57.60	0.010-0.10
		Mean	6.41	7.42	294	0.46	182	48	0.45	239	0.60	5	29.62	0.033
		SD	2.46	0.56	52	0.08	31	93	0.31	65	0.39	4	13.69	0.030
2	S2	Range	2.18-41.24	6.50-8.00	209-450	0.33-0.72	180-300	6-100	0.20-0.93	120-480	0.30-1.40	5-15	19.20-58.4	0.010-0.10
		Mean	8.32	7.27	300	0.47	237	46	0.53	279	0.75	9	43.44	0.032
		SD	10.01	0.47	78	0.12	46	17	0.20	78	0.37	2	22.13	0.025
3	S3	Range	5-28.10	6.00-8.00	220-280	0.34-0.44	180-250	10-195	0.20-1.50	75-315	0.20-1.50	3-5	40-57.60	0.010-0.10
		Mean	8.49	6.92	246	0.38	223	70	0.53	223	0.62	5	29.62	0.033
		SD	7.24	0.62	24	0.04	26	46	0.33	51	0.46	1	13.69	0.030
4	S4	Range	5-10	6.50-7.50	180-420	0.36-0.42	180-310	25-50	0.20-0.50	150-270	0.30-1.00	5-10.2	40-45	0.010-0.050
		Mean	5.28	7.00	280	0.39	245	49	0.31	223	0.65	8.389	28	0.023
		SD	1.118	0.38	60	0.05	28	6	0.16	43	0.25	2.253	20	0.017

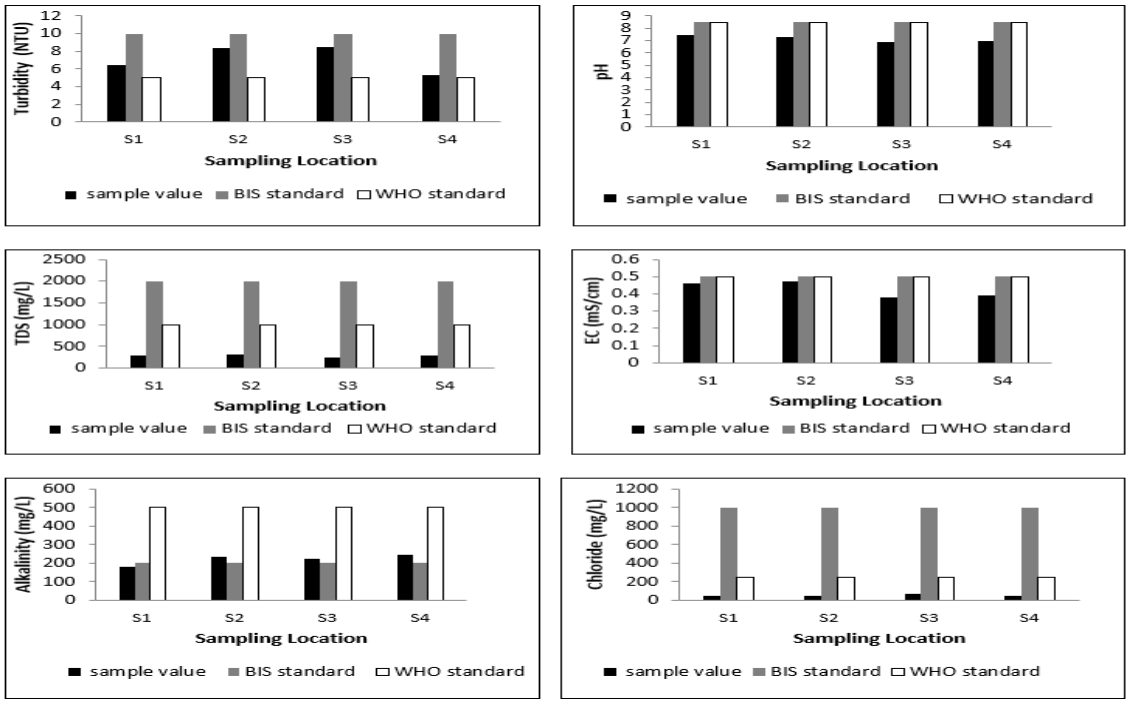

Fig.1. Comparision of physicochemical parameters (Turbidity, pH, TDS, EC, Alkalinity, and Chloride) with BIS standard and WHO standard

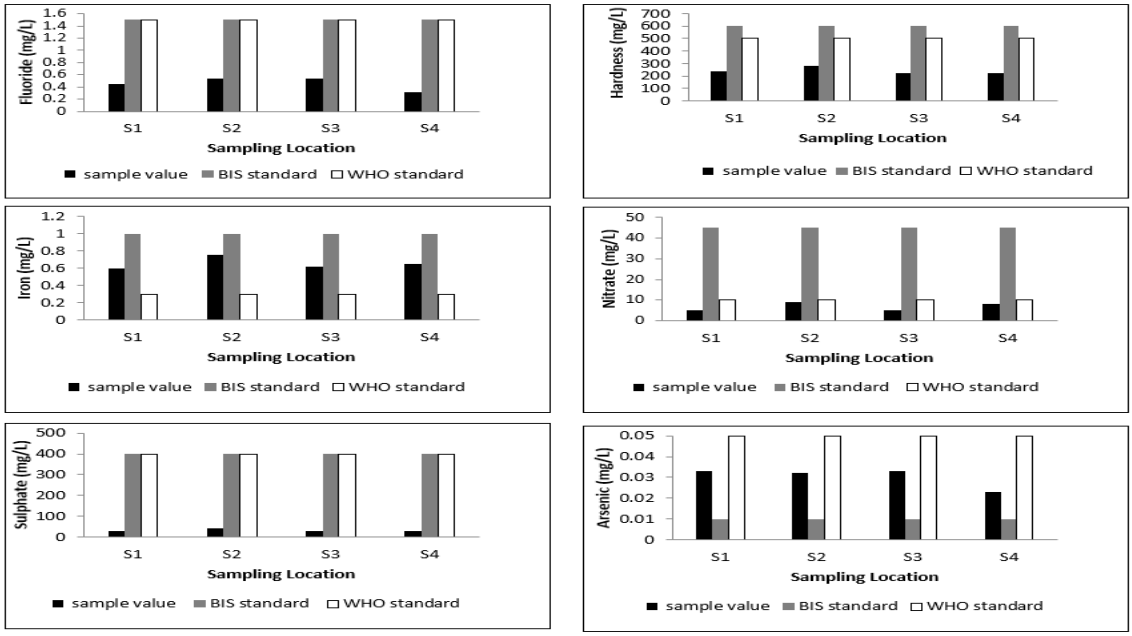

Fig.2. Comparison of physicochemical parameters (Fluoride, Hardness, Iron, Nitrate, Sulphate, and Arsenic) with BIS standard and WHO standard

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RESEARCH ARTICLE

Phytochemical and Pharmacological Importance of *Indigofera tinctoria* Linn

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ABSTRACT

Thousands of years, nature has given many useful medicines to mankind. Many biologically active molecules have been developed from plant. Herbal medicinal preparations are more attractive because having many advantages than modern medicines like less cost, easy availability, minimal side effects etc. Nearly 122 chemical entities are listed as an important drugs having definite pharmacological activities where derived from medicinal plants. One fourth of drugs are derived from medicinal plants and also several others are manufactured analogues frame on preliminary version of compounds isolated from medicinal plants. *Indigofera tinctoria* is one of the important medicinal plants belonging to the family Fabaceae. *Indigofera tinctoria* Linn is an erect medicinal shrub found in most part of the India, China, Thailand, and Sri Lanka. In Indian traditional system of medicine, IT is used to cure bacterial infection, liver protective, rejuvenating agent, antidote, diuretic, nerve disorders and peptic ulcer. The present review will focus on the traditional information, phytochemistry and biological importance of IT. The data has been collected from reputed indexed journal. The papers used for this review have been published earlier to December 2019. Phytochemical investigations clearly evidenced that the plant is a rich source of flavonids, tannins & phenolic compounds, saponin glycosides and alkaloids. Pharmacological activities comprise hepatoprotective, antioxidant activity, antidyslipidemic activity, anticancer activity, effect on immune system, antidiabetic activity, analgesic activity, nephroprotective activity, antibacterial activity, anthelmintic activity and antiulcer effects. The main objective of this review is anticipated to encourage help to forward the research on *Indigofera tinctoria*, and its pharmacological effect to provide a novel or innovative therapeutic agents.

Keywords: *Indigofera tinctoria*, 2, 2-diphenyl-1-picryl-hydrazyl-hydrate, Deoxyribonucleic acid, Ferric reducing antioxidant power, Reactive oxygen species, Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Gamma-Glutamyl Transferase and Lactate dehydrogenase.

INTRODUCTION

In worldwide, medicinal plants are plays the important role in finding a new biologically active molecules to treat various diseases. Nearly four billion of the world population still depends on tradition system of medicine for their primary health care and treatments. Medicinal plants are producing the thousands of active metabolites or ingredients which are having the beneficial effect on biological system. Treatments with medicinal plants are having more merits then allopathic treatment such as low cost, easy availability and lesser side effect. Many decades medicinal plants derived medicaments have been used for the treatment of various illnesses. Many semi-synthetic derivatives are derived from medicinal plants such as teniposide and etoposide are used as anticancer agents from the root of *podophyllum hexandrum*, vincristine & vinblastine from whole plant of *Catharanthus roseus* and many more produces physiological action on the human system.

Traditional and Medicinal uses

Indigofera tinctoria is a medicinal plant which belongs to the family Fabaceae. IT is small erect shrub found throughout India. It is a source of Indigo dye, widely cultivated in northern parts of India especially states like West Bengal, Orissa, Punjab and Bihar. The vernacular names of IT are tabulated in Tab No: I. Plant also cultivated in the countries like China and Japan. The main intention of the cultivation of this plant is for production of Indigo dye. In 1897, interest in cultivation settled due to the invention of artificial indigo dye [1], The common plant morphological characters of IT are reproduced in Tab No: II. Indigo is one of the oldest natural dyes belong to the group of carbonyl dye. It is a derivative of the colourless glucosides of the enol form of indoxyl, e.g. indicant. Indigotin, a colourless glycoside responsible for the blue colour dyes [2]. It is the one of the important drug which plays significant role in Indian traditional system of medicine possessing much medicinal importance. In Ayurveda it is used as stimulant and alterative. In Indian traditional system of medicine the extract of the medicinal plant is used to control the liver and nervous disorders [3]. In the Siddha system of medicine, this medicinal plant is considered to be effective medicament for the treatment of Peptic ulcer [4].

Also used as an antidote due to its bitterness, rejuvenate property due to anti-oxidant activity, diuretic and antiperiodic activity. Root is used to treat sexually transmitted bacterial infections like gonorrhoea, anti-poison and giddiness. Juice of leaves is used for whooping cough [5]. In Unani system of medicine plant has been used as haemostatic, sedative, piles, healer of ulcers, diuretic and dropsy. In Unani medicine, a seed paste with water is applied externally on blemishes, freckles, pimples and other skin disorders [6]. In homoeopathy medicine, IT is authorized medicament for the treatment of epilepsy and other nervous disorders [7]. The juices extracted from the leaves are used to treat hydrophobia [8]. Leave decoction to manage excessive secretion and discharge of mucus. Indigo leaves powder mixed with honey was used for enlargement of liver and spleen. Traditionally dried leaf powder is used to treat asthma [9]. Antiseptic and astringent property of the whole plant is due to the presence of Indigo dye. The root is used to treat liver disorders, snake bites, and urinary troubles. The whole plant is also used to treat and stimulate the hair growth, skin disorders, stomach disease, chronic bronchitis, asthma, ulcers and epilepsy [10, 11].

Chemical constituents of *Indigofera tinctoria*

Preliminary phytochemical screening of the plant extract shows the presence of alkaloid, terpenoids, flavonoid, sugar and tannins [12]. Indirubins is the indole-derived molecules are present in a greater number of indigo-producing plants. Indirubins is the main constituents of IT L. (*Mu Lan*) (Leguminosae) a product from the Chinese Materia Medica [13]. Indigtone is a bioactive pale yellow coloured fraction obtained from the petroleum ether extract of whole plant of IT [14]. The entire plant contains glycoside namely indican, indigotine, indiruben and galactomannan composed of galactose and mannose [8]. Three furano-flavones and one flavonol glycoside are isolated from the aerial parts of IT [15]. Rotenoids are naturally occurring substances and most of them possessing insecticidal activity. Six different rotenoids are isolated from aerial part of the plant such as deguelin, dehydro deguelin, rotenol, rotenone, tephrosin and sumatrol [16]. The presences of phytochemical and different phytoconstituents are shown in Tab No: III. Phenolic and flavonoidal compounds like Myricetin, Gallic acid, Rutin and Caffeic acid are also reported [17]. The methanolic leaf extracts of plant shows the presence of three compounds namely 9,12, octadecadienoic acid, Octadec-9 enoic acid and 9,12 actadecadienoic acid are found by GC-MS [18].

Pharmacological action of *Indigofera tinctoria*

Hepatoprotective activity

Singh et al (2001) was investigated the hepatoprotective activity of Indigtone, a bioactive fraction isolated from petroleum ether extract of the aerial parts of IT. The Indigtone does not cause any mortality even at the higher dose level of 2gm/kg in mice. This revealed that Indigtone is safer at the higher dose level. Pre and post-treatment against carbon tetrachloride induced acute hepatic injury in rats which elevate the serum levels of SGPT, SGGT and bilirubin. Treatment with Indigtone significantly reversed the majority of the serum parameters altered by the carbon tetrachloride on dose dependent manner [14]. Muthulingam et al (2010) evaluated the anti-hepatotoxic efficacy of aqueous extract of IT against paracetamol induced liver damage in rats. It was observed that significant rise in serum levels of AST, ALT, ALP, GGT, LDH, bilirubin and cholesterol and decreases the protein level compared to control which shows clear sign of liver damage in rat. Histopathological examination of paracetamol treated rat liver showed fatty changes, necrosis, vacuoles, space formation and loss of cell boundaries. By oral administration of rats with IT at two different doses for 28 days which brings the elevated serum level in to normal level and increase the protein level and this was further confirmed by histopathological examination of drug treated rat liver [19]. Sreepriya et al (2001) analysed the protective effect of alcoholic extract of IT on D-Galactosamine induced hepatotoxicity in rats. Oxidative stress can induce by treatment with D-Galactosamine and endotxoin which leads to alter the enzymatic level in rat. The pre-treatment with alcoholic extract of IT which develops the hepatic enzymic and nonenzymic antioxidant level and reduce the degree of lipid peroxides in rats [20]. Chitra et al (2003) investigated the anti-hepatoxicity activity of IT on albino rats. Hepatotoxicity induced by tuberculosis drug Isoniazid. Treatment with high doses of IT extract will bring down the normal level of protein, AST, ALT, and ALP. The result clearly demonstrates that the hepatoprotective effect is present in a dose-dependent manner [21].

Anti-oxidant activity

Rashmi Singh et al (2015) compared the presence of tannin and flavonoid content of different extracts and found that hydroethanolic extract hold relatively high quantity of above mentioned phytochemicals. Plant polyphenols possess potent antioxidant properties and having the ability to reduce the DNA damage caused by oxidative stress. Correlation analysis between bioactive constituents and scavenging assays like DPPH and FRAP clearly shows that hydroethanolic extract of whole plant having better antioxidant activity due to the presence of different classes of phytochemicals [22]. Sakthivel Srinivasan et al (2016) was investigated the free radical scavenging activity of IT by in-vitro. The results are clearly showed that aqueous extract of IT are exhibit a very good antioxidant and free radicals scavenging activities NO, O^{2-} and DPPH. HPTLC chromatograms are shown different bands of varying

intensities which evidently point out that presence of various phytochemicals like phenols, flavonoids, saponins and terpenoids. These phytochemicals may responsible for antioxidant and free radicals scavenging activities [23].

Antidyslipidemic activity

Anju puri et al (2007) evaluated the antidyslipidemic activities of alcoholic extract and chloroform fraction of IT. The risk of heart diseases is depending on elevated level of total cholesterol and lower level of high-density lipoprotein cholesterol. High-density lipoprotein cholesterol arbitrates the elimination of reverse transport from the peripheral tissues to the liver by excretion of the bile. This process prevents the slow acquisition of lipids in the arteries, which eventually produce atherosclerosis. In this study antidyslipidemic activity is compared with fenofibrate. The chloroform fraction of IT is lowering the total cholesterol and increasing the level of HDL-C in hamster model similar to fenofibrate [24]. Tadigoppula Narender et al (2006) were isolated three furano-flavones and one flavonol glycoside from the aerial parts of IT. It has been scientifically proven that naturally occurring flavonoids are plays a significant role in reducing the risk of coronary heart disease. Platelets play an important role in the pathogenesis of atherosclerosis and the development of severe thrombotic events. It was already reported that isolated compounds possess inhibitory effect on human platelet aggregation. Platelet aggregation is closely connected with the threat aspect for CVD. The treatment with diastereomeric flavonoid mixture 1 and 2 (80:20) significantly reduced the plasma triglycerides, total cholesterol, glycerol, and free fatty acid. In another side, treatment with flavonoid mixture leads to increase in high density lipoproteins-cholesterol and HDL-C/TC ratio in high fat diet fed dyslipidemic hamsters [25].

Anticancer activity

Paitoon Aobchey et al (2007) was isolated Indirubin from methanolic extract of *Indigofera tinctoria* Linn. Highly purified Indirubin shows better inhibitory effect on human breast cancer cell line (MCF-7 cells). Even 30mM Indirubin retard 42% of cell growth within 24 hours. Extended incubation time and increasing the concentration of Indirubin in higher level which will lead to crystallization, formation of slight needle shape crystals which will affect the inhibitory effect followed by reduce the cytotoxic effect on cancer cell lines. The main mechanism of Indirubin is to inhibit the CDKs and GSK-3 and by interaction with the aryl hydrocarbon receptor which leads to block cancer cell proliferation [26]. Thiruvanmiyoor ravichandran kameswaran et al (2008) was investigated the antiproliferative activity of flavonoidal fraction of IT. Indirubin and quercetin are the major flavonoids present in the plant. The flavonoidal fraction shows the significant effect on human lung adenocarcinoma cells (A-549). The hindrance effect of flavonoidal fraction happened at G1/S transition remarkably increase the number of cells at G1 phase and followed by decrease in S phase. This may be due to the presence of flavonoidal components [15]. Magesh et al explored the antiproliferative efficacy of methanol extract of IT on HCT-116 colon cancer cell line. The various concentration of plant extract shows growth inhibition and induced apoptosis in a dose-dependent manner on HCT-116 cell lines. The HCT-116 cell lines are exposed to methanol extract of IT revealed morphological and biochemical changes. Since apoptosis is characterized by loss of cell viability, chromatin condensation and DNA fragmentation. The induction of apoptosis is may be due to the presence of different classes of phytochemicals [27].

Madakkannu Boothapandi et al analysed the antiproliferative activity of chrysin isolated and purified from chloroform extract of IT on Human epidermoid carcinoma cells. Chemically chysin is 5, 7- dihydroxy flavone. Cells were treated with chrysin inhibited the proliferation of A431 cells with IC_{50}value of 23.52µg/mL. Minimum two hydroxyl position necessary to produce apoptotic activity. Structurally chrysin having two hydroxyl positions 5 and 7 of the ring A. Apoptotic death through caspase dependent manner [28]. Remya Vijayan et al were synthesized silver and gold nanoparticles from leaf extract of IT (AuNP-tinctoria and AgNP-tinctoria) by fast microwave-assisted method. Comparison of cytotoxic effect of leaf and synthesized nanoparticles were studied on lung cancer cell line A549 by MTT assay. Cell viability is directly proportional to the concentration of nanoparticles. IC_{50} values of leaf extracts, AuNP-tinctoria and AgNP-tinctoria (71.92 ± 0.76 µg/ml, 59.33 ± 0.57 µg/ml and 56.62 ± 0.86 µg/ml) are

clearly indicate that AgNP-tinctoria are more cytotoxic against lung cancer cell line than others. The reason behind this is ROS induced by nanoparticles; it is the origin of damages to the cellular component and assists to the cell death. More in spherical shape of AgNP- tinctoria having higher in cytotoxicity [29].

Effect on Immune system

Madakkannu Boothapandi et al evaluated the effect of aqueous extracts of IT on chronic noise induced stress conditions in Wistar albino rats. The steady exposure of noise by human beings can influence the physiological and psychological process leading to many chronic diseases. The study results were well explained that aqueous extract of IT have the potential to prevent the immune irregularity produced by chronic noise stress in Wistar albino rat [30].

Antidiabetic activity

Verma et al check the efficacy of antidiabtic potential of methanolic extract of IT leaf, where diabetic induced by alloxan in rabbits. The results are clearly showing that significant ($p<0.001$) total percentage reduction in glucose level. The blood glucose level is compared between controls, drug and extract treated animal group. The antidiabetic potential of IT may be due to the presence of complex phytochemicals [31].

Analgesic activity

Saravana kumar et al estimated the antinociceptive properties of IT leaves extracts in mice. The ethanolic extract of IT given orally at three different doses of 100, 200 and 400 mg/kg. Treatments with ethanolic extract are shows the positive responses in acetic acid-induced writhing, formalin induced pain and hot plate reaction time in dose-dependent manner. Earlier research reports evidence that secondary metabolites like flavonoids and tannins may be responsible for analgesic activity. Since the preliminary phytochemical screening of IT reveals that presence of those phytochemicals. The antinociceptive properties of IT may be due the presence of above mentioned phytochemicals [32]. Sundaram et al studied the analgesic and behavioural activities of IT in experimental albino rats. Four different potencies (3x, 6x, 12x and 30c) of homeopathic formulations were dispensed by orally for 30 days. The analgesic activity of the homeopathic formulations was evaluated by Hot plate, Ice plate, Randall - Selitto teat and behavioural activity evaluated by Rota - rod and Open - Field test. The results of analgesic activity, drug treated group animals are show the increase the latency times for both thermal noxious stimulus and cold sensation and also increased the quantum of threshold pressure to mechanical induced pain. The results of behavioural activity show the decrease the locomotors and motor coordination is the sign of central nervous system depression. The presence of flavonoids and saponins are responsible for analgesic activity of homoeopathic formulation [33].

Nephroprotective Activity

Priyadarsini et al analysed the nephroprotective activity of siddha preparation avuri kudineer against cisplatin-induced nephropathy in rats. In Siddha, avuri kudineer was prepared with leaves and roots of IT. Cisplatin is a chemotherapeutic agent used to induce the nephrotoxicity in male Wistar rats. Fourteen days of treatment with avuri kudineer lowered the serum creatinine, urea and uric acid. Similarly, the other side of the treatment increases the animal body weight and urine output. Further it was supported that histopathological examination of treated group shows the minimal changes compared with toxic group. Higher doses of avuri kudineer made with both root and leaf exhibit noticeable nephrocurative activity but both preparation (leaf and root & leaf alone) displayed the significant nephroprotective activity. The above data proved that Siddha preparation possessing nephroprotective activity [34].

Antibacterial activity

Magesh Vijayan et al evaluated the antibacterial and mutagenicity of leaves of IT. The methanol extract of IT exhibited powerful activity against various bacterial stains. The minimum inhibitory concentration of methicillin sensitive *Staphylococcus aureus* 0.125 μg/ml, methicillin resistant *S.aureus* 0.25 μg/ml, coagulase negative *S.aureus* 0.25-0.5 μg/ml, vancomycin sensitive *Enterococcus faecalis* 0.5 μg/ml, vancomycin resistant *Enterococcus* species 0.125-0.5 μg/ml, *Moraxella catarrhalis* 2 μg/ml, Streptococcus species 0.125-0.25 μg/ml, *H. influenzae* 2-4 μg/ml and anaerobes is 1-2 μg/ml against standard drugs. The results of Time kill assay of methanol extract of IT exhibits a very good bacteriostatic and nonmutagenic activity. Antibacterial activity of the extracts may be due to the presence of secondary metabolites like alkaloid, flavonoid and saponins and the extraction done with polar solvents like methanol. Methanol having the ability to liberate the more numbers of active components from leaves of IT [35].

Renukadevi et al evaluated the antibacterial activity of IT leaf. The antibacterial evaluation is done by agar well diffusion method with gram positive bacteria like *Bacillus pumilus*, *Staphylococcus aureus* and gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*. Among all the four bacterial stains highest zone of inhibition found in *Staphylococcus aureus* (17mm) and *Bacillus pumilus* (16mm) both are gram positive bacteria. At the same time, both gram negative bacteria do not show the zone of inhibition [18]. Remya Vijayan et al performed green synthesis of silver and gold nanoparticles of IT leaf by fast microwave-assisted method. Agar well diffusion method is used to evaluate the antimicrobial property of synthesized nanoparticles. Different gram negative, gram positive bacterial strains and fungal strains were used to evaluate the potency of leaf extract, AuNP and AgNP-tinctoria. The presence of biologically active secondary metabolites, aqueous leaf extract, synthesized AgNP- tinctoria and AuNP- tinctoria are shows some mild antimicrobial activity [29].

Anthelmintic activity

Ambalathaduvar Meenakshisundaram et al examined the anthelmintic activity of ethanolic extract of IT leaves against gastrointestinal nematodes of sheep by both *in-vitro* and *in-vivo*. Results of *in-vitro* assays shows significant ($p<0.05$) hindrance of egg hatching at two different doses (40 and 80 mg/ml) and also values are demonstrating that increasing the egg hatching is directly proportional to the drug concentration. The efficacy of the anthelmintic drug evaluated by faecal egg count reduction. Ethanolic extract of IT at the dose of 500 mg/kg shows highest percentage of faecal egg count reduction in sheep infected with gastrointestinal nematodes. Mechanism of anthelmintic activity of IT leave is unknown. But this may be due to the presence of complex phytochemicals [36].

Antiulcer activity

Manimekalai et al evaluated the Gastro protective effect of standardized ethanolic leaf extract of IT in Indomethacin and ethanol induced ulcer rats. The results of the experiments explained that oxidative injuries caused by ulcer inducing chemicals. Ethanolic extract of IT having ROS scavenging activity followed by gastric mucosal protection. Flavonids and phenolic compounds are the major constituents in IT. In earlier, many experimental data disclosed that IT is very good anti-oxidizing agent. The above results give more nourish to the ethanomedicinal importance of IT [37]. Pawmitha et al assess the efficacy of root decoction of IT on peptic ulcer by quasi-experimental clinical trial in ten ulcer admissions. Root of IT is highly bitter in taste. In Siddha, the drugs having bitter taste has enabled and improves the digestion. In another side it also minimises the ulcer inflammation. Totally forty days of treatment and patients are made six visits. The data has been collected from the patient in questionnaire format. The results of the study highly encourage the development ($p<0.001$) in ulcer related complications such as heart burn, epigastric pain, indigestion, nausea& vomiting and eructation. These results highly support to the Indian system of medicine [38].

CONCLUSION

Medicinal plants are the influential origin of phytoconstituents and liable for biological activities. Herbal medicinal products are also known as phytopharmaceuticals. Many biologically active phytopharmaceuticals are derived from the medicinal plants to treat various diseases. World Health Organization estimates that nearly 70 to 80% of world population still trust and use the medicinal plants for their primary health care. Many countries are following their own system of medicine. In India, Ayurveda, Siddha, Unani, Homeopathy etc. are the traditional system of medicines and still effectively followed by indigenous people without knowing the phytoconstituents responsible for the pharmacological activity. The unknown value of medicinal plants, many pharmaceutical companies are currently conducting extensive research on plant materials collected from wild and knowledge acquired from traditional healers. The outcome of various experimentation results leads to the conclusion that *Indigofera tinctoria* having many biological activities. Due to their wide range of biological importance, *Indigofera tinctoria* is the key ingredient in the preparation of Indian traditional formulations. In this present review summarizes the knowledge with respect to the traditional uses of phytochemicals, pharmacological activities of crude extracts in addition to pure compounds, evaluation of biologically active compounds, and clinical trials related to the phytochemical and biological importance of *Indigofera tinctoria*. The exact mechanism action of IT is still not clear, but it has been possessed many biological activities. Exploration of the exact mechanisms of action is necessary to be scrutinized for further development and conversion of medicinal plant into therapeutics.

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Table 1. Vernacular names of *Indigofera tinctoria*

S.No	Vernacular names
1.	commonly known as: Bengal indigo, common indigo, Indian indigo, true indigo
2.	Tamil: avuri, nili
3.	Sanskrit: gandhapushpa, maharasa, nilaka. nilini, rangapushpi, ranjani, shyamalika
4.	Telugu: aviri, nilichettu
5.	Malayalam: amari,nilayamari
6.	Kannada: anjooraneeli, hennuneeli, neeligida, olleneeli
7.	Konkani: neeli
8.	Bengali: neel
9.	Hindi: nilika, neel
10.	Odia: nila, rangapushpa,shyamalika
11.	Rajasthani: neel
12.	Marathi: neel,nili
13.	Assamese: neel
14.	Urdu: neel, nilika
15.	Tulu: neeli
16.	Punjabi:lil
17.	Mizo: chi-cha
18.	Manipuri: nim
19.	Tibetan: ni la
20.	Pali: nili
21.	Thai: kram
22.	Chinese: Mu Lan

Table 2. General description of *Indigofera tinctoria*

S.No	General description
1.	Habitat: Plant is an erect medicinal shrub; attain the height of 50-100 cm tall. Found in most parts of the India. Pakistan, Sri Lanka. Cultivated in countries like China and Japan.
2.	Parts Used: Whole plant
3.	Leaves:Leaves are compound, 2.5-11 cm long, with 9-13 leaflets. Leaf-stalk 1.3-2.5 cm; leaflet-stalks about 2 mm; leaflet blades opposite, obovate-oblong to obovate, 1.5-3 x 0.5-1.5 cm, both surfaces with appressed modified trichomes, above sometimes hairless, base broadly wedge-shaped to rounded, tip rounded to notched.
4.	Flowers: Flowers are borne in racemes 2.5-5 cm, laxly flowered; flower-cluster-stalk absent; bracts bristlelike, 1-1.5 mm. Flower-stalks are 4-5 mm, reflexed in fruit. Calyx is about 1.5 mm, with trichomes; teeth triangu-lar, as long as tube. Flowers are red; standard broadly obovate, 4-5 mm, outside with brown trichomes; wings about 4 mm; keel as long as wings. Stamens 4-5 mm; anthers heart-shaped. Ovary hairless.
5.	Pods: Pods are linear, deflexed and straight to semi-circular but never sickle shaped, 2.5-3 cm, hairy or hairless; endocarp purplish red blotched.
6.	Seeds: Seeds are 5-12 per legume, cubic, about 1.5 mm.

Table 3. Phytoconstituents found in *Indigofera tinctoria*

S.No	Classes of Phytochemicals and Phytoconstituents found in *Indigofera tinctoria*
1	Phytochemicals - Flavnoids, Tannins&Phenolic compounds, Steroids, Saponin glycosides, Alkaloids
2	Phytoconstituents - Indirubins, Indigtone, Indican, Indigotine, 5, 7-Dihydroxylflavone, Deguelin, Dehydrodeguelin, Rotenol, Rotenone, Tephrosin, Sumatrol. 9,12, Octadecadienoic acid, Octadec-9 enoic acid, 9,12 actadecadienoic acid Myricetin, Gallic acid, Rutin and Caffeic acid

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RESEARCH ARTICLE

Tannery Pollution and its Effect on the People's Life in Dindigul District

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ABSTRACT

During the tanning operation direct contact with chemicals can cause disability, allergy, asthma, various skin diseases and permanent illness and even death. Chromium is a controversial on account of the persistent and potentially toxicity of some of its chemical forms. The health status of the tannery workers was carried out by clinical laboratory findings involving the haematological and bio-chemical analysis of blood specimen from the tannery workers and analyzed in detail.

Key words: TLC, DLC, ESR, Biochemical parameters.

INTRODUCTION

Health is a multi-dimensional term. When an individual's health fails it is not only due to genetic or biological reasons, but his environment. Man is shown to be the vital factor of his own environment.Immune system is structurally and functionally complex. Tannery waste water pollution causes a serious health hazard to man. The occupational health hazards caused in the long run are even more jeopardizing. The health hazards among tannery workers are intestinal, respiratory and neurological disorders. Concentration of gases is poisonous and a great threat to the health of the workers. Spillage of chemicals on the workers body occurs during the transfer of materials between different operations. Improper drainage of chemicals from the pits, flood the floor with corrosive chemicals. Slipperiness due to wet and greasy floor is a potential danger to the general movement of workers. Toxic chemicals cause chronic ailments. The heavy metal exposure causes a variety of complications and serious health hazards, like contract dermatitis. The well water near the tannery industry and the sewage nearby causes gastroenteritis, amoebiosis, giardiasis, T.B and other dysentery associated diseases. Lower respiratory diseases are more common among the industrial workers. Long term exposure to heavy metals leads to liver cancer, eyelid ulceration and

asthma among the workers.Decreases in leucocytes counts may be caused by overwhelming inflammation, peripheral leukocyte destruction, bone marrow toxicity, loss of lymph, and stress. Factors such as age, gender [1], ethnicity [2] and environment including altitude and geo-chemicals [3] affect the measurements determined in different populations. A detailed study on the impact of tannery environment on occupational health status of the tannery workers working in Dindigul is necessary for the health department to draft future plan to prevent the occupational health risks and also to improve the health status of the tannery workers.The present study was performed on 5 patients who have been clinically diagnosed to the following four investigations i.e. TLC, DLC, ESR & Hb.The Clinical and Laboratory Standards Institute (CLSI) [4] and the International Federation for Clinical Chemistry (IFCC) [5] recommend that each laboratory establishes its own reference values.

EXPERIMENTAL

The study was carried in Dindigul, an important developing city in Tamilnadu state. The study area lies between 77°15 and 78°15 E longitude and 10°0 to 10°45 N latitude. It covers a total area of 6,066 km^2. The elevation of Dindigul varies from 158 to 2,529 m above MSL. The runoff from precipitation all throughout the year flow towards main river Kodaganar in small streams. The average annual rainfall is in the order of 915.5 mm and now it has been reduced to 813mm.Dindigul district is bounded by Palani hill ranges of Western Ghats in the West, Sirumalai hills in the South and East. The northern part of the district covers plain terrain. This study area enjoys sub-tropical climate with the temperature (°C) ranges from 21.8 to 41.80. The study area chiefly consists of hard crystalline rocks of Archean age, which include charnockites, granite gneiss, calcagneiss and quartzite.

Dindigul is well known for its tannery units. It has more than 80 tannery units in and around the city and nearly 50 units are under processing of leather. The occupational environment can have an adverse effect on haematological, bio-chemical parameters of the tannery workers. This makes an investigator to focus the attention on the immunologic effect of the environmental pollutants on humans. Biochemical and hematological parameters are optimized.Hematological parameters should be monitored during this period, with prompt dose modification or switch if abnormalities are observed. There are about 10 permanent workers employed in the tannery industries. They undergo treatment in ESI hospital at Begampur in Dindigul .Five tannery workers who have been working for more than 20 years were selected for blood analysis. he parameters analysed were Total Leukocyte count (TLC), Differential Leukocyte count (DLC), Erythrocyte sedimentation rate (ESR), Haemoglobin levels (Hb). The bio-chemical parameters analysed are Total serum protein, Albumin, Globulin and Albumin/globulin ratio. The techniques of haematology are concerned mainly with the cellular formed elements of blood, their number or concentration, the relative distribution of various type of cells and structural or bio-chemical abnormalities that promote diseases.

Collection and Storage of Blood Samples

For Haematological parameters, venous blood (6 ml) was drawn from the antecubital vein by means of sterile disposable syringe after surface sterilization of the area with ethanol. In order to avoid the contamination, separate disposable syringes and needles were used for each collection of blood. For determination of total and differential leukocyte count and haemoglobin content, 2 ml of blood was collected in sterile tube containing EDTA (Ethylene Diamine Tetra Acetic Acid), a powerful anti-coagulant. To carry out ESR (Erythrocyte sedimentation rate) 2 ml of blood was collected in a sterile test-tube containing 0.5 ml of 3.8 per cent Trisodium citrate as anticoagulant. Similarly 2 ml of blood was delivered into another tube without any coagulant and kept for serum separation.[21]Blood samples collected for serum separation were immediately transported to the laboratory within an hour or two. The blood after clotting was stored in refrigerator overnight at 4°C.Serum was then separated using centrifuge on the following day and stored in storage vials at 20°C for further analysis.

Procedures Followed for Hematological Parameters

Procedure for the Total Leucocyte Count (TLC)

The freshly drawn blood samples with EDTA from both experimental and control subjects were brought to the laboratory within an hour after bleeding. Using a WBC pipette and haemocytometer, the total leucocytes count (TLC) was made as outlined.

Procedure for The Differential Leucocyte Count (DLC)

The differential leukocyte count was done by following a standard procedure followed in most of the clinical laboratories. In brief, a thin blood film was made by spreading a drop of blood across a clean slide using smooth edged slides and stained with using Leishmen's stain. For every blood sample, two or three slides were made and the good ones were used. For the differential leukocyte count the area where the morphology of the cell is clearly visible is chosen. Based on the standard morphology criterion, the different leukocyte populations namely Neutrophil, Basophil, Lymphocytes and Monocytes were counted by moving the slide in the order. A minimum of 200 nucleated cells were counted and the results expressed as per cent population in each category.

Hemoglobin Estimation

Estimation of blood Hemoglobin (Hb) of both experimental and control subjects was done using photo-electric colorimeter.

Erythrocyte Sedimentation Rate (ESR)

ESR is the rate at which erythrocyte sediment on their own weight when anti- coagulated blood is held in a vertical column. It is expressed as the fall of RBC's in man at the end of the first hour.ESR was measured by Westergren's method.

Procedure for Bio-Chemical Estimation

Estimation of the total protein and albumins by modified Biuret and Dumas method

Total proteins and albumins in the serum sample were estimated by modified Biuret and Dumas method, using a diagnostic reagent kit supplied by M/s. Span Diagnostics. Serum globulin and A/G ratio were calculated from the values of total protein and albumin.

RESULTS AND DISCUSSION

To assess the health hazards associated with chemicals used in the leather industry various blood samples were collected from the workers in tannery industry in order to find the diseases like lungs disorder, gastro intestinal tract infection. Hexa valent chromium is the main cause of dermatitis, allergic skin reaction and skin veneration. The acceptable standard level of Blood Parameters for human beings as given below in table-1. The results obtained for the blood samples of the tannery workers, given above is then compared with the following table-2 and discussed. The pictorial comparison of blood samples with standard values are shown in fig-1,2,3,4 & 5. The present study that reveals the following findings will be of very much useful for the abatement methods for the occupational health of the tannery workers. The nature of the toxic chemicals used within the tannery industries is not good for the health of the workers. Bare handling of chemicals like sulphides, acids, bases, chromium compounds and others organic compounds namely dust of hides, hair and chemicals which are inhaled by the workers lead to bronchitis and lung diseases.

Tannery workers are exposed to various types of skin diseases and anthrax. It causes lung diseases, nausea, respiratory tract diseases, skin allergies and dermatitis. Chemicals used in tanning industry attack mucous membrane of nose, throat, liver and kidney. They also cause asthma, bladder cancer and tumours. As per the ESI report concentrated gases from pits are poisonous and they cause respiratory tract problems and damages the lungs. Acute Pharyngitis is caused by obnoxious gaseous air pollutants like Hydrogen sulphide. H_2S causes respiratory diseases. Asthma is also caused by isocynates and formaldehyde. T.B is caused by inhalation of toxic vapours and dust.

Acid burns in tannery workers are caused due to improper handling of concentrated acid .A significant increase of total count in blood (T.C) and (D.C) differential leukocyte counting blood indicate various diseases like dermatitis, T.B, lung diseases, etc., A significant decrease in Haemoglobin content blood leads to anaemia. A significant increase in (ESR) Erythro Sedimentation Rate indicates TB Tuberculosis among tannery workers. An increase of Eosinophils indicates skin allergy conditions and eosinophilia. Based on the analysis of blood samples which were collected from the laborers exposed to the tannery environment it is observed that the tannery workers face a lot of serious health problems due to continuous exposure of toxic chemicals. The common health hazards are respiratory disorders, vomiting, skin rashes and diarrhoea.

Toxic chemicals used in tanneries contaminate the surface water. The use of such contaminated water for drinking purpose causes various types of illness. The contaminated water in the polluted area will cause the disease like diarrhoea respiratory diseases and skin diseases. Exposure to toxic gases also causes many disease conditions.

CONCLUSION

The study of the various health hazards caused by occupational exposure to various chemicals used in tannery industry directly was also screened in order to evaluate the health hazards of the people working in the tannery industries. The results obtained during the investigations of blood samples and feces collected from the tannery workers who were constantly exposed to the variety of chemicals volatile in nature and highly toxic. If they are inhaled or handled without proper gloves, the workers may suffer from various types of diseases like allergy, asthma and bronchial disorders.

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Table 1. The acceptable standard level of Blood Parameters for human beings

S. No	Blood Parameters	Standard level
1.	Age Groups	40-60
2.	Total leucocytes count (TC) No. of cells/mm^3	4000-10000
3.	Differential count (DC)% P	40-60%
	L	20-40%
	E	Up to 6%
	M	2-10%
	B	Up to 2%
4.	Erythrocyte sedimentation rate (mm/hr)	5-20 (mm/hr)
5.	Hemoglobin (gms %)	14-16 gms %
6.	A/G ratio	1.2:1-2.5:1

TC: Total Count L: Lymphocytes B: Basophile
DC: Differential Count E: Eosinophiles P: Polymorphous M: Monocyte

Table 2. Comparison of the blood samples with the standard values

Blood Parameters		Standard Value	Blood samples from the Tannery Industry Workers				
			S1	S2	S3	S4	S5
Age Groups		40-60	47	52	53	48	46
TC (No of cells/mm^3)		9500	8,500	9,800	10,300	8,500	9,800
DC (%)	P	40-60%	65	70	70	64	68
	L	20-40%	30	28	28	30	40
	E	Up to 6%	5	2	2	6	2
	M	2-10 %	1	1	1	2	1
	B	Up to 2%	1	2	1	1	2
ESR(mm/hr)		5-20	15/30	20/40	30/60	20/40	15/30
Hemoglobin		14-16gms%	10.5	10.8	10.2	11.8	11
Protein			7.0	6.8	6.8	6.9	6.9
Albumin			4.5	4.0	3.5	3.4	4.5
Globulin			2.5	2.8	3.3	3.5	2.4
A/G Ratio			1.8	1.4	1.0	0.9	1.8

TC: Total Count L: Lymphocytes B: Basophile
DC: Differential Count E: Eosinophiles P: Polymorphous M: Monocyte

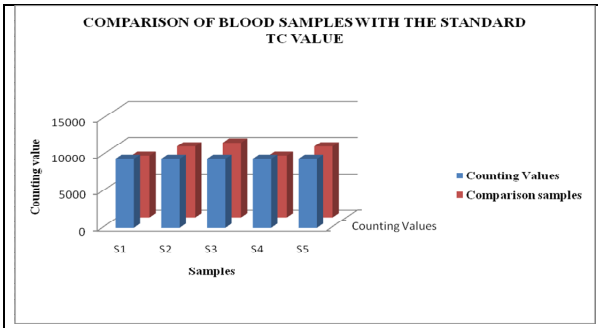

Fig. 1.Comparison of blood sample with the standard TC value

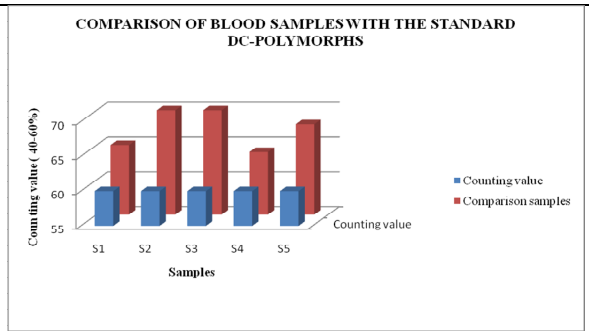

Fig. 3. Comparison of blood sample with the standard DC-polymorphs

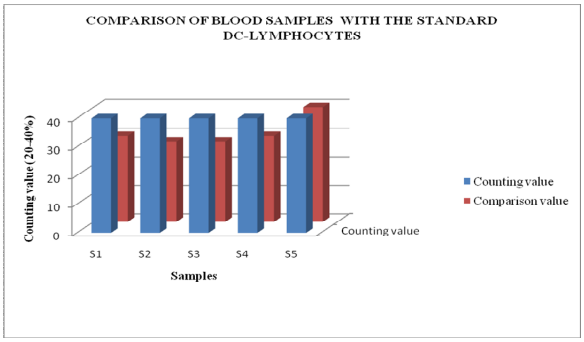

Fig. 3. Comparison of blood sample with the standard DC-Lymphocytes

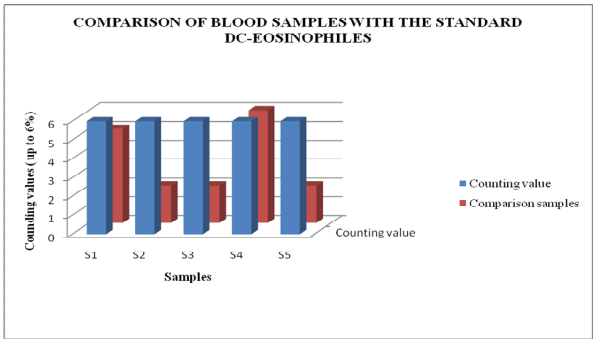

Fig. 4. Comparison of blood sample with the standard DC-Eosinophiles

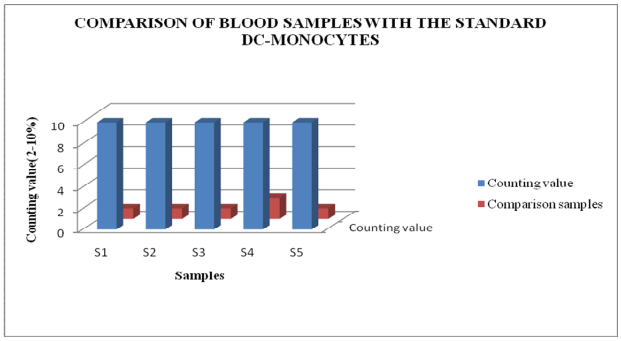

Fig. 5. Comparison of blood sample with the standard DC-monocytes

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RESEARCH ARTICLE

Sowing time Regulates Quality Chickpea (*Cicer arietinum*) Production by Mediating Morphological, Biochemical and Functional Traits

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an imperative pulse crop with diverse potential nutrition and health benefits. The present research work was conducted to determine the appropriate sowing time of chickpea by assessing the maximum yield (through number of shoot, number of pod per plant) and quality of chickpea (through phenol, protein, crude fibre, ash and functional properties like gelation, foaming capacity and water absorption capacity) production. Four chickpea varieties (BARI chhola-3, BARI chhola-5, BARI chhola-6 and BARI chhola-9) were sown with triplicates along with four treatments (different times) in pots. Each time of sowing intervals was one month such as 15 October (T1), 15 November (T2), 15 December (T3) and 15 January (T4). Plants sown on 15 January could not survive after initiation of flowering due to high temperature and sudden rainfall at the reproductive stage. Flowering initiation were observed with different duration like 55 DAS, 53 DAS, 50 DAS and 43 DAS at T1, T2, T3 and T4 treatments, respectively. Shoot number per plant and pod number per plant were increased significantly in all varieties at T2 treatment compare to other in all varieties. Both T2 and T3 showed significant decrease in total phenol content compare to T1 treatment. However, phenol accumulation was significantly higher in T2 compare to T3. Meanwhile, there was no significant difference in protein content (%) among all the chickpea varieties under different treatments. Fibre content was significantly increased in T2 and T3 compare to T1. Furthermore, T2 treatment increased relatively higher fibre content than T3. T2 showed significant increase in ash accumulation in all varieties. Gelation was commonly occurred in all the varieties at 12 to 14% flour concentration for each treatment. At T2, all the varieties showed higher specific volume compare to their respective varieties grown in different treatments. Moreover, their respective volume increases were higher even after 4 hours which indicates the good

quality of the protein structure. There was no remarkable variation of water absorption capacity in all the chickpea varieties under different treatments. From the above study, it could be suggested that chickpea varieties sown in mid November (15 November) might perform the best compare to other sowing times.

Key Words- Sowing time, Morphological, Biochemical, Functional traits, Chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum*) is the largest produced food legumes in South Asia and it occupies sixth position among pulses in Bangladesh [1]. Chickpeas are rich in protein, complex carbohydrates, and fiber, while low in fat and cholesterol, and its protein quality is considered to be better than other pulses [2].These days chickpea are greatly consumed on account of their therapeutic value in various lifestyle diseases such as CVD (cardiovascular disease), type 2 diabetes, bone health, digestion, weight management and cancer [2]. Considering all, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits.

There is an increasing focus on chickpea production in Bangladesh for meeting the domestic demand and diversification of rice-based cropping system with legumes, which can help in improving soil fertility and system productivity [3]. Rice based cropping system is prominent in Bangladesh where chickpea as a minor crop is cultivated after harvesting rice. In the northwestern Bangladesh about 800,000 ha land of the high *Barind* tract remains fallow after rice cultivation. This can potentially be brought under chickpea cultivation. However, chickpea sowing is often delayed (up to December) due to late harvest of *Aman* rice. As a result, the chickpea crop faces heat stress during its reproductive phase. Thus, the major constraint to chickpea production in Bangladesh is heat stress which adversely affects pollen viability, pod set and grain yield [3].

Functional properties of a legume crop indicate the physical and chemical properties of the crop protein which affect the behavior of proteins in food systems during storage, processing, preparation and consumption [4]. The production of legume protein concentrates is of growing interest to food industry because of their functional properties and ability to improve the nutritional quality of food products [5]. According to the mechanism of action these functional properties could be divided into three groups like (i) properties related to hydration (water absorption), (ii) properties related to protein structure and its rheological characteristics (gelation) and (iii) properties related with the protein surface characteristics (foaming capacity) [6]. Legume proteins have gained increasing importance because of desired functional properties, including water absorption, gelation and foaming capacity and could be proposed as a potential supplement in a greater number of food applications [5].

The present research work was undertaken to select an appropriate sowing time, on other word, judicial temperature range which could be achieved through assessing:

1. Proper growth and development of the plant (shoot number, phenology, number of pods per plant) that promotes maximum yield.
2. Quality chickpea production (phenol, protein, crude fibre, ash, gelation, foaming capacity and water absorption capacity) through avoiding hot and humid conditions in the reproductive stages.

MATERIALS AND METHODS

Plant material and culture conditions

The experiment was conducted at the laboratory of the department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh during the period from October, 2015 to April, 2017. Seeds of four Chickpea varieties such as BARI chhola-3, BARI chhola-5, BARI chhola-6 and BARI chhola-9 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. Earthen pots were selected for planting all the four

Chickpea varieties following complete randomized design (CRD). For each variety, pots were prepared as triplicates. At 18 DAS (days after sowing) thinning (keeping 3 plants in each pot) and fertilization (Urea 4g; MOP 2.5g; & Boric acid 1.5g) were done accordingly. Four different sowing times on 15th of October, November, December'2015 and January'2016 were assumed as four treatments like T1, T2, T3 and T4, respectively. Daily temperature was recorded during the study period with a centigrade thermometer.

Determination of growth parameters

Number of shoots per plant were counted and collected after 40 DAS to analyze different parameters. Flowering initiation time that is phonology was observed for different treatments subsequently. Number of pods per plant were counted and collected after 70 days of sowing time of each treatment to analyze different parameters.

Sample preparation for biochemical and functional parameters analysis

After reproductive stage, three replicate numbers of pods were recorded and collected. Collected shoots and pods were labeled and stored at -20^{o}C for further for biochemical and functional analysis. Samples were oven dried at 70^{o}C until they were sufficiently moisture free. Then the shoot samples were grinded manually using mortar and pestle till they produced fine powder. Pod samples were grinded using electric grinding machine to produce fine powder. Then the materials were preserved in sealed container for further analysis.

Preparation of plant extracts

0.5 g of dried ground leaf samples was taken in weighing paper and measured accurately. Then it was poured into 25 ml test tubes and 80% 10 ml alcohol was added. Then the mixture was shaken vigorously for minutes and again shaken on vortex machine for 2-3 minutes. Then the sample mixture was settling downed for 24 hours. After 24 hours, the sample mixture was centrifuge at 5000 rpm for 20 minutes. Then the supernatant was collected in a fresh vial and stored at 4^{o}C temperature. 80% 10 ml alcohols was added into the remaining pellet and settle downed for another 48 hours. Before settle downed, samples was shaken like previous manner.

Again, after 48 hours, the pellet mixture was centrifuge at 5000 rpm for 20 minutes. Then the supernatant was collected and the pellet was discarded. Then both days supernatant was mixed and filtered through Whatman No.1 filter paper and stored the extract at 4^{o}C temperature for vacuum dry evaporation. Then the combined supernatants were evaporated to dryness under vacuum at 40^{o}C using Rotary evaporator (Model: DSB-1000E). After evaporation 5ml distilled water was added and sonicated using ultra sound sonicator to make concentrated 5ml extract. Then the extract were filtered again using Whatman No.1 filter paper to remove any remaining debris and kept in sterile tubes and stored at refrigerator at 4^{o}C.

Determination of total phenolic contents

The concentration of phenolics in shoot extracts were determined using spectrophotometric method [7]. The standard curve was prepared by plotting the catechol concentrations on the X- axis and the absorbance values on Y-axis. From the standard curve, concentrations of total phenols in terms of mg phenols/100 g plant material were estimated and converted to per cent.

Determination of protein contents

The estimation of nitrogen in seed samples were made by modified micro-Kjeldahl method [8] which was further converted into protein content(%) by multiplying with conversion factor 5.5.

Determination of crude fiber

The crude fiber content of the chickpea pod samples were determined by sequentially acid and alkali hydrolysis followed by ignition of the hydrolysate as described the method [9].

Determination of ash

The dried seed samples of chickpea (3g) contained in the crucible and pre-ashing of the sample was done by placing the crucible in a muffle furnace maintaining 300°C for 3 hrs. The temperature of ashing was increased up to 600°C and operated for 9 hrs. Then the crucible was cooled, kept for some time in a desiccator and weighted. From the weights recorded the per cent of ash contents was calculated as % Ash = $\frac{\text{Weight of Ash (g)}}{\text{Weight of sample (g)}} \times 100$.

Determination of functional properties of seed samples

The gelation properties of chickpea seeds flour were evaluated by the prescribed method [10] with some modifications [11]. Foaming capacity and stability were studied accordingly [10]. Water absorption was measured using a modification of the centrifugation technique [12].

The statistical analysis

The statistical analysis of the experimental data from one way ANOVA of CRD with 3 replications for varieties and 4 treatments of sowing time were carried out using MS Excel 2010 and MiniTab 17.0 data processing software. Statistical levels of significances among different treatments were carried out by $p \leq 0.01$ or $p \leq 0.05$ values.

RESULTS AND DISCUSSION

Observation on temperature variation during study periods

The chickpea varieties were sown from 15th October, 2015 to 15th January, 2016 with one month interval. The last treatment group survived until April, 2016. Accordingly, these six months' temperatures were recorded in the laboratory. The temperature variations are shown in the Figure 1. The temperature ranged from 31°C to 16°C throughout the experiment. High temperature (28°C) was observed in 15 October that is at the first sowing (T1). After that, temperature gradually decreased till the month of January (17°C). Then, it also increased with the progress of time till April (28.5°C).

Effect of sowing time on the morphological attributes of chickpea varieties

The important yield attributing traits of chickpea such as the number of shoot and the number of pods per plant of different chickpea varieties at different sowing times are presented in Figure 2 and 3, respectively. There were significant (1% to 5% level) increase in the shoot and pod number of all the four chickpea varieties when they were sown on mid November (T2). The productivity of the winter crop chickpea is constrained by several abiotic stresses [13]. High temperature stress could decrease photosynthesis and increase transpiration rate. Generally, chickpea adapts to high temperature through an escape mechanism. However, heat stress during reproductive phase can cause significant yield loss by affecting pod and seed setting [14]. It was previously observed in chickpea [15] that the best temperature for pod development is between 20 to 26o C and more than 30° C could be harmful. These findings also coincide with our research study and may be the reason for lower vegetative growth and pod number. Thus the plant grown on the mid January experienced high temperature at its vegetative and reproductive stage. Finally, after first initiation of flowering they could not survive.

Early phenology (especially first flowering) could be a heat escape mechanism. However, in this study after giving the first flowering with T4 treatment all the chickpea varieties could not survive in further high temperature with heavy rainfall. From Table 1, it is confirmed that the varieties showed early phenology with the gradual temperature increase. High temperature and photoperiod can regulate plant phenology, particularly if the crops are exposed to warn temperature and long days in the summer as proposed by [16, 17]. Thus high temperature may shorten the duration of vegetative growth which in turn, cannot ensure sufficient metabolite accumulation for desired production.

Effect on biochemical parameters

Patterns of total phenol content (%) are presented in the Figure 4. Compare to the sowing (T1) of October month, T2 and T3 showed a dramatic decline of phenol content (1% level significance) at all the chickpea varieties. Phenolic compounds play important role in plant defense system against pathogens and insect herbivores [18]. From the figure it is clear that, high temperature could be an effective determinant of total phenol content (%) reduction in chickpea leaves. Thus, the reduction of total phenol content could induce more vulnerable condition of insect attack and pathogen infestation in the varieties which could eventually lead to lower production. Interestingly, there was no significant variation in protein content (%) of different varieties chickpea at different sowing time. In a previous research work [19], researchers obtained that total protein content was decreased by heat stress in strawberry. However, we measured the protein content by percentage. Therefore, it seems that if the production of the pod number decreases or even the decrease in pod size persists, total protein content would be subsequently decreased. However, in its proportional point of view the protein percentage could remain similar as it is genetically stabilized.

In the present study, crude fibre (%) was increased (1% level of significance) the most at T2 in BARI chhola-3, BARI chhola-6 and BARI chhola-9 varieties compared to that of T3 and T4 trial (Figure 5). Researchers showed that cold stress could reduce fibre content in chickpeas [20]. They further recommended that the similar mechanism could be implied to reduce fibre content even in heat stress. Their conclusions offers the understanding of reduced fibre content could be due to less accumulation of other different intermediates that might not help in more fibre accumulation due to stress conditions. Ash content (%) represents the mineralization of a biological sample. In a proper growth condition due to having sufficient metabolites, mineralization takes place spontaneously. Present study showed the sufficient increase of ash content (%) of BARI chhola-3 (1% level of significance), BARI chhola-5 and BARI chhola-6 (5% level of significance) chickpea varieties at T2 trial (Figure 6). However, the later trial (T3) experienced high temperature at their vegetative and reproductive stages. Thus it could hamper the metabolite accumulation in all the varieties grown later on. Ash content was reduced by 50% in oil seed plant like Andean Lupin under heat stress [21].

Effect on functional properties

This experiment showed the gelation (Table 2) was commonly occurred with slight variations in all the varieties at 12% to 14% flour concentration with any treatment. Protein gelation is one of the most important functional properties when it comes to modify the structure and texture of foods. For gelation, it is important that the functional groups (e.g. hydrophobic groups) within the protein are exposed. This makes it easier for the groups to interact and form a three dimensional work [22]. Gel formation is complicated, and affected by the concentration of protein, amount of water, ionic strength, time and temperature as well as pH and interaction with other components in the food systems [23]. Proteins containing a high frequency of non-polar amino acid residues tend to form coagulum type gels [24].

Foams are formed through unfolding and absorption of the protein, at the air water interface, as well as film formation around the air bubbles. For a protein to have superior foaming properties it must process high solubility in the liquid phase as well as the ability of quickly forming a film around the air bubbles in the food system [25]. The extrinsic factors that affect the foaming properties are pH, temperature and ionic strength. In this study, it was shown that the chickpea varieties grown in mid November and achieved their mature stage at January (lowest temperature) had higher specific volume compare to others grown on different time. Moreover, their respective volume increases even at after 4 hours were higher (Table 3).

There were no remarkable variations of water absorption capacity in all the chickpea varieties due to different treatments (Table 4). However, their respective values in 5% NaCl solution were subsequently reduced. The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. Other functional properties like foaming and gelation are dependent on the solubility of proteins [26].

The experiment was conducted to ensure the qualitative and quantitative yield of chickpea varieties through the selection of judicial sowing time, in true sense, proper temperature range for their optimum growth and production. Since, chickpea is a short duration crop, high precaution should be maintained for its growing time as temperature and day length play vital role on its growth and development. From the study, it is notable that the morphological parameters, biochemical parameters and functional properties of the chickpea varieties sown in mid November performed the best compare to other sowing times. From the result, it can be concluded that the favourable sowing time of chickpea cultivation in Bangladesh should be mid November which could ensure the vigorous vegetative stage at 12^{o}C to 20^{o}C, proper reproductive stage at 22^{o}C to 25^{o}C and fruitful harvest stage at 25^{o}C to 28^{o}C.

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Table 1. Effect of sowing time on the phenology duration of different chickpea varieties at different treatments

Treatments	Duration of Phenology (DAS)
T1	55
T2	53
T3	50
T4	43

DAS means Days after sowing

Table 2. Effect of sowing time on gelation of different chickpea varieties at different treatments

Flour solution % (w/v)	Gelation status				
	Treatments	BARI Chhola-3	BARI Chhola-5	BARI Chhola-6	BARI Chhola-9
2	T1	Liquid	Liquid	Liquid	Liquid
4		Liquid	Liquid	Liquid	Liquid
6		Liquid	Liquid	Liquid	Liquid
8		Viscous	Liquid	Liquid	Viscous
10		Gel	Viscous	Viscous	Gel
12		Gel	Gel	Gel	Gel
14		Gel	Gel	Gel	Gel
16		Firm gel	Gel	Gel	Firm gel
18		Firm gel	Firm gel	Firm gel	Firm gel
20		Firm gel	Firm gel	Firm gel	Firm gel
2		Liquid	Liquid	Liquid	Liquid

4	T2	Liquid	Liquid	Liquid	Liquid
6		Liquid	Liquid	Liquid	Liquid
8		Liquid	Liquid	Viscous	Viscous
10		Viscous	Viscous	Gel	Gel
12		Gel	Gel	Gel	Gel
14		Gel	Gel	Gel	Gel
16		Gel	Gel	Firm gel	Firm gel
18		Firm gel	Firm gel	Firm gel	Firm gel
20		Firm gel	Firm gel	Firm gel	Firm gel
2	T3	Liquid	Liquid	Liquid	Liquid
4		Liquid	Liquid	Liquid	Liquid
6		Liquid	Liquid	Liquid	Liquid
8		Liquid	Liquid	Viscous	Viscous
10		Viscous	Viscous	Gel	Gel
12		Gel	Gel	Gel	Gel
14		Gel	Gel	Gel	Gel
16		Gel	Gel	Firm gel	Firm gel
18		Firm gel	Firm gel	Firm gel	Firm gel
20		Firm gel	Firm gel	Firm gel	Firm gel

Table 3. Effect of sowing time on the foaming capacity and stability of different chickpea varieties at different treatments

Treatments	Varieties	Volume after whipping (ml)	Volume increase (%)	Specific volume (ml/g)	Volume (ml) at room temperature (25°C) after whipping time (hr)				
					0	1	2	3	4
T1	BARI Chhola-3	134	34	1.43	134	130	126	122	118
	BARI Chhola-5	138	38	1.47	138	132	128	123	119
	BARI Chhola-6	135	35	1.44	135	130	125	120	116
	BARI Chhola-9	139	39	1.49	139	133	128	124	120
T2	BARI Chhola-3	138	38	1.52	138	133	129	125	120
	BARI Chhola-5	140	40	1.54	140	134	130	125	120
	BARI Chhola-6	139	39	1.53	139	133	128	123	119
	BARI Chhola-9	143	43	1.57	143	135	130	126	122
T3	BARI Chhola-3	127	27	1.34	127	123	120	116	112
	BARI Chhola-5	128	28	1.35	128	122	118	115	110
	BARI Chhola-6	125	25	1.32	125	120	117	112	107
	BARI Chhola-9	130	30	1.38	130	124	120	116	112

Table 4. Effect of sowing time on water absorption capacity of different chickpea varieties at different treatments

Treatments	Varieties	Water absorption (g/g flour)	Water absorption in 5% NaCl (g/g flour)
T1	BARI Chhola-3	1.74	1.57
	BARI Chhola-5	1.72	1.56
	BARI Chhola-6	1.77	1.60
	BARI Chhola-9	1.76	1.59
T2	BARI Chhola-3	1.73	1.58
	BARI Chhola-5	1.74	1.55
	BARI Chhola-6	1.76	1.59
	BARI Chhola-9	1.75	1.58
T3	BARI Chhola-3	1.73	1.56
	BARI Chhola-5	1.72	1.55
	BARI Chhola-6	1.74	1.57
	BARI Chhola-9	1.73	1.56

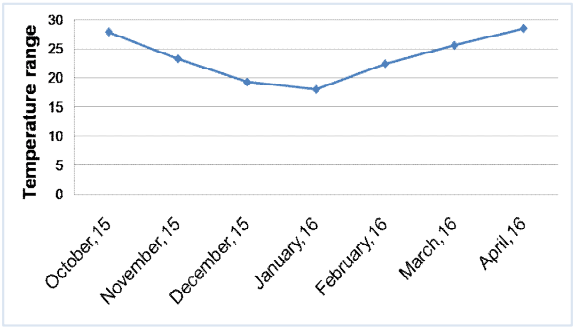
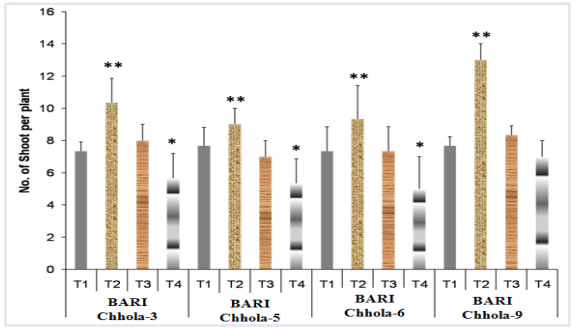
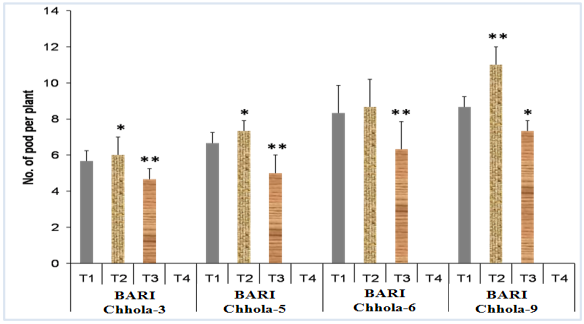


Figure 1. Average temperature variations throughout the experiment period



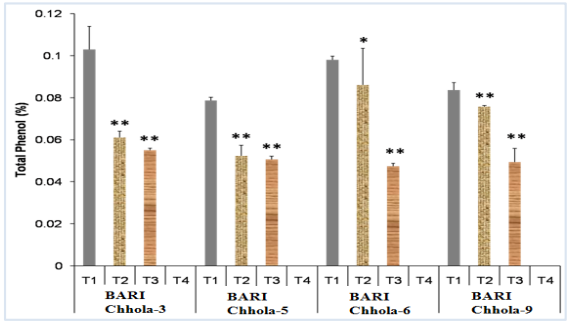
** = Significant at 1% level of probability, * = Significant at 5% level of probability

Figure 2. Number of shoots of different chickpea varieties at 40 days after sowing in different treatments



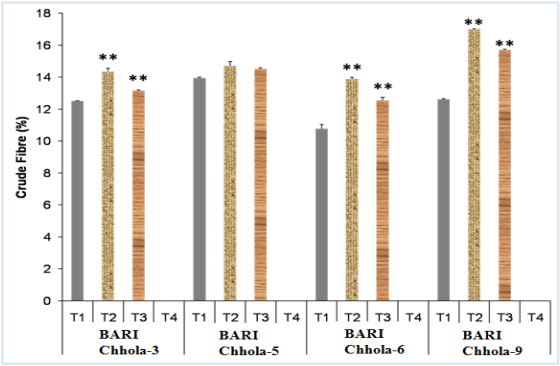
** = Significant at 1% level of probability, * = Significant at 5% level of probability

Figure 3. Number of average pod per plant of different chickpea varieties at 70 days after sowing in different treatments



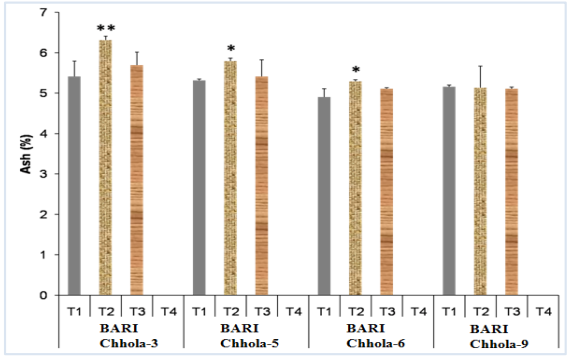
** = Significant at 1% level of probability, * = Significant at 5% level of probability

Figure 4. Effect of sowing time on phenol content (%) of different chickpea varieties at different treatments



** = Significant at 1% level of probability

Figure 5. Effect of sowing time on crude fibre content (%) of different chickpea varieties at different treatments



** = Significant at 1% level of probability, * = Significant at 5% level of probability

Figure 6. Effect of sowing time on ash content (%) of different chickpea varieties at different treatments

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RESEARCH ARTICLE

Influence of Nitrogen Flow Rate on Structural, Optical and Wettability Properties of Mixed Chromium Oxide-Nitride Films

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ABSTRACT

The influence of nitrogen flow rate is examined on structural, optical and wettability properties of chromiumoxide-nitride films deposited by reactive magnetron sputtering. The films were characterized by X-ray diffraction, scanning electron microscope (SEM), and contact angle measurementsystem.The optical properties of the films were measured by UV-Vis-NIR spectrophotometer and transmittance of ~70% in the visible region of the spectrum was achieved. The band gap increases with increase in nitrogen gas flow rate. The refractive index and packing density of the deposited films increases gradually with increase of nitrogen gas flow rate.The deposited films were hydrophobic and the contact angle increaseswith increase in nitrogen flow.

Keywords: Chromium oxide-nitride; Sputtering, Contact Angle; Optical Properties; Band gap.

INTRODUCTION

Chromium nitride thin films have efficient mechanical property and a better corrosion resistance. Chromium oxide thin films also have interesting properties, such as hardness [1] and a good decorative green color, thus they have industrial significance and used as hard and decorative thin films. Chromium oxide-nitride films should have

improved properties that can be adjusted in a defined manner by varying the reactive gas flow rates.The physical deposition of chromium oxide-nitride films in a mixed reactive gas (oxygen and nitrogen) atmosphere has not been widely published. One of the reasons for this is the dissimilar sticking coefficients for oxygen and nitrogen and the resulting difficulties for the deposition process. Usually a very high nitrogen gas flow in comparison to the oxygen flow is reported [2].

In this study, we have deposited chromium oxide-nitride films using RF reactive magnetron sputtering process and characterized their structural, optical and wettability properties. The effect of nitrogen flow rate on the properties of chromium oxide-nitride films is investigated. In this work, an array of experimental techniques wasutilized, including X-ray diffraction (XRD), scanning electron microscope (SEM), Contact angle measurement and UV-vis-NIR spectrophotometer.

Experimental Details

RF reactive magnetron sputtering was used to deposit chromium oxide-nitride films in a cylindrical chamber (Excel Instruments, India), using a chromium target (99.99% purity, 50.8mm × 6.5mm). Vacuum chamber was evacuated up to ~ 6 x10^{-4}Pa with the help of a turbo molecular pump backed with a rotary pump. Argon, nitrogen and oxygen gases of high purity (99.999%) were injected into the chamber. The flow of argon and oxygen gases was kept constant and controlled by Mass Flow Controller (MFC), (ALICAT instruments, USA). Sputtering was carried out at constant flow rates of argon and oxygen kept at 10sccm and 1.5sccm respectively for 60 minutes. The nitrogen flow rate was varied at 10,20, 40 and 80sccmrespectively represented by samples names I10, I20,I30 and I40 respectively [3]. The RF powerwas kept constant for all sample as 175W and the distance of target to substrate was kept constant at 50mm.

X-ray diffractometer (XRD) using Bruker D2 phaser, advance diffractometer with Cu-K α radiation having wavelength 1.54Å, characterized the structural properties of chromium oxide-nitride films. The morphology of films was investigated by Nova Nano FEG-SEM 450. Optical transmission and absorption were measured in the 200-800nm wavelength range using UV-vis-NIR spectrophotometer (Shimadzu, model: UV3600 plus). To determine whether the films are hydrophilic or hydrophobic by nature, contact angle meter (Rame-Hart model 290) was used to find contact angle of water with the films.

RESULTS AND DISCUSSION

The XRD patterns of chromium oxide-nitride films deposited at different nitrogen gas flow of 10, 20, 40 and 80sccm is shown in figure 1. When the nitrogen gas flow was 10sccm, sharp (104) peak of chromium oxide (Cr_2O_3) having good intensity was observed along with (024) and (128) peaks and (111) peak ofchromium nitride (CrN). As the flow of nitrogen gas of coating was increased to 20sccm, the evolution of (104), (024) and (128) peaks for chromium oxide (Cr_2O_3) diminishes A further increment of nitrogen gas flow rate to 40sccm and 80sccm shows negligible variation in these textures for deposited films.

With increasing N2 gas flow the crystallization of the CrN phase in the films seems to be improved, since the intensity of the (200) diffraction peak decreases. At the two highest flows a texture transition from the (128) to the (111) orientation can be observed which has also been reported by Barata et al.[4] In 2015, SuguruIkeyama et al. were observed the epitaxial growth of Cr(N,O) thin films on (001) and (011) oriented on substrates as well as a (200) oriented polycrystalline Cr(N,O) thin film(111) and all substrates exhibited greater hardness than a comparison specimen consisting of epitaxial CrN (001) orientation. Scherrer formula [5] was used to calculate the average crystallite size (d) of the samplesas shown in equation (1).

$$\frac{0.9\,\lambda}{\beta\,\cos\theta B} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where λ, and β are the X-ray wavelength (1.54056 Å), Bragg diffraction angle and line width at half maximum ofthe most dominant peak, respectively. The average crystallite size increased from 8 to 13nm with increase in nitrogen gas variation from 10 to 80sc cm. The similar observation was reported by Louse et al [6] and Wang and Oki [7].

The SEM images of the chromium oxide-nitride films at different nitrogen gas flow rate is shown in figure 2. The surface of the thin film prepared at 10sccm of nitrogen flow was relatively smooth. On the other hand, the surfaces of the thin films prepared At 80 sccm of nitrogen flow is rough[8]. It is considered that the smooth surface obtained under lower flow is due to direct deposition of the ablation plasma. Under higher flow of nitrogen, however, the ablated material is cooled while colliding with the gas molecules, and as a result, starts to aggregate before arriving at the substrate. The deposition of fine solid particles may have caused the rough surface of the thin films obtained under higher gas flow[9]. The surface of the thin film prepared at 10 SCCM nitrogen flow was relatively smooth than the surfaces of the thin films prepared at nitrogen flow at 40 and 80 sccmare rough. It is considered that the smooth surface obtained under lower flow rate is due to direct deposition of the ablation plasma. Under higher flow rate, however, the ablated material is cooled while colliding with the gas molecules, and as a result, starts to aggregate before arriving at the substrate. The deposition of fine solid particles may have caused the rough surface of the thin films obtained under higher gas flow rate. A crystalline size of CrON, according to Scherrer formula and SEM image was revealed that nitrogen flow flux is gave major influence on the grain size and it increases with rise in nitrogen flow rate.

UV–vis transmittance and absorbance spectra were obtained for all samples deposited on the glass substrates. Figure 3 shows the spectral transmittance of chromium oxynitride films at different Nitrogen gas flow. The oscillations in the spectrum with wavelength are due to interference effect [10]. The transmission of chromium oxide films decreases with the increase in the Nitrogen gas flow. Initially the films were amorphous at low Nitrogen gas flow. When the Nitrogen gas flow was increased, films were crystalline. These may have led to decline in the transmission of the deposited films with increase in nitrogen gas flow. The refractive index increases with increase in nitrogen gas flow. The increase nitrogen gas flow leads to more striking of the metal atoms that result in formation of crystalline films and increase in the deposition rate of the deposited films. The optical band gap of the films was determined by the absorption coefficient (α) from the absorption spectra of the films using the Tauc relation [11], which is described in detail elsewhere [12]. The band gap of the deposited chromium oxynitride films are shown in figure 4. The band gap of chromium oxide films is increased in the nitrogen flow rate of respective targets, the Cr atoms. This also favors the formation of the respective phases as evident from the XRD graph in figure 1. Hence with the increase in the nitrogen flow rate of metal targets, a change in atomic concentration and structure of the deposited films are observed.

Effect of nitrogen gas flow variation on wettability of mixed chromium oxide-nitride thin film

Contact angle and surface energy of the deposited chromium oxynitride films are shown in figure 5. The contact angle increased from 89.5° to 92.0°.A transformation from hydrophilic to hydrophobic behavior was observed for chromium oxynitride films with the gradual increase of respective nitrogen gas flow. Surface energy of chromium oxynitride coatings increases with increase in nitrogen gas flow as shown in figure 5.It was observed that as contact angle of chromium oxynitride increases surface energy decreases.[13].

CONCLUSION

When the nitrogen gas flow was low (10sccm) the deposited chromium oxynitride films were amorphous, as the nitrogen gas increases films were observed crystalline. With the gradual increase of respective nitrogen gas flow a transformation from hydrophilic to hydrophobic behavior was observed for chromium oxynitride films. The transmission of chromium oxide films decreases with the increase in the Nitrogen gas flow. It was observed that

nitrogen flow flux gave major influence on the grain size and it increases with rise in nitrogen flow rate.The band gap values of the deposited chromium oxide increased from 1.67 eV to 1.95 eV as nitrogen gas flow increased from 10sccm to 80sccm. The refractive index (n) of chromium oxynitride films increased from 1.71 to 1.83 with increased in Nitrogen gas flow.

ACKNOWLEDGEMENTS

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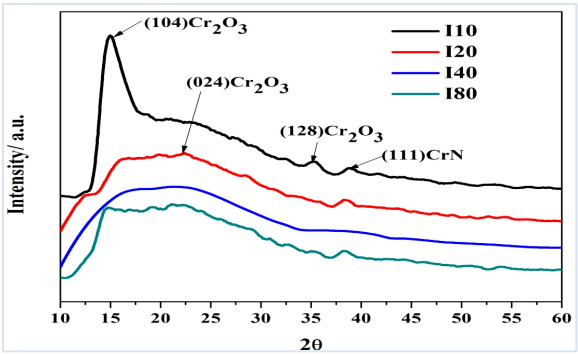

Figure 1:The XRD pattern of chromium oxide-nitride deposited at varying nitrogen gas flow rate.

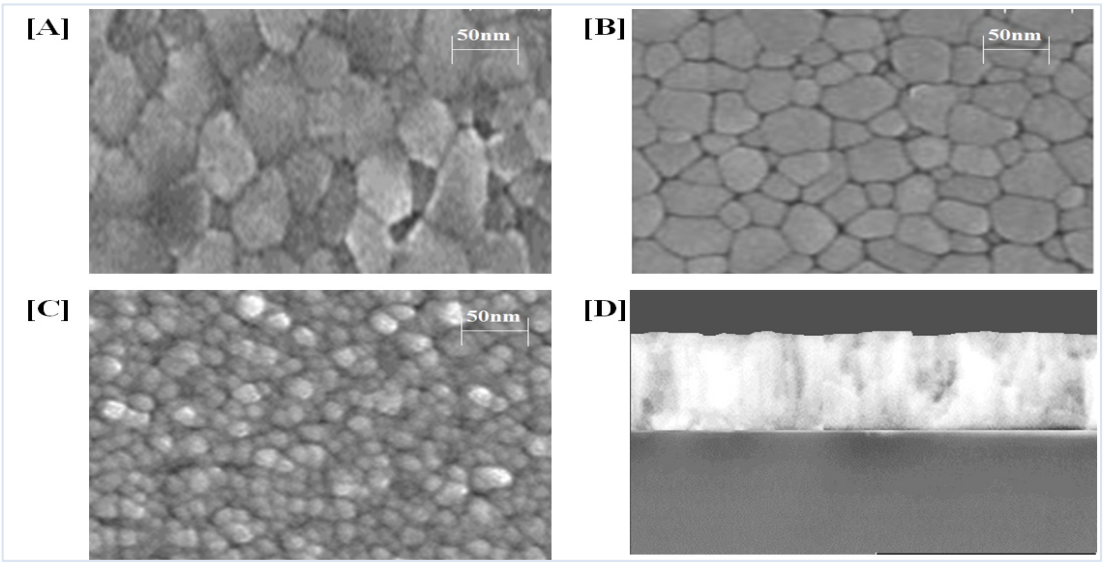

Figure 2. SEM image of the mixed chromium oxide-nitridethin film prepared under different nitrogen gas flow [A] 80 sccm [B] 40 sccm [C] 10 sccm [D] Cross section image of chromium oxynitride film deposited at 10 SCCM nitrogen flow

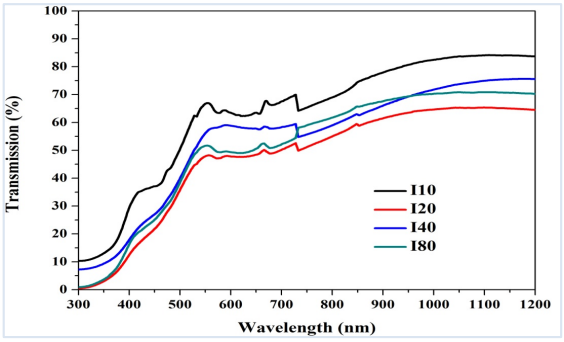

Figure 3: Effect of Nitrogen flux on transmission spectra of mixed chromium oxide-nitridethin film

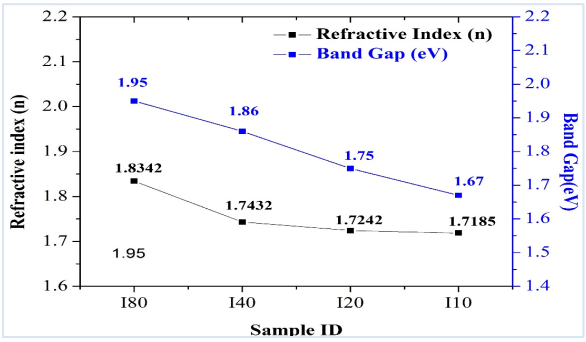

Figure 4. Effect of nitrogen flow rate on refractive index and band gap of mixed chromium oxide-nitridethin film

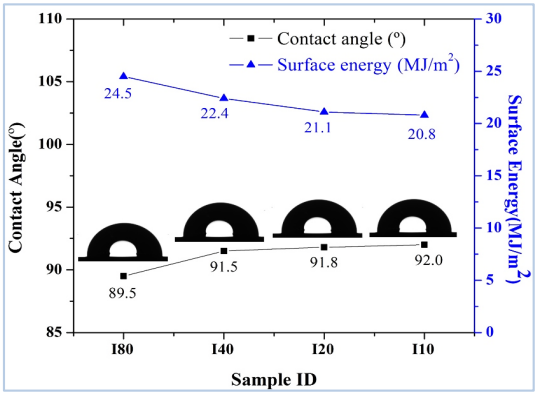

Figure 5. Effect of nitrogen flow rate on contact angle and surface energy on mixed chromium oxide-nitridethin film

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REVIEW ARTICLE

Mobile Phone Radiations as an Alarming Tool for Human Health: A Review

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ABSTRACT

The extensive uses of mobile phones have increased the risk of creating health problems among human beings. These problems were due to production of microwave radiation by mobile phones. Electromagnetic fields (EMF) radiations are produced from cellular phones which showed a relationship with biological effects on human tissues, particularly the brain and the human immune system. The relation between the two showed an increase in intimation because of growing use of mobile phones in day-today life. Large numbers of scientists are working to find out the effects of mobile phone radiations on human health and any possible means of making ourselves safe from these effects. Various studies so for done are concentrated on the single negative effects and there is not a single study to with the aim to find out the overall ill effects of mobile phone radiations. Keeping these facts in consideration the present study was aimed to investigate the total possible negative and positive biological effects of cell phone radiation on different human organ systems. The current study is in the form of review of literature and tried to assemble all possible positive as well as negative health risks to public due to excessive use of cell phones. It was concluded that long-term exposure to EMF radiation due to excessive use of mobile phone caused number of health effects, including brain cancer. In addition to this some positive health effects were also noticed which include improved bone healing and reduced toxic effects of chemotherapy etc. No major effect on human health due to use of mobile phones had also been shown by some workers. Taken all these facts in consideration there is a need for long-term studies and analysis. From above discussion we can conclude that EMF exposure altered in neuro behavioral activity, cytokine level, oxidative stress and DNA integrity.

Keywords: Mobile phone radiations; Radio frequency; Human Health.

INTRODUCTION

Modern telecommunications are growing continuously due to increased growth in use of cellular phones and are now becoming its integral part. The market of mobile phones is growing too fast as in various countries more than half their population use cellular phones. As per reports nearly 6.9 billion subscriptions for mobile phones have been recorded globally up to 2014[1]. There are 14 countries in the world with over 100 million mobile subscriptions in which India is in the second position. According to Telecom Regulatory Authority of India 987.30 million subscribers are using mobile phone in India [2]. As the use of cell phone technology has grown throughout the world in recent years, the tendency for determining its potential harmful impacts on human health has also increased significantly [3].

The mobile phones operate in microwave region where the frequency varies from 900 MHz to 2.4 GHz [4,5]. Previous studies showed that these frequency components may changes in the biological system. Most2G phones use the global system of mobile communications for 2G cellular phones is GSM standard, which signals at 217 Hz having pulses between 890-960 MHz and 1710-1880 MHz. Where as universal mobile telecommunication's system (UMTS) is used for3G phones with W-CDMA2 air interface standard, which operates at higher frequency range of 1900-2170 MHz [6]. Although the familiarization and dependency of mobile phones is growing at an alarming pace, the biological effects due to the exposure of radiations have become a subject of intense debate.

Mobile Phone Antennas and Base Stations

There's been a lot of controversy about radio antennas, mobile phone antennas and their emissions causing leukemia and other diseases. It is the actual antenna itself that emits radio waves, not the structure that supports it. The truth is, there is no conclusive evidence one way or the other, but here are few facts about levels of radiation transmission from mobile phone antennas: To get a dose of radiation considered dangerous from a mobile phone transmission antenna, you'd have to be almost touching it. The antennas don't beam signals directly down, so they don't 'blow' radiation directly onto us below. The towers that support the antennas don't emit radiation. Radiation dramatically and rapidly decreases as you move away from the mast - 10 meters away, the dose is 0.1% of what it was at 1 meter away; 0.0125% at 20 meters away and so on [7].

How Mobile Phone System Works

The mobile phone system works like a two-way radio, includes the individual handset and the base stations. Base station antennae are mounted high off the ground (on a tower or roof) to get the widest coverage. A mobile phone has a radio receiver and a transmitter. When you make a call, your phone uses radiofrequency (RF) radiation via its antenna to 'talk' to a nearby base station [8]. Once the base station has received your signal, your call is directed through the landline phone system. Mobile phone base stations emit relatively constant levels of RF radiation. The handsets emit levels of RF radiation that vary depending on three things:

1. How long you use the phone
2. How close you hold the phone to your body
3. How close you are to the base station. If the link to the base station is weak, the handset increases its radiation level to compensate.

The levels of RF radiation from the handset, to which your head is exposed, are around 100 to 1,000 times more intense than exposure from base stations. The newly introduced smartphones are with extensive and high standard features which distinguishes them from other simple phones. The International Union for telecommunication measures those with Internet connection, which it calls Active Mobile-Broadbandsubscriptions (which includes tablets, etc.). Theusage of earlier simple mobile phones has now been mostly replaced by the modern smartphones in developed countriesand nearly 50 percent in developing countries [9].

Mobile Phone Radiations

The electromagnetic energy that travel with the speed of light in space constitute radiations. They are also referred to as electromagnetic radiation (EMR)and are classified into two broad groups1) Ionizing radiations (IR) which are capable of causing changes in atoms or molecules in the body that can result in tissue damage such as cancer e.g., x-rays and gamma rays. 2) Non-ionizing radiations (NIR) are thosewhich doesn't cause these changes, but can prompt molecules to vibrate. This can lead to rises in temperature, as well as other effects e.g., infrared radiation, visible light, light bulbs, ultraviolet radiation in sunlight, microwave energy and radiofrequency energy [10]. In other words a radiation is energy that's travelling through space in the form of waves or particles. It occurs naturally and has always been around, we've evolved with it and we're bombarded with it in one form or another every day of our lives - from the earth, from space and even within our own bodies. Mobile phones emit electromagnetic radiation and use and make and receive calls by utilizing radio frequency (RF) waves. . EMRcan also be defined as the waves with electromagnetic radiant energy propagating (radiating) through space and having a definite electromagnetic field [11].

Classically, electromagnetic radiation consists of electromagnetic waves, which are synchronized oscillations of electric and magnetic fields that propagate at the speed of light.Such oscillations of both electric and magnetic fields are perpendicular to each other as well as to the direction of energy and wave propagation under homogeneous, isotropic mediathus forming a transverse wave [12]. The wave front of electromagnetic waves emitted from a point source (such as a light bulb) is a sphere. Within the electromagnetic spectrum the position of an electromagnetic wave can be localized by its frequency of oscillation or its wavelength. These waves were given different names depend upon the difference in their frequencies as produced by different sources. They are known with the names such as radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays in order of ascending frequency and descending wavelength. Electromagnetic radiation can travel through empty space. Most other types of waves must travel through some sort of substance. For example, sound waves need either a gas, solid, or liquid to pass through in order to be heard.The speed of light is always a constant. (Speed of light: 2.99792458 x 10^8 m s^{-1}). Wavelengths are measured between the distances of either crests or troughs. It is usually characterized by the Greek symbol λ [13].

Facts related mobile radiation exposure

1. There are more than 100 million mobile subscription in the world in which India is the second most user [14, 1].
2. A 2014 survey by Ipsos report that India accounts for the highest among 24 countries, in the number of child cyber bullying cases (32%) compared to the U.S (15%) or Great Britain (11%). About 70 % of Indian teens spend over five hours on the Internet in a normal week, out of which 27% kids use smartphones, says a McAfee Intel survey report[15].
3. About 78% of population is using 3G and 35% is using 4G network [16].

Mobile Phone Radiation: Human Health

The introduction of the Universal Mobile Telecommunications System (UMTS) hasincreased the use of mobile phone technology which indirectly increased the number of mobile phone base stations (MPBS) all over the world [17]. This modern technology development raised public concerns and substantial controversy about the potential health effects of the radiofrequency electromagnetic field emissions.Due to electromagnetic exposure a chunk of population showed some non-specific symptoms of unhealthiness which include sleeplessness or headache. During 1930s scientists began to claim that high-frequency electromagnetic fields (EMFs) may cause health problems [18]. It was the beginning of the debates on human health and microwave radiations. One of the famous person 'Kundi' writes, "Because of the enormous increase in mobile phone use starting in the mid-1990s and reaching almost 100% prevalence in many countries worldwide by now, concerns have been raised that even small risks for developing chronic diseases such as cancer from mobile phone use may have substantial impact on public health". It was

observed that EMR cause severe damageto different essential organs of human body viz., reproductiveorgans, lungs, Kidney, liver and heart [19-22], and affected negatively the motility of spermatozoa [23].The intracellular pathway may get altered by EMR through changes in permeability of Ca^{+2} ions across cell membranes and cellular calcium levels [24]. According to ICAR microwave radiation is damaging endocrine, reproductive and nervous system, but in human being it is not confirmed and retained in the category of possibly carcinogenic. Although many toxicological symptoms have been shown in different studies but all these effects are not reported in human being so further study is needed to have conclusive remarks.

The effects of mobile phone radiations on human health are the subject of recent study and interest, as the enormous increase in mobile phone usage throughout the world [25]. Mobile phone radiations are electromagnetic radiations in the microwave range. Digital wireless system, such as data communication networks, also produce similar radiations. Health symptoms of mobile phone radiation have been investigated by many scientific studies. These scientific studies are occasionally reviewed by some scientific committees to see overall health risks. WHO will conduct a formal risk assessment of all studied health outcomes from radiofrequency field's exposure [26].The make the assessment of number of risks by use of mobile phones the European Commission Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) was published in 2007[27].

Physical Effects

The non-ionizing electromagnetic waves affect the human health directly and indirectly. When high frequency radiation is absorbed by human body, then power is absorbed it. The most common complaints due to the power absorption are memory loss, behavior change, headache, sleep disruption, discomfort, depression, nausea, dizziness, irritability, appetite loss, numbness, muscle spasms, tingling, altered reflexes, subjects reported buzzing in the head, light headedness, palpitations of the heart, heat, cardiovascular problems, visual disorders, agitation, nervousness, respiratory problems, etc [28]. More severe reactions include paralysis, seizures, stroke and psychosis. Mobile phone radiation can damage hair cells whose age is between 18-25 years. These hair cells do not regenerate causing hearing problems in human being. All these changes are related to electrical activity of the human brain [29].

Thermal Effects

The physiological mechanism of mobile phone radiation related health effect is not well known. The effect of high frequency radiation which may lead to an increase in temperature of human body tissues is called thermal effect. These effects may cause disruption of cell function and their development [30]. The tissue damage could occur in human beings due to the inability of body to dissipate the excessive heat. The testis and the eye are vulnerable due to lack of blood flow to dissipate the excessive heat [31]. The electromagnetic field induce dielectric heating effect by rotation of polar molecules which is responsible for ill health. At the time of using mobile phone there is increase in temperature of head by some fraction as most of heating effect is on the surface of head but is magnitudelower than theobtained during the exposure of head with direct sunlight. The blood brain circulation is capable of disposing of excessive heat. However the cornea of human eye does not have such mechanism of temperature regulation and exposure of 1–2 hours duration, SAR values from 100-140W/kg produce cataracts in rabbit's eyes which mayproduce temperatures of 40°C [32]. Microwave causes dielectric heating effect in the human body. Human tissue is rich in water and exhibit dielectric property (+ and –ve ions). Such as water living tissue heat up through the rotation of polar molecules. This friction causes heating of tissue. Head is in the near field of radiation so that most of the heating effect occurs in the head. Temperature in the internal ear brain etc increases to 1 degree or more. Mobile phone base station power is usually limited to ensure that neighboring cells do not interfere with each other, not for health reasons. Typical base station power levels are really low compare to an analog radio or television transmitter that radiates tens or hundreds of kilowatts even megawatts in some cases.

Blood Brain Barrier Effects

Researchers from Lund University in Swedish have studied the effects of microwave radiation on brain of rat. During their research they found a leakage of albumin into the brain via a blood brain barrier [33-35]. This study on the blood brain barrier was confirmed by H. Allan et. al [36]. Other researcher groups have not confirmed these findings in whole animal studies or vitro cell studies.

Male Fertility and Semen Effects

The electromagnetic wave transmitted by the mobile phone handset can have an effect on not only the general body but more specifically on the male reproductive organs such as effects on sperm functions, sperm motility, count, morphology. Sertoli cells and Leydig cells have been studied, as well as an analysis of the blood testis barrier and pituitary gland are frequent in studies related to mobile phone radiations [37,38].

Pregnant Women and Risks to Children

The effect of mobile phone radiations on children are more as they absorb more energy than adults from the same phone owing to their smaller brain size and head, thinner skin and thinner cranial bones, lower blood more elastic ears, lower cell volume, greater conductivity of nerve cells and the energy penetration of energy is more deeply. Brain tumors are more deadly as compared to temporal lobe. Immune system of children is not as well developed as compared to adults; therefore they are less effective against fighting cancer growth than children. It was found that mobile phone radiations are harmful for women during pregnancy and greater likelihood for congenital malformations, spontaneous abortion and behavioral problems in their children. It also found that the eggs which form the embryo are damage and affect will become apparent after the child reaches puberty [39]. According to Russian National Committee of Non-Ionizing Radiation Protection use of mobile phones by pregnant women and children should be limited. It is seen that children talk on the mobile phone are likely to suffer from decline of attention, disruption of memory, cognitive abilities and diminishing learning increased irritability in the short term and long term hazards include degeneration of the nervous structures of the brain and depressive syndrome [40].

Effect on Skin

Mobile phone and their tower radiations affect human skin. Those People who often talk on mobile phone handset have higher concentration of Transtyretin protein than who do not. Transtyretin protein is formed in the liver which helps transport of vitamin A in the body and plays an important role in nervous diseases such as Alzheimers [41].

Ear Damage and Tinnitus

Tinnitus is the psychological disease of sensation of mobile phone ring and hearing phantom sound and it has been reported among millions of mobile phone users in the world. Tinnitus is also known as Ringxiety. People with tinnitus may have trouble of hearing or even sleeping. Mobile phone radiations may damage the delicate workings of the inner ear and excess use mobile phone for more than four years and for longer periods than 40 minutes in a day are at higher risk of developing hearing loss [42]. Such auditory perception has been occurring when a human head is exposed to high energy microwave. When human head absorbs microwave pulse it launches a thermo elastic wave of acoustic pressure that travels by bone conduction to the inner ear [43]. Now a day more and more young people between 17 and 24 years of age are suffering from hearing loss, which doctors say this is due to excessive use of mobile phones . Good hearing depends on the health of some 16,000 hair cells present in each inner side of ear. But doctors have treating the people whose hair cells have been damaged by mobile phone radiation. Hearing problems occur because these hair cells do not regenerate. Person who spends three to four hours on the mobile phone every day are at risk of partial deafness within four to six years.

Effect on eye

Excess use of mobile phones can damage the visual system of the eye which may cause the tumors. Tumors involve in choroid (97%), iris (1.5%) and unknown parts of the uveal (1.5%). Experiments and computational modeling with

several laboratory animals show that mobile phone radiation of frequencies 800, 900, 1800 and 2450 MHz can cause chromosomal breaks in the corneal epithelial cells and increase the temperature of human eye [44]. Temperature of eye lens increases as low as 2oC can result in lens opacities. When human eye lenses were exposed to mobile phone radiation it cause macroscopic damage and affect the optical function of the lens. The damage of lens increased to reach a maximum level after a number of days.

Cell Phone Weaken Bones

Bone density at upper rims of the iliac wings in men has been measured by researchers who were using mobile phone and carried on their belts. Iliac wings are widely used as a source for 9 bone grafting, so any reduction in bone density leads to reconstructive surgery. It is better to keep the mobile phones away from the body during daily lives [45].

Effect on Heart Beat

Heart rate or pulse rate has been regarded as an important part of human health and high pulse rate is regarded as not good for health. Several observation and experiments a establish that radiation emitted from mobile phones handset may influence heart rate or pulse rate and change the autonomic balance. The heart beat or pulse rate in age groups 10 to 20 the pulse rate varies 76.84 to 78.20 age group 21 to 30 pulse rate varies from 80.46 to 83.45, age group 31 to 40 pulse rate varies from 83.02 to 85.30 and age group 41 to 80 pulse rate varies from 79.78 to 82.84 beats per minute during mobile phone call duration. It is also observed that the pulse rate increased very rapidly when mobile phone is used in vibration mode which is harmful for human being but the influence of speaking cannot be excluded. It has been observed that vibration on mode has highest pulse rate, and pre- exposure mode has lowest pulse rate as compared to other two modes of vibration [46-48].

Although lot of work has been carried out to ascertain the actual effects of mobile phone uses on the human health, no factual evidences were concludedabout its harmful effects in humans.Due to the advancing of mobile phone technologies at faster pace it looks that research for it development is far ahead of the research done for finding out and ascertaining the effects it causes on human health. Lot of research is to be needed to finalize the main problems and adverse effects onhuman health caused by its use.In thisreview paper we try to mention all the possible effects found so far due to exposure to EMRemitted from mobile phone. Some of important effects are as under,

1. When a person is on call the blood pressure slightly increased while as it returns to normal as we disconnect the call. That means our blood pressure will continue to fluctuate throughout the day and is much affected when a persons is speaking on call [49].
2. The prolonged use may cause increase in brain temperature which may disperse as we a person stops phone thus may not cause harm [50].
3. The persons which are using mobile phones are have been found suffered with benign tumors' of the acoustic nerve two times more as compared to those persons which don't use phone as per the recent studies done at Sweden. On the other hand there were no confirmationsthat persons using a mobile phone were suffering from some fetal disorders like cancer, tumor, brain damage or memory impairment[51].
4. As per some of the studies the health risks due to mobile phone use were low but some persons get more affected than others thus are more susceptible tomobile phone radiation. The mobile phone radiationsemitted during use of phones were very low doses so precautions should be taken at the time of phones use and shouldreduce our exposure to phone radiation[52].
5. The mechanism of relationship between RF energy and the human body is heating of body tissue.So at the time person's tissue got directly exposed to microwaves some of the violent deformations may lead to microwave sickness. Number of disorders like Insomnia, night sweats, sleeplessness, headaches, dizziness, Increased heart rate, blood pressure, behavioralchanges, Swollen lymph nodes and a weakened immune system, Impaired

cognitive function, finally depression, irritability, Vision and eye problems, Frequent urination and extreme thirst may be found in persons which high exposure to microwave radiations [53-56].

Precautionary Measures to Lower the Exposure to Radiations

Moving your phone 20cm away from your head reduces radiation doses by about 98%. So instead of placing it under your pillow when going to bed, leave the phone at a bedside table.Hands free headsets dramatically reduce radiation emissions into the Brain.Try not to chat for hours on end or, if you must, get a hands free kit.There are a few devices on the market that you can fit to your phone that reduce the emissions of radiation or allow the body to neutralize the effects, but beware of over-hyped promotions by these manufacturers using scare tactics to market their products.It's always sensible to take precautions where children are concerned as their developing brains and bodies are far more susceptible to radiation effects than adults, absorbing radiation at three times an adult's rate [57-60].The population is using different frequencies at unlimited durations as the earlier studies reported the toxicological effects microwave radiation. So present investigation of mobile phone frequency radiation toxicity will strengthen our understanding on toxicological manifestation in human being. Further research work could be conducted, it is necessary to conduct laboratoryexperiments to prove the effects of mobile phone radiation on human body with the help of Faraday Cage. Explore the new mechanism inside the mobile phone this can filter the harmful radiations automatically. Scientists have to develop new device (chip) in our homes which may be installed in walls or any type of material in the form wall paint this can reflect only harmful radiations of the mobile phone towers. Prepare new handset gives alarm automatically when the radiation exceeds the safe limit. It could be develop online software for measuring of radiation level in cities and the data to be sent to the central information centre [61-62].

CONCLUSION

Mobile phone transmits and receives the electromagnetic wave, so, it can be considered as the transmission tower as well as receiver. The electric fields are computed around the mobile phone and mobile phone tower. After comparing the theoretical computational with the safe limit of electric fields and specific absorption rate, we came to the conclusion that frequencies of mobile phone radiation are harmful for general public exposure at various distances. It is cleared from this reviewis that electromagnetic radiations of mobile phone frequencies may affect the health of the human body. Further research is needed to determine the human health effects and their possible relevance if any. Very little work has been done in the mobile phone frequencies which need further investigations. The penetration of electric field inside different tissues of human body has not been estimated. Specific absorption rate (SAR) has been observed only for some tissues. There is need to compute SAR for more tissues. The safe distances from mobile phone tower and safe frequency range needs further investigation. There is also need to compute the increased temperature in tissues of human beings.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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RESEARCH ARTICLE

Novel Approach to Caffeine Extraction Recrystallization and Findings using Liquid-Liquid Method

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ABSTRACT

Caffeine belongs to class of organic compounds called as alkaloids.The study performed on the extraction and characterization of caffeine from tea leaves. The caffeine extraction was executed throughliquid-liquid extraction methodusingdi-chloromethane acting as extracting agent. It involves four steps: steeping, evaporation, liquid-liquid extraction and recrystallization. The UV-Visible spectral characteristics of extracted caffeinewasevaluated using different solvents. The results indicate caffeine has higher absorption in dichloromethane as compare to other solvents.

Keywords: Caffeine, Tea Leaves, UV Spectroscopy.

INTRODUCTION

Tea refers to the agricultural products of the leaves, leaf buds and internodes of the Camellia sinensis plant. Over 2000 years have passed since tea has been consumed as beverage. After water tea is the most loved and consumed beverage across the world. In order to understand the composition structure and effect of tea on human bodies a great deal of scientific effort has been made to isolate and identify the active components in tea [1]. Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class. It is the world's most widely consumed psychoactive drug. It is white and bitter in nature. It is basically a methylxanthine alkaloid and has found out that it is chemically similar to adenine and guanine bases DNA and RNA.

Caffeine is a bitter, white crystalline purine, a methylxanthine alkaloid, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA. It is a natural pesticide. Caffeine does not counteract the effects of alcohol. As Caffeine is a xanthine alkaloid compound it acts as a stimulant in humans. There are several known mechanisms of action to explain the effects of caffeine. The most prominent is that it reversibly blocks the action of adenosine on its receptors and consequently prevents the onset of drowsiness induced by

adenosine [2,3].Many consumers prefer to avoid caffeine partially or altogether, due to its stimulant effects and others, still on health concern. This makes decaffeination of tea an important industrial process. In addition, caffeine has a slightly bitter flavor. As a result, decaffeinating coffee beans and tea leaves will leave the flavor slightly changed, even if no other components are lost. It should be noted that, decaffeinated coffee and tea are not caffeine free. Caffeine also stimulates certain portions of the autonomic nervous system.

The present study presented in extracting caffeine from tea and characterized it by melting point and UV spectral analysis. The adopted method provides qualitative way to extract and analyze caffeine. The rest of paper is organized as follows: section -2 presents literature review; section-3 experimental; section-4 results and discussion; section-5 conclusion.

Literature Review

There have been several works by various investigators on extraction and characterization. In a research done by Huet al. [4], using heat flux and ethanol caffeine was extracted from tea. A solution of water and ethanol was refluxed at 85^{o}C for 45 min.Using filter paper, the solution was filtered and then centrifuesed at 400rpm. Ramli et al. [5] had used different types products like cocoa powder, liquor & beans etc. to find the caffeine content in it using High Performance Liquid Chromatography (HPLC). Mumin et al. [6] had used High-Performance Liquid Chromatography (HPLC) on tea, coffee and soft drinks for extraction and characterization of caffeine present in it. Insolation, purification and characterization of extracted Caffeine from tea and coffee was performed and analyzed. Abourashed and Mossa [7] had presented a research on presence of caffeine in herbal products using HPTLC. They analyzed the caffeine content in selected herbal products and energy drinks available in the Saudi market by HPTLC–UV densitometric.

EXPERIMENTAL SECTION

Plant Materials & Chemical

The caffeine extraction was performed on the tea leaves that were purchased from the local market. In general, the caffeine contain in tea leaves is about 2 to 5% and 10-35% of polyphenols. All then chemicals used in the experiment are of analytical reagent grade chemicals

Spectroscopic Characterization

The UV visible spectroscopy analysis is done to find the useful information regarding composition. The visible absorption spectra of extracted caffeine samples were recorded. For the identification and characterization of extracted compound, the resultant compound was mixed with alkaline compound. Visible spectrophotometric measurements were made between 350 and 650 nm

Standard solution preparation

Different types solvents were used in order to characterize the extracted caffeine. The extracted compound was dissolved in different solvents to determine the caffeine content in it. The extracted caffeine was immersed into dichloromethane, water and chloroform. The solution was then magnetic stirred for 45mins. The absorption spectroscopy were recorded using UV visible spectroscopy.

Extraction of Caffeine

About 10 gm of red tea was taken in a clean beaker then it was treated with one base to extract caffeine i.e 1gm of sodium carbonate. Then 100 ml of distilled water was added to it. It was stirred some time and the total mixture was placed on a heat mantle for 10 to 15 min, by filtering the residue is being separated. Again, to extract remaining amount of caffeine was boiled with more distilled water for 10 minutes.

Now all extract was mixed and transformed into another beaker and again it was filtered by the help of Whatman filter paper. For extraction of caffeine all filtrate was transferred into a separating funnel and 10 ml of dichloromethane / chloroform was added to it for formation of two immiscible layer. After agitating it was stand for 15 to 30 minutes. It was observed two distinct layers. Now bottom layer was collected which consist of caffeine. This process was repeated for 2 to 3 times for better production of caffeine. All the organic layer that has been collected was transferred to a Petridis which was covered with a perforated aluminum foil. After the evaporation of methylene chloride, the product which was left that is caffeine which was characterized by UV-visible spectroscopic method.

RESULT AND DISCUSSION

Two sample were prepared for extracting caffeine from tea. For sample-1 dichloromethane is used for the extraction and sample-2 was extracted using chloroform. Both the sample weigh 5gm each. The Table-1 show the amount of caffeine extracted from samples. It is evident that the extraction efficiency of caffeine using dichloromethane is superior as compare to using chloroform. As shown in table -1 the amount caffeine obtained from using dichloromethane is 1.22mg as compare which is higher than that of amount extracted when chloroform was used. The absorption rate of extracted caffeine was measured using UV-visible spectroscopy within the spectral range between 200nm to 380nm. The extracted caffeine was dissolved in three different solvents water, chloroform and dichloromethane. Table-2 represents the experimental result of the absorption rate of caffeine in different solvents.
In each solution one peak is observe at 280nm.As the obtained caffeine was not in the pure form so its Melting point value was little less from its actual value. From 5 gm of tea leaves about 1.22 mg of caffeine was obtained. Which is then proceed for characterization process for confirmation of caffeine. The figure -1 show the UV-visible spectroscopy of the extracted caffeine taken after dissolving it in three different solvents.

From the spectra, it can be observed that caffeine absorbs in the spectral range between 200 nm to 340 nm in dichloromethane and in water the spectral range is between 200 nm to 300 nm with max at 280. For chloroform caffeine absorbs in the spectral range between 200 nm to 320 nm with max at 300 nm. Hence it can be concluded that the dichloromethane is far better solvent as compare to any water and chloroform

CONCLUSION

Aninnovative procedure has been developed and followed for the extraction of caffeine from tea. The caffeine was extracted using liquid-liquid extraction followed by recrystallization. The extracted caffeine was effectively characterized using UV- Visible spectroscopy which is found to be relatively easy and cheap and extremely sensitive for the determination of caffeine content in tea leaves. The result of the experiment also indicates the potential use of dichloromethane as solvent has higher efficacy compare to other solvents.

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Table 1. Amount of caffeine extracted from Tea

Extraction Caffeine from Tea			
Sample	**Solvent used for Extraction**	**Amount of sample in gm**	**Amount of Caffeine Extracted in gm**
I	Dichloromethane	5	0.122
II	Chloroform	5	0.075

Table 2. Absorption of Caffeine in caffeine in dichloromethane, water, chloroform

Absorption rate of Extracted Caffeine			
Wave Length in nm	**Solvents**		
	Water	**Chloroform**	**Dichloromethane**
200	0.6	0.73	0.84
220	0.62	0.64	0.74
240	0.5	0.62	0.72
260	0.3	0.72	0.81
280	1.5	1.02	0.25
300	0.2	0.02	1.12
320	0	0.01	0.11
340	0	0	0.01
360	0	0	0
380	0	0	0

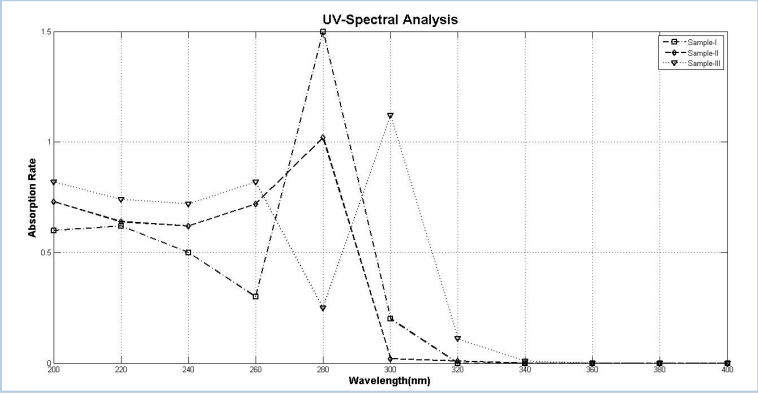

Figure 1. Absorption rate of Extracted Caffeine

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RESEARCH ARTICLE

Tooth Size Discrepancy in Class II Division 1 malocclusion among Orthodontic Patient

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ABSTRACT

The aim of this a retrospective study is to determine tooth size discrepancy of class II division 1 malocclusion among orthodontic patients.This study was carried out using 126 orthodontic study models. The sample was selected using random sampling technique based on the orthodontic patient's list in the Orthodontic Department. Right first molar to left first molar for upper and lower arch were measured by two examiners at the largest mesiodistal dimension, using digital caliper recorded up to 0.01mm. Comparison were made intra-arch using individual tooth size measurement and inter-arch using Anterior Bolton Index (ABI) and Overall Bolton Index (OBI). All teeth had no significant differences ($p>0.05$) in mesiodistal width between left and right except for upper first molar, upper first and second premolar, upper lateral and central incisor and lower lateral incisor. Inter-arch assessment showed that OBI was 91.83±4.48% and ABI was 78.12±5.19%. In class II division 1 malocclusion, upper second premolar and upper first molar were significantly different between left and right. Based on ABI and OBI, no inter-arch tooth size discrepancies detected in class II division malocclusion.

Keywords: Tooth size discrepancy, Bolton discrepancy, Class II division 1 malocclusion, mesiodistal widthy.

INTRODUCTION

An excellent orthodontic treatment result with optimal occlusion, overjet and overbite is often affected by tooth size discrepancies (TSDs). TSDs conventionally have been described as a relative excess or small of tooth structure in one arch in relation to the other arch. It can also be defined as a disproportion among the sizes of individual teeth. We can do a few procedures such as composite build up, prosthetic reconstruction, stripping and crown recontouring for aesthetic compensation. An appropriate balance of mesiodistal tooth width between maxillary and mandibular arches are needed to achieve the possible aesthetic and a good functional results at the completion of orthodontic treatment (1). Tooth size that is within the acceptable range is needed to achieve a good optimal occlusion. This optimal occlusion has been widely accepted as outlined in Andrew six-keys for normal occlusion. He stated that optimal occlusion should have correct inter-arch relationship, correct crown angulation, correct crown inclination, no rotations, tight contact point and flat curve of Spee (0.0-2.5mm). Many studies have been carried out to determine the prevalence of TSDs in various populations. A comprehensive diagnosis and treatment planning should allow us to predict whether we can achieve good interdigitation or not in the end.

A lack of information about tooth size could also compromise the final result in extraction cases if the chosen extraction pattern leads to a clinically significant intermaxillary TSD (2). When comparing with the Bolton's original sample of excellent occlusions, there was more relative tooth size excess in the mandibular arch. Other than that, 17.4% had anterior ratios and 5.4% had total tooth-width ratios greater than 2 standard deviations from the Bolton's mean (3). Clinically significant tooth size discrepancy has generally been defined as 2 standard deviations outside Bolton's mean ratio recently. For anterior discrepancy, there was a greater mandibular excess than the maxillary teeth while the overall discrepancy was almost equally to be an excess in the maxillary or the mandibular teeth (4).The aim of this research was to determine tooth size discrepancy among orthodontic patients in Faculty of Dentistry UKM. The objective for this study were to evaluate the size of the teeth in patient with class II division 1 malocclusion and to assess tooth size discrepancy in class II division malocclusion using Bolton analysis.

MATERIALS AND METHODS

Sample's selection

This was a retrospective study of orthodontic study models taken from Orthodontics department, Faculty of Dentistry, University Kebangsaan Malaysia. The subjects for this research were all study models of patients who have come for their orthodontic treatment in UKM.The UniversitiKebangsaan Malaysia (UKM) Institutional Review Board for Research and Ethics approved this study. 126 study models of class II division 1 malocclusion were selected using stratified random sampling technique based on the orthodontic patient's list. The inclusion criteria for selecting the samples are shown in Table 1.

Tooth width measurement

The right first molar to left first molar for upper and lower were measured at the largest mesiodistal dimension using a digital caliper accurate to 0.01mm (ABSOLUTE Digimatic, Mitutoyo USA) by two examiners. The readings were recorded at the 0.01mm level manually. The measurements of all 126 orthodontic study models were done within one month of data collection with average of 30 study models per week. The anterior and overall tooth size discrepancies were calculated based on the formula described by Bolton as shown below:

$$\frac{\text{sum mandibular “3-3”}}{\text{sum maxillary “3-3”}} \times 100 = \text{Anterior Bolton Index \%}$$

$$\frac{\text{sum mandibular “6-6”}}{\text{sum maxillary “6-6”}} \times 100 = \text{Overall Bolton Index \%}$$

All statistical analyses were performed using the SPSS software package (Statistical Package for Social Sciences, version 24.0). All the data were analyzed descriptively using percentages and frequencies. Comparison tests were performed using independent T-test ($p>0.05$).

RESULTS

Intra-arch assessment

Table 2 shows overall number of teeth, mean and standard deviation of upper teeth. Central incisor was 8.74±0.63 mm, lateral incisor was 7.25±0.62 mm, canine 8.13±3.12 mm, first premolar 7.23±0.57 mm, second premolar 6.90±0.51 mm, and first molar 10.59±0.49 mm. Table 3 shows overall number of teeth, mean and standard deviation of lower teeth. Central incisor was 5.61±0.31mm, lateral incisor was 6.14±0.39mm, canine 6.95±0.47mm, first premolar 7.40±0.51mm, second premolar 7.39±0.49mm, and first molar 11.22±0.55mm. Table 4 shows the p-value between left and right of upper teeth.Central incisor was 0.002, lateral incisor was 0.007, canine 0.219, first premolar 0.000, second premolar 0.038, and first molar 0.000. Table 5 shows the p-value between left and right of lower teeth. Central incisor was 0.664, lateral incisor was 0.046, canine 0.752, first premolar 0.672, second premolar 0.281, and first molar 0.142.

Inter-arch assessment using Anterior Bolton Index (ABI) Overall Bolton Index (OBI)

The ABI for all samples was 78.12 ± 5.19% while the OBI was 91.83 ± 4.48% as shown in Table 5. The ABI and OBI in this research were almost the same with the Bolton analysis (ABI = 77.2 ± 1.65%;OBI = 91.3 ± 1.93%).

DISCUSSION

The importance of tooth size discrepancy in orthodontic diagnosis had been widely reported in literature because the relationship between the upper and lower arch was related to orthodontic treatment outcome. The prevalence of tooth size discrepancy served as an indicator of how important it is to perform a thorough diagnosis before orthodontic treatment. In this research, we wanted to determine whether there is the difference in size of teeth from right first molar to left first molar on upper and lower in class II division 1 malocclusion.

Intra-arch assessment

For maxillary arch, the largest mesiodistal width was first molar followed by central incisor, canine, lateral incisor, first premolar and second premolar. While for mandibular arch, the largest mesiodistal width was first molar followed by first premolar, second premolar, canine, lateral incisor, and central incisor(5). Class II division 1 malocclusion had the largest mesiodistal width of anterior tooth size for both maxillary and mandibular teeth for all classes of malocclusion teeth except for the lower central incisor. The result correlated with our study where we found that lower central incisor had the smallest mesiodistal width while lower first molar had the largest mesiodistal width. Interestingly in maxillary and mandibular arch, central incisor, lateral incisor, canine and first molar on right side were larger in mesiodistal width than left side. While left first and second premolar were larger in mesiodistal width than right. In comparison between left and right, all teeth had no significant differences ($p>0.05$) in mesiodistal width except for upper first molar, upper first and second premolar, upper lateral and central incisor and lower lateral incisor.

Inter-arch assessment using Anterior Bolton Index (ABI) Overall Bolton Index (OBI)

In class II division 1 malocclusion, the ratio of mesiodistal widths of mandibular teeth to maxillary teeth was assessed using ABI and OBI. Bolton had recommended the ABI of 77.2 ± 1.65% and OBI of 91.3± 1.93%. In our study, the ABI was 78.12 ± 5.19% while the OBI was 91.83 ± 4.48% for the whole sample. The ABI and OBI were slightly similar to the Bolton's sample. Study done among Brazilian population, the ABI was 78.16 ± 2.21% (6). Recently, Malaysian study found that the ABI's and OBI's value were slightly similar to the Bolton's value which were 77.7 ±

2.89% and 91.6 ± 2.20% respectively (7). However, there were studies which found that there were different from the Bolton's norms. There were significant differences between the Spanish's and Bolton's values in Spanish population (8).No significant inter-arch tooth size discrepancies were noted between the malocclusion groups in Japanese orthodontic population (Endo et al, 2008).

CONCLUSION

This study found that in Class II division 1 malocclusion among orthodontic patient in Faculty of Dentistry UKM, lower right first molar had the largest mesiodistal width while lower left central incisor had the smallest mesiodistal width. However, the Anterior Bolton Index (ABI) and Overall Bolton Index (OBI) had no significant difference compared to Bolton's value.

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Table 1. Inclusion and exclusion criteria

Inclusion criteria
• All study models with complete pre-treatment models. • Good quality study models which unbroken model, non-fractured tooth and absence of bubble. • Fully erupted and complete permanent dentitions from first molar to first molar.
Exclusion criteria
• Clinically visible dental caries, gross restorations, proximal restorations (Class II amalgam or composite), build up, crown and onlay that affect the tooth's mesiodistal diameter. • Obvious interproximal or occlusal wear of teeth.

Table 2. Total number of samples (N), mean ± standard deviation of each upper teeth

Teeth	Central incisor	Lateral incisor	Canine	First premolar	Second premolar	First molar
N	126	126	126	126	126	126
Mean	8.74 ± 0.63	7.25 ± 0.62	8.13 ± 3.12	7.23 ± 0.57	6.90 ± 0.51	10.59 ± 0.49

Table 3. Total number of samples (N), mean ± standard deviation of each lower teeth

Teeth	Central incisor	Lateral incisor	Canine	First premolar	Second premolar	First molar
N	126	126	126	126	126	126
Mean	5.61 ± 0.31	6.14 ± 0.39	6.95 ± 0.47	7.40 ± 0.51	7.39 ± 0.49	11.22 ± 0.55

Table 4. The p-value between left and right of upper teeth

Teeth	11 & 21	12 & 22	13 & 23	14 & 24	15 & 25	16 & 26
p value	0.002	0.007	0.219	0.000	0.038	0.000

Table 5. The p-value between left and right of lower teeth

Teeth	31 & 41	32 & 42	33 & 43	34 & 44	35 & 45	36 & 46
p value	0.664	0.046	0.752	0.672	0.281	0.142

Table 6. Mean and standard deviation of ABI and OBI in class II division 1 malocclusion

	Anterior Bolton Index (ABI) (%)	Overall Bolton Index (OBI) (%)
Mean	78.12	91.83
Standard deviation	5.19	4.48

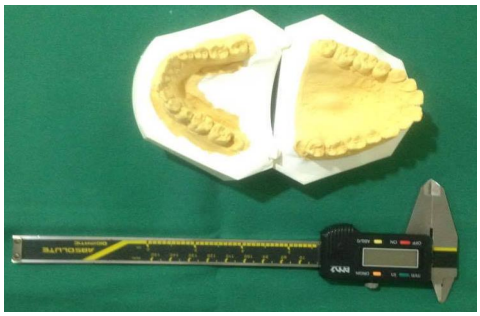

Figure 1. Digital caliper (ABSOLUTE Digimatic, Mitutoyo USA) and orthodontic study model

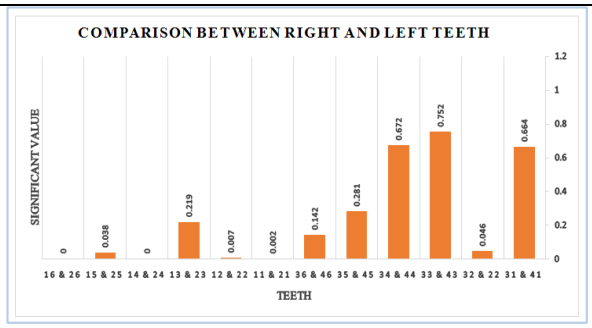

Figure 2. Comparison between right and left teeth

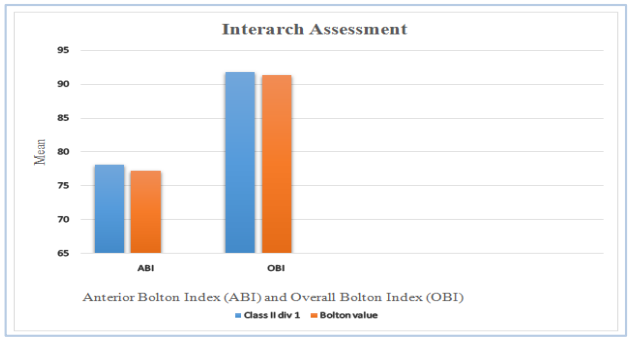

Figure 3. Inter-arch assessment using Anterior Bolton Index (ABI) Overall Bolton Index (OBI)

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RESEARCH ARTICLE

Novel Structural and Optical Properties of Eu doped ZnO Nanoparticles Prepared by Chemical Co-Precipitation Method

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ABSTRACT

We have carried out study to understand the structural and optical properties of nanoZnO by doping rare earth element Eu into it. The synthesis was carried by chemical co-precipitation method taking the precursor materials. The material property is investigated by using techniques like X-ray diffraction, Field emission scanning electron microscopy, Fourier transferred Infrared spectroscopy and photoluminescence spectroscopy. XRD gives information regarding the crystallographic phase and existence of Eu in ZnO lattice. The size of ZnO is obtained as 48 nm and for ZnO-Eu it is 57 nm. FESEM study shows the agglomerated form of ZnO nanoparticles. EDAX confirms the Eu presence in ZnO. So both XRD and FESEM studies give proof of Eu doping into ZnO. FTIR spectra for ZnO and ZnO-Eu illustrate the existence of different elements in different form (single bond/double bond/triple bond) along with metal oxygen bond. The characteristic emissions from ZnO are seen from the PL spectra which are modified using Eu doping.

Keywords: Europium, optical property, Photoluminescence, structural property, ZnO.

INTRODUCTION

ZnO is a novel material having adopted by scientific community for its application in search for new device or industry/technology. ZnO with very small size (0-100 nm) shows very unusual properties as shown in bulk form [1]. Due to quantum confinement effect, the effective widening of band gap leads to new optical properties which find application in optoelectronics. ZnO has some special feature like direct band gap (3.37eV) which is quite high in comparison to others in the same community along with higher value of excitonic binding energy [2]. These two unique features have made ZnO suitable for application in different domains. The list of novelty goes on as it also

shows high breakdown strength. ZnO has gained considerable interest due to its high impacting applications such as UV light emitters, sensors, transistors, solar cell, photocatalyst, varistors, nanogenerators and antibacterial agent. Doping is as innovative way to enhance some the structural, electrical and optical properties [1, 2]. Doping of selective rare earth elements into ZnO has been proven as a successful method for enhancement of photo-luminescent property of ZnO [3].

Change in size, shape and surface morphology of materials yield to modified physical and chemical properties. This concept was utilized by Rodrigues-Paez et al. and by changing parameters like concentration, pH and washing medium ZnO with different morphology was synthesized [4]. To obtain ZnO with novel structure (size/ shape/ morphology), abundant number of methods such as; sonochemical, hydrothermal, electro-deposition, sol-gel, spray-pyrolysis, micro-emulsion, thermal evaporation, chemical/physical vapour deposition, microwave synthesis, pulsed laser deposition, molecular beam epitaxy, sputtering etc. have been developed and undertaken [1-3]. Most of these techniques are complicated and not used in large scale except the chemical methods. Among these, chemical co-precipitation method is very simple and of low cost giving stable nanostructures with reproducibility.

MATERIALS AND METHODS

The samples were synthesized by wet-chemical method as stated above. The Zn and Eu sources as chosen as ($Zn(CH_3COO)_2.2H_2O$) and (Eu_2O_3) respectively. These are ultra pure chemicals of analytical grade. The above materials were taken in distilled water with calculated proportions. To completely dissolve the reagents, it was continuously stirred with a magnetic stirrer. To the above solution, ammonia solution was added to obtain the precipitates. Measurement of pH of the mixture was carried out once before and after the precipitation and all the experiment was carried out in alkaline medium. A centrifuge was used to collect the precipitates. It was washed with distilled water and acetone several times before collection. An oven was used for drying the samples overnight at 100 ^{0}C to eliminate the hydroxide phases. The process of the formation of the materials can be explained in terms of the following chemical reactions.

$NH_3+H_2O \rightarrow NH_3.H_2O \rightarrow NH_4^+ + OH^-$

$Zn(CH_3COO)_2 \rightarrow Zn^{2+} + 2CH_3COO^-$

$Zn^{2+} + 2OH^- \rightarrow ZnO + H_2O$

$NO_3^- + H_2O + 2e^- \rightarrow -NO_2^- + 2OH^-$

The samples obtained such as ZnO and ZnO-Eu were subjected to different analytical measurement techniques such as powder X-ray diffraction (PXRD), Field emission scanning electron microscopy, Fourier Transformed Infrared Spectroscopy, and Photoluminescence spectroscopy.

RESULTS AND DISCUSSION

Characterization method like PXRD is truly significant in ascertaining the structural/crystallographic information of a material. We performed the measurement using Cu source of wavelength 1·5418 Å. The diffractometer gave data which are plotted (2θ vs intensity) and shown in Figure-1. It shows a number of diffraction peaks at different 2θ values in 20–80° range. These are all belongs to hexagonal ZnO. Amongst all the peaks, the (101) reflection shows highest intensity. The polycrystalline nature of the synthesized sample is also verified from the XRD data. Scherrer equation is used to calculate the average crystallite sizes of ZnO and ZnO-Eu samples which are found to 45 and 51 nm. A small peak is seen at 27.96° which belongs to the phase of Eu_2O_3 phase as matched from the JCPDS Card: 36-1451. Structural parameters other than size such as; strain and crystal lattice parameters are also been calculated and shown in Table-1 [5]. Doping of Europium leads to a change in the lattice parameters as observed from the table below.

The FESEM images of the sample ZnO-Eu was captured using the electron microscope and have been illustrated in Figure-2. The images show the agglomerated form of ZnO nanoparticles and a clear picture cannot be obtained for individual nanocrystallites. These nanoparticles are grown adjacent to each other to form multi-structures. To confirm the doping of Eu, we carried out EDAX measurement along with FESEM and the spectrum is shown in the same figure. It gives us information about the existence of elements like Zn, O and Eu which is evident as per our study. So, our experiment for successful doping of rare earth ions like europium into ZnOis fruitful.

The transmittance (%) of infrared light through the sample by different chemically active compounds is studied by FTIR spectroscopy. The FTIR patterns of zinc oxide and europium doped zinc oxide are presented in Figure-3. The pattern of ZnO shows a sharp peak at 480 cm^{-1} which is the Zn-O stretching modes present in ZnO [6, 7]. Further, peaks over the range of 1700-600 cm^{-1} are due to compounds like C=O, C–O and C–H [7]. The other peaks beyond 1700 cm^{-1} are may be due to hydroxyl groups present in the sample [7]. With Eu doping the FTIR pattern changes significantly. One can see one broad peak in the region 500-400 cm^{-1}. This may be the overlap of two peaks corresponding to Zn-O and Eu-O stretching vibrations in that region absorbed infrared radiation with close frequencies. So, the FTIR measurement also gives information on Eu doping supporting to XRD and FESEM results.

ZnO shows good photoluminescence pattern when excited with suitable wavelength of light. The nanopowders of ZnO and ZnO-Eu are excited with 350 nm light and the emission spectra were recorded. These have been presented in Figure-4. The ZnO sample exhibits near UV emission around 400 nm as a clear peak which is suppressed with Eu doping. The intensity of this emission is significantly decreased after Eu incorporation into ZnO. Two more peaks in the visible region are also observed at 428 nm and 456 nm. These emissions in the violet and blue region along with another broad peak at 550 nm are related to deep level emissions linked to defects like zinc interstitials/zinc vacancy/oxygen interstitials [8-11]. With Eu doping the position of these emissions slightly shifted to higher wavelength side. A new peak around 581 nm is observed in the PL spectra of ZnO-Eu. The peak at 581 nm in the emission spectrum of ZnO-Eu is due to the $^5D_0$-$^7F_1$ transition [8, 9, 11]. The energy level diagram of ZnO and ZnO-Eu is depicted in Figure-5

CONCLUSION

In this study, the Eu^{3+}doped ZnO had been successfully synthesized using precipitation method. Characterization of the nanopowders of ZnO and ZnO-Eu exhibit good crystallinity and belongs to hexagonal wurtzite phase. The size of the ZnO crystallites is obtained in nanometer dimension which increased with Eu doping. The FESEM and FTIR spectra support the XRD result on Eu doping. Zn-O and Eu-O stretching modes are observed in low frequency range in the FTIR spectra confirming Eu doping. PL properties of undoped and Eu-doped ZnOnanopowders are studied and explained. The ZnO-Eu sample exhibits emissions due to Eu^{3+} which is feasible due to the energy transfer from the host ZnO to Eu^{3+} ions.

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Table 1. Crystallographic parameters of ZnO and Eu doped

Sample Name	Crystallographic parameters					
	Size (nm)	*Strain*	*a (A^0)*	*c*	*u (A^0)*	*BL (A^0)*
ZnO	48	0.0011	3.249	5.209	0.379	1.9355
ZnO-Eu	57	0.0062	3.247	5.199	0.380	1.9793

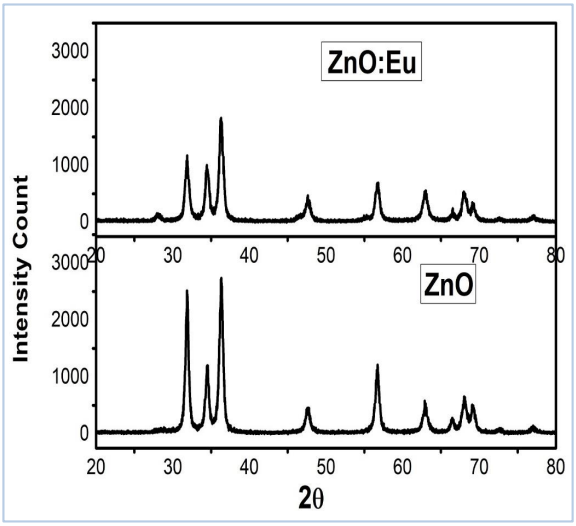

Fig. 1. XRD patterns of ZnO and Eu doped ZnO

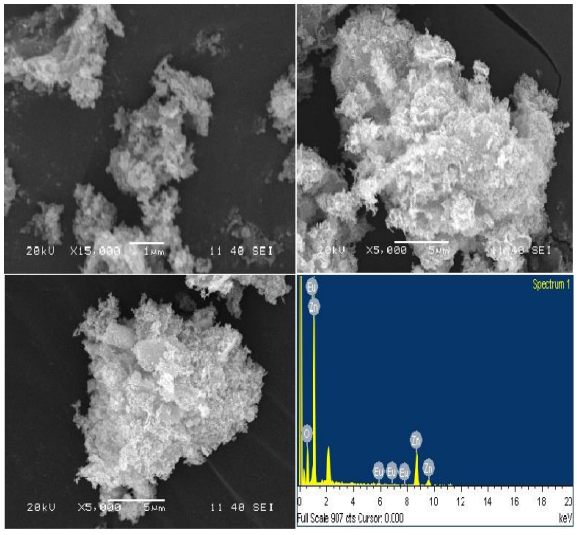

Fig. 2. FESEM images of Eu doped ZnO

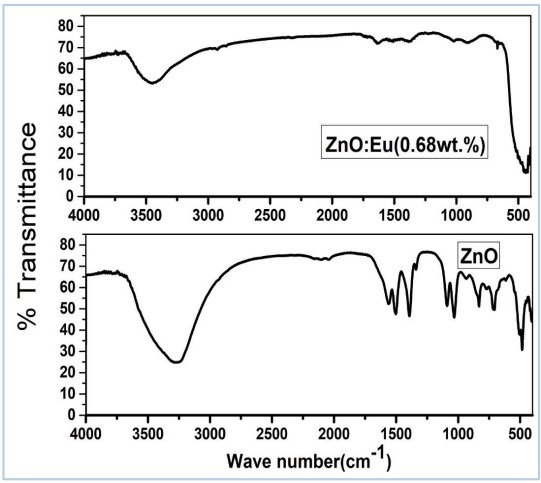

Fig.3. FTIR spectra of ZnO and Eu doped ZnO

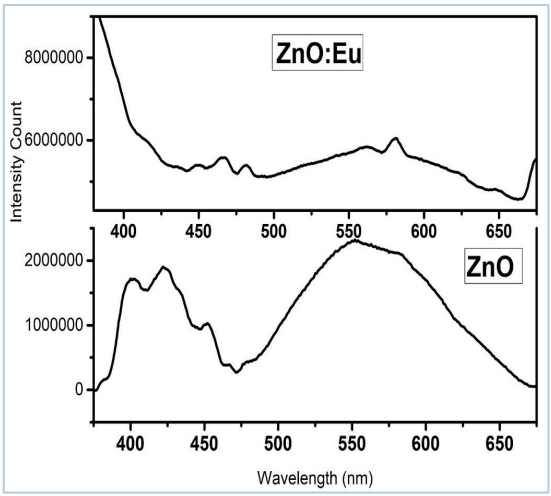

Fig.4. Photoluminescence spectra of ZnO and Eu doped ZnO

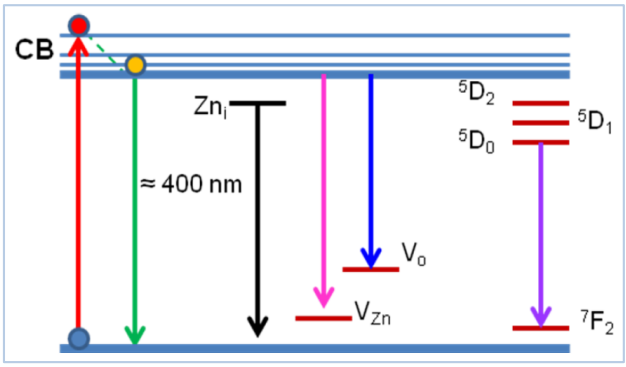

Fig.5. Energy level diagrams for ZnO-Eu

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RESEARCH ARTICLE

Synthesis and Study of Mechanical and Biodegradation Properties of (Chitosan-g-PMMA) / Cockle Shell based Bionanocomposite Materials

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ABSTRACT

The focus on organic–inorganic materials is to form a polymer bionanocomposites, such as organic polymer chitosan grafted with Poly (methyl methacrylate)(PMMA)–inorganic (nano-CaO)spheres of cockle shell(CS) powder. The calcium carbonate nanoparticles have been observed to be biocompatible for use in medicine, pharmaceutical industries, and drug release systems. The significant Chitosan-g-PMMA/nano-CaO is a promising new antibacterial bone cement bionanocomposite polymeric biomaterial. The CS composites were improved the mechanical property strength and functionalized as bone cement composite metrial. It was prepared via emulsion polymerisation technique and physiochemically characterized as bone graft substitute. The so prepared grafted bio based bonecement (BBC) bionanocomposites (BNCs), chitosan-g-PMMA/ nano-CaO was characterized by FTIR, XRD, FESEM and TGA. The water uptake, retention ability and the nanosize particle arrangement in the polymeric BBC-BNCs were studied along with the mechanical property. BNCs exhibited better tensile strength of 37.5MPa and compressive strength of 45MPa. Bionanocomposites also make them good choices to use as bone cement martial implants in future.

Keywords: Chitosan, cockleshell, Bone cement, Bionanocomposite.

INTRODUCTION

Currently, research with respect to cockle shells, biowaste is strongly encouraged in our society for green environmental and economic reasons. In which the primary component is approximately 98-99% of calcium carbonate, is in its infancy [1,2]. Calcium carbonate nanoparticles are abundant inorganic biomaterials with different

morphological structures that have attracted the interest of researchers in different fields.The valuable calcium carbonate nanoparticles have been observed to be biocompatible for use in medicine, pharmaceutical industries, and drug delivery systems [3,4].The chemical composition and availability makes CS a potential source of filler for polymer composites. Better attention has been given to the study of bio-filler reinforced bio-polymer composites. Particularly, large bone defects are a major clinical problem since autologous bone grafts are not available up to 40% of these patients [5]. Therefore, there is a pressing need for more reliable and abundant bone substitutes to replace or repair bone filling defect with either PMMA or bio-absorbable bone graft substitutes – ceramics – in cement defects in clinics. PMMA individually has not gained wide acceptance when used for fracture treatment in the extremities because of its inability to remodel and possible inhibition of fracture healing [6]. Among many potential bone cement materials, PMMA or its derivatives has been used successfully in orthopedic surgeries. PMMA was first introduced as bone cement in the early 1960s by Charnley and Smith [7]. It was weaker than cortical bone [8].

It has a high exothermic reaction temperature [9,10] and exhibits the monomer toxicity [11]. To address the concerns regarding PMMA, several investigations of different bone cements (BC) have been conducted [12] modified PMMA by adding hydroxyapatite (HA) powder. Other researchers have added bone particles and growth hormones to PMMA cement [13,14]. Chitosan based PMMA composite exhibits biological properties such as biodegradability, biocompatibility, immunological and antibacterial activities. Although improvements have been realized, many fundamental problems with PMMA remain unresolved. Polymer-layered nano-CaOis represent a new class of materials with high performance and is of great academic and industrial interest. The important properties of nanocomposites are greatly dependent on the filler's aspect ratio, surface area and interactions between the filler and polymer matrix. For example, layered silicates and fibers exhibit good reinforcing effects on many polymeric matrixes due to their large aspect ratio, but this kind of filler with a high aspect ratio does not obviously improve the toughness, and sometimes even decreases it [15].

In contrast, spherical mineral nano particles are quite different. Their low aspect ratio but large surface area could result in a strong interfacial interaction between filler and polymer matrix. Different techniques for the research of calcium carbonate nanoparticles have been reported, with the precipitation of homogeneous solutions [16], the emulsions of oil-in-water [17], the most significant aspect with respect to the synthesis of nanoparticles is control of the particle size, polymorphism and morphology of the desired material. Control of this parameter has led to the improvement of new materials with unique properties that differ from those in the bulk material [18]. These particles were reported to be able to greatly increase strength, modulus, as well as toughness. Nano-$CaCO_3$ is one of the most common spherical nanoscale fillers. The nano $CaCO_3$ is known as nano-CaO composition of $CaCO_3$ used in preparation of nanocomposites [19]. Exfoliated structures, however formed because of extensive penetration of polymer into the nano-CaO resolution in the determination of the nano-CaO layers, and there fore, a loss of the ordered structure.The purpose of this study was to develop novel BC, composed of nano-CaO of CS and chitosan, for use in orthopedic surgeries such as a bone filler. The addition of nano-CaO of CS to a BBC is annovel study that has not been reported in the literature [20].

MATERIALS AND METHODS

Materials

The deacetylated chitosan and the initiator, ammonium persulfate, were purchased from HIMEDIA, Mumbai, India. MMA was purchased from SRL India Ltd., sodium hydroxide, hydrochloric acid, ethanol, orthophosphoric acid, diethylether and sorbitol were purchased from Qualigen India Ltd. The CS were collected from a sea beach and washed wish demineralised water for 3 times and then oven dried at 120 ^{0}C for 2 h.The filer nano-CaO of CS was prepaired in Laboratory. Deacetylation of chitosanto increase the amine content of chitosan , chitosan with 76% of deacetylation degree was further deacetylated according to the modified procedure to increase the deacetylation degree above 90% . Degree of deacetylation (DD) or % NH_2 group is determined by potentiomeric titration method

as per the report [21]. The DD chitosan sample prepared, 0.14g chitosan into 35mL NaCl solution (0.1mol/L) and the add 14ml HCl(0.106mol/L)of the standard solution to get protonation and thus increase its solubility. Adjust the pH value to 6 by adding NaOH (0.01mol/L) of the standard solution and calculation the total volume of NaOH solution consumed for the other replicates and then add 0.3mL cellulase solution (160U/mL). After 40^{0}C water bath incubation for 6 h, chitosan solution is taken out and replenished with HCl solution and the pH is adjusted back to about 2.5. By adding NaCl the total volume maintains at about 50ml.with the aid of the pH meter, chitosan is titrated with NaOH and then after the two abrupt pH changes (pH>10.5),retitrated with HCl solution making the the pH back to <3. In process of two abrupt pH changes, both the pH values and the consumption of NaOH (or HCl) are recorded. The degree of deacetylation is calculated by using the following formula.
The following formula

$$DD=\frac{203.196\times w(NH2)}{16.02262+40.47037\times w(NH2)}$$

$$W(NH_2)=\frac{V\times C\times 100\times 0.016}{Wdry}$$

Where V, C and W dry stand for volume of NaOH (or HCl) consumption between two abrupt change of pH, concentration of NaOH(or HCl), and the dry weight of chitosan sample (derived from the moisture content) respectively. DD and w (NH_2) are based on the percentage.

Synthesis of Calcium Carbonate Nanocrystals

The clean and dry CS were dried in oven once again over 24 h at 120 ^{0}C. Then the dried. CS were crushed and grinded in a mechanical attritor. The CS powder was reduced by adding 10% o-phosphoric acid and then the sample was heated in a furnace at a temperature of 900^{0}C for 8 h. Upon cooling, the sample was stored in a descicator. The $CaCO_3$ at about 900^{0}C was completely decomposed and turned to nano-CaO [22].The Samples of the CS were dried in an oven at 60^{0}C for 5 days, which was sieved through a 90-m laboratory stainless steel sieve (Endecott, London, England). The calcium carbonate powders were finally packed into a sample holder for further analyses.The synthesis of calcium carbonate nanocrystals was performed through oil-in-water (O/W) microemulsions. In this technique, the particles sizes are reduced after leaving the homogenising gap by cavitations, particle collisions, and shear forces [23]. The calcium carbonate nanocrystals were prepared by the dissolution of 3gm of dry cockle shell powder in a formulated oil-in-water (O/W) microemulsion, which was moderately stirred by a magnetic stirrer for 5 min at 100 rpm to form a calcium carbonate suspension. The product of the crystal suspension was filtered and dried in an oven at 105^{0}C for 24 h.This result indicates the total decomposition of calcium carbonate to nano-CaO and signifies the release of CO_2 from calcium carbonate, leaving only the product, that is, “ash”[24].

Preparation of chitosan-g-PMMA and Chitosan-g-PMMA/nano-CaO of

To make the grafted composite i.e. chitosan-g-PMMA (BBC1): chitosan (0.20gm), monomer (MMA), initiator (APS) along with complex initiator $CuSO_4$ and glycine (1:1) and surfactant sorbitol (0.05gm) were added sequentially to the reaction vessels. Then a different set of kaolin based graft copolymer samples were prepared, taking the same quantity of monomer MMA and chitosan in the reaction vessels followed by addition of CaO (BBC2 = 0.25 %, BBC3 = 0.5%, BBC4 = 0.75%, BBC5 = 1.0% and BBC6 = 1.5%) to each vessel as shown in Table 1. The mixtures were stirred overnight around 14 h for complete insertion of monomer into the chitosan at 25^{0}C. Under N_2 atmosphere desired quantity of initiator APS, complex initiator $CuSO_4$ and glycine (1:1) along with the surfactant were added at 55^{0}C. Then the reaction was continued with stirring for 3h and then ceased by quenching the vessel in ice water. The graft copolymer and the additive based graft copolymer were washed with hot water and acetone thrice and were oven dried at 70 ^{0}C for 3h and kept in the desiccators for 1h to make moisture free and then weighed. The material so prepared was shown in the Scheme-I

Calculation of Grafting Parameters

For chitosan copolymers, the grafting parameter was obtained by using the following expression:
Yield of grafting (%) = [(wt. of graft copolymer – wt. of chitosan)/wt. of chitosan] x100 (2)

Characterization

The PMMA grafted chitosan sample and nano-CaO based bionanocomposite BBCs were characterized by FTIR, XRD, FESEM and TGA. The insertion of PMMA into the chitosan was confirmed by using an XRD monitoring diffraction angle 2θ from 10^0 to 90° on a Philips PW-1847 X-ray crystallographic unit equipped with a Guinier focusing camera with CuKα radiation (λ= 0.15059 nm) with a 0.02 2θ step size and a 2-s count time. Nanoscale structure of grafted samples was investigated by means of a FESEM accelerating voltage of 5.00 kV. The ultrathin section (the edge of the sample sheet perpendicular to the compression mold) with a thickness of 1µm and 200 nm was microtomed at -80°C on Zeiss Mevlin SEM Instrument (FESEM). Thermal properties were measured by using a Shimadzu DTA-500 system. It was carried out in air from room temperature to 600°C at a heating rate of 10°C/min. The IR spectra of samples, in the form of KBr pellets, were recorded with a Perkin–Elmer model Paragon-500 FTIR spectrophotometer.

PROPERTIES

Testing of mechanical properties

The prepared BBCs were pressed between two glass plates for 1 h. After the cement had hardened, it was pulled out of the plates and stored at room temperature. The specimens for the tensile tests were prepared of (75x5x3) mm dimension [25].The tensile test and three-point bending test were performed on the Instron universal materials testing machine (Model 5544). The tensile test (according to ASTM D638-03) was conducted at a cross-head speed of 1 mm/min. The BBCs were poured into the mould for the compression and bending test, and fixed with PTFE end plates and C-shape fixture for 4h. After the bone cements were hardened, the endplate and C-shape fixture were removed and then the cylindrical test specimens were taken out for the compressive strength test. The compressive strength of each specimen was determined according to ISO 5883-2002 standard using the same universal testing machine, operating at a crosshead speed of 5 mm/min.

Equilibrium water content (EWC)

Equilibrium water content of BCC nanocomposite with different nano-CaO content had been studied to evaluate the effect of nano-CaO content on the size stability of material. The EWC,
Waof BCC –BNCs at time t was calculated using the equation

$$Wa\ \% = \frac{wt - wo}{wo} \times 100 \qquad (3)$$

Where wt and wo are the weights of sample at time t and the dry state at 23°C respectively.

Biodegradation by activated sludge

The activated sludge water was collected from domestic waste water as the sludge water contains many different types of microorganisms (bacteria, fungi, yeast, etc) which are responsible for the biodegradation of waste materials. The sludge was collected in a polypropylene container, which was filled completely and then fully closed [26]. Then, the waste water was brought to the lab immediately. After settling for about 1 h, the total solid concentration was increased to around 5000 mg/L. The activated sludge water and a polymer sample (0.2 g) were incubated together in a sterilized vessel at room temperature (28 ± 2°C) for 21 days, 45days, 2months and 4months. Duplicate samples were removed at time intervals for biodegradation study via weight loss. Vessels containing polymersamples without sludge water were treated as controls.

RESULTS AND DISCUSSION

FTIR spectra of the BBC- BNC

The grafting of PMMA onto chitosan was confirmed by the FTIR spectral study as shown in Fig.1. Chitosan-g-PMMA /nano-CaO segments the ester function group($CO\text{-}O\text{-}CH_3$) gives C=O function group at 1745 cm^{-1} and Ca-O-Ca peak at the range 979 cm^{-1}which confirmed the insertion of nano-CaOlayer into the copolymer layer Fig. 1(C).The copolymer indicates the typical spectra for amide bands of chitosan-g-PMMA located 1685cm^{-1},the slight shifting of the peak to lower frequency might be due to the hydrogen bonding between the amino group of chitosan and the carbonyl group of the pendant graft and C-N stretching frequency at 1280cm^{-1}Fig1 (B). The nano-CaO ,theCa-O bond indicates the stretching frequency between the range at1485 $cm^{-1}$and the bending frequency region indicating at 980cm^{-1} Fig1 (A).

XRD of the BBC- BNC

The crystallinity of samples like CS, chitosan-g-PMMA (deacetylated) and chitosan-g-PMMA/ nano-CaO were investigated by X-ray diffraction study. In the X-ray pattern in Fig. 2, the (A) is the X-ray pattern of nano-CaO showing sharp peaks due to partially crystalline in nature. But, in Fig. 2(B) chitosan-g-PMMA, the chitosan shows a broader pattern at around $2\theta = 20^0$, indicating lower degree of crystallinity [27, 28]. It has been reported that the crystalline structure of chitin derivatives depends on the degree of deacetylation i.e. the crystalline structure of shrimp chitosan is retained up to 70% of the deacetylation degree. But, on further deacetylation, the crystalline regions of chitosan are destroyed and become amorphous. In Fig. 2(C), the chitosan-g-PMMA/nano-CaO sample confirms the insertion of polymer in the layered structure of nano-CaO.

FESEM of the BBC-BNC

The FESEM micrographs of the copolymers without and with nano- CaO are shown in Fig. 3 at different magnifications like 50KX, 15KX, 20KX, 50µm. The micrographs FESEM of nano-CaO (A) 50.00KX (B) 15.00KX , Chitosan-g-PMMA (C)50.00KX (D)20.00 ..KX, Chitosan-g-PMMA/nano-CaO composite (E) 50.00KX (F) 20.00KX and Chitosan-g-PMMA/nano- CaO .confirmed the nano range homogeneous insertion of the graft copolymer into the matrix of the nano-CaO. It provides the mechanical strength to the chitosan based graft copolymer for its biomedical application. The surface morphology after biodegradation by activated sludge has been confirmed as shown in Fig. 3(G) 50µm composite after biodegradation This might be due to the decomposition or colony growth by the micro-organisms on the chitosan-g-PMMA/ nano-CaO BNC resulting in the rough surface as compared to that of before biodegradation (Fig. G). Hence, the prepared novel nanocomposite is eco-friendly in nature.

TGA of the BBC-BNC

The thermal decomposition of chitosan-g-PMMA and the first decomposition of chitosan-g-PMMA/nano-CaO of CS were studied by the TGA analysis as shown in Fig. 4. The initial decomposition of both the samples is due to the presence of little bit of moisture in the samples but, moisture content is found to be more in chitosan-g-PMMA/nano-CaO of CS showing the enhancement of hydrophilicity . Later, the decomposition of the chitosan-g-PMMA copolymer at temperature 155^oC and that of chitosan-g-PMMA/nano- CaO of CS BNC at 280^oC is explained on the fact that on the higher thermal decomposition of the BNC chitosan-g-PMMA/nano-CaO of CS might be due to the insertion of the copolymer into the layered structures of the nano-CaO of CS. This is an added advantage for this nanocomposite as it can resist the higher temperature.

Mechanical Properties of BBC-BNCS

The mechanical properties like tensile strength and compressive strength of the BNC samples were studied in freeze-dried condition for 48 h.

Tensile strength

From the test results it has been observed that the tensile strength of the BBC-BNCs is gradually increasing from BBC1 to BBC4 and then decreased till BBC6. The increased trend of the tensile strength might be ascribed to the gradual enhancement of the crosslinking of the PMMA with chitosan up to 37.5MPa and after that the BBC-BNCs might become brittle due to higher crosslinking density, as a result after 37.5MPa there is a decrease trend of tensile strength as shown in Fig. 5.[29].

Compressive Strength

The compressive strength results shown in Fig. 5 are similar to as that of tensile strength and the highest compressive strength found for BBC4 might be due to optimum croslinking density. Here, the BBC1 to BBC4 there is increase in compressive strength i.e., up to 45MPa and further it decreased upto sample BBC6 as shown in Fig. 5.[30].

Equilibrium Water Content

The equilibrium water content depends on the amount of nano-CaO present in the BNCs. The maximum percentage of water content is found in BBC4 due to getting more space in the composite matrix. After that, the decrease in waterabsorbency suddenly uptoBBC6 might be due to the increase in rigidity and crosslink density of the polymer inside the BNC. As a result, penetration and retaining capacity of the water might decrease. EWC for samples BBC5 and BBC6 with more nano-CaO might be ascribed to their microstructures by decreasing the porosity. This is in accordance with the observation that the water absorbency decreases by using more divalent ions like Ni^{+2},Pd^{+2} in the swelling medium[31].

Biodegradation by Activated Sludge

From the Fig.7 it is clear that from BBC1 to BBC4 there is increasing trend of biodegradation than that of other BNCs. It might be ascribed to more water retention capacity of the BBC4-BNC, as a result it might be getting more chance for growth of microorganisms. For other BNCs, due to less equilibrium water content, the biodegradation was found to be less.

CONCLUSION

The copolymer, chitosan-g-PMMA and the BNC chitosan-g-PMMA/nano-CaOofCSwere synthesized via emulsion technique using APS as an initiator under N_2 atmosphere and were characterized by XRD, FTIR, FESEM and TGA. The grafting of PMMA onto chitosan was confirmed by FTIR as well FESEM micrographs and the homogeneous insertion of kaolin was also confirmed from XRD and FESEM study. The mechanical properties like tensile strength and compressive strength show similar results and the sample BBC4 was found to be more suitable BNC. The EWC was also in agreement with biodegradation results for good water retention capacity of BBC4. Finally, the BNCs would be considered as an excellent material for bone substitute.

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Table 1. Variation of [MMA] and (%) of grafting

Sample code	Chitosan gm	MMA ml	APS gm	$CuSO_4$ gm	Glycine gm	Sorbitol, gm	Cockleshells (nano-CaO) gm	% of Grafting
BBC1	0.25	3	0.684	0.32	0.15	0.05	-	89.6
BBC2	0.25	1	0.684	0.32	0.15	0.05	0.25	-
BBC3	0.25	2	0.684	0.32	0.15	0.05	0.5	-
BBC4	0.25	3	0.684	0.32	0.15	0.05	0.75	-
BBC5	0.25	4	0.684	0.32	0.15	0.05	1.0	-
BBC6	0.25	5	0.684	0.32	0.15	0.05	1.5	-

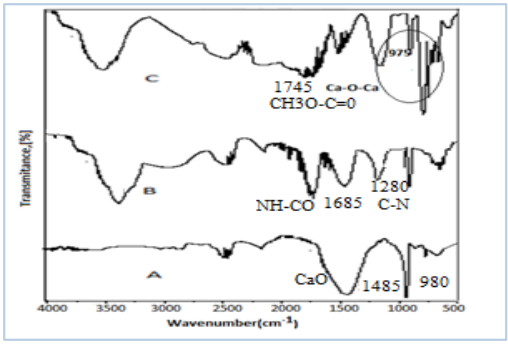


Fig. 1. FTIR spectra of (A) nano-CaO (B) chitosan, (C) PMMA (D) chitosan-g-PMMA and(E) chitosan-g-PMMA /nano-CaO BNC

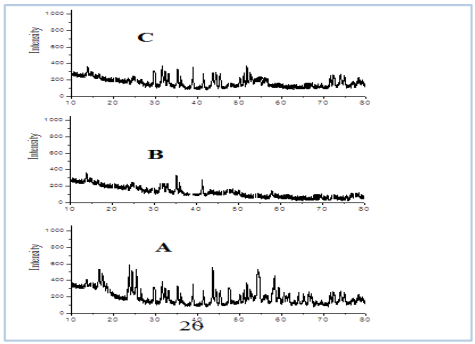
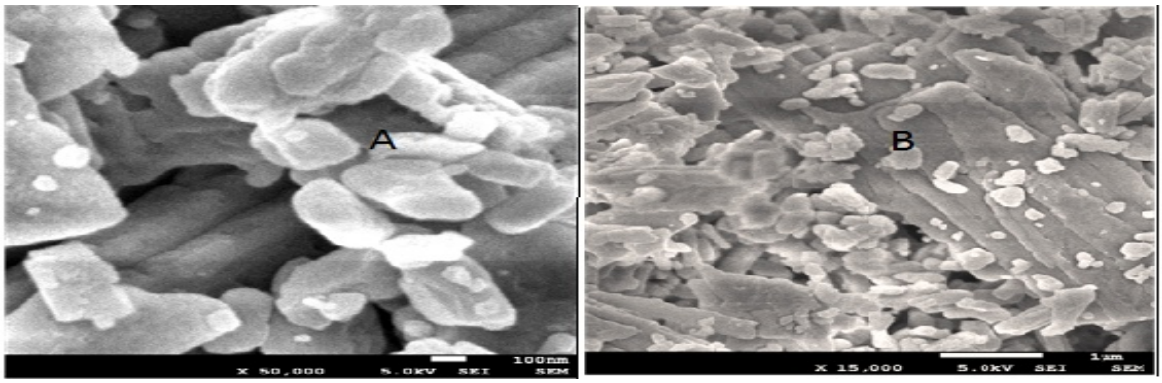


Fig.2. XRD of (A) nano-CaO (B) chitosan-g-PMMA and (C) chitosan-g-PMMA/nano- CaO BNC



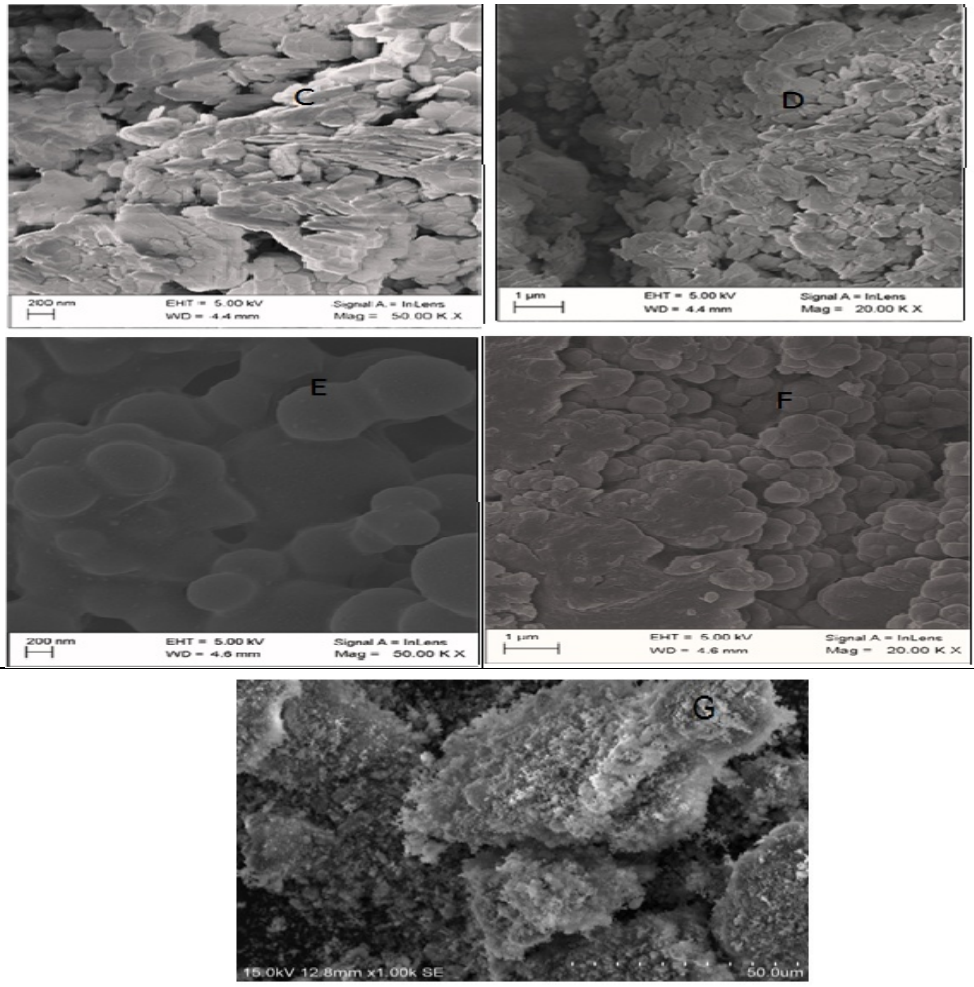


Fig. 3. FESEM of nano-CaO (A)50.00 KX (B) 15.00KX , Chitosan-g-PMMA (C)50.00KX (D)20.00KX, Chitosan-g-PMMA/nano-CaOcomposite (E)50.00KX (F)20.00KX and Chitosan-g-PMMA/nano- CaO (G) 50µm composite after biodegradation

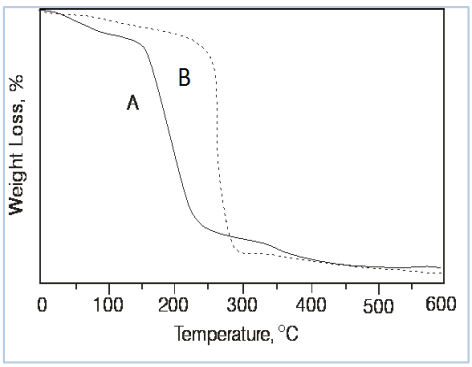


Fig. 4. TGA thermograms of (A) chitosan-g-PMMA and (B) chitosan-g-PMMA/nano-CaO

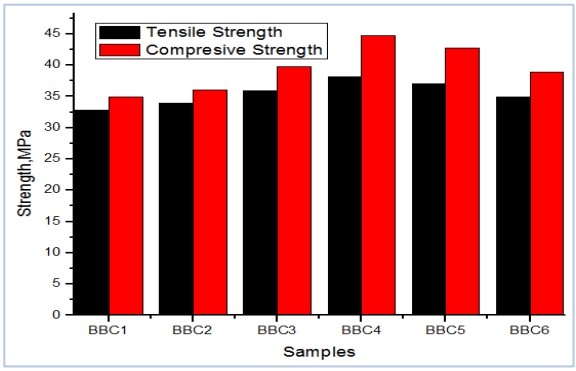


Fig. 5. The tensile and compressive strengths of Chitosan-g- PMMA/nano-CaO (BBC 1 to BBC 6)

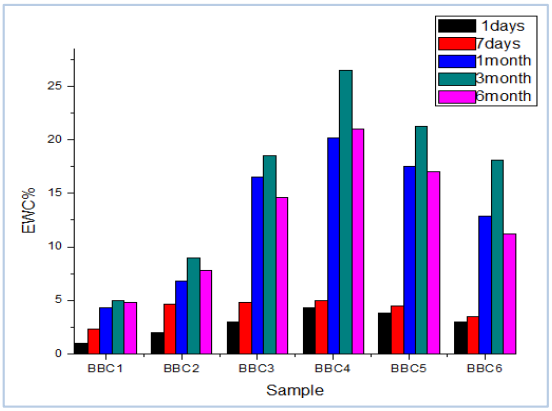


Fig. 6. EWC study of Chitosan-g-PMMA/nano- CaO (BBC1 to BBC6) BNCs

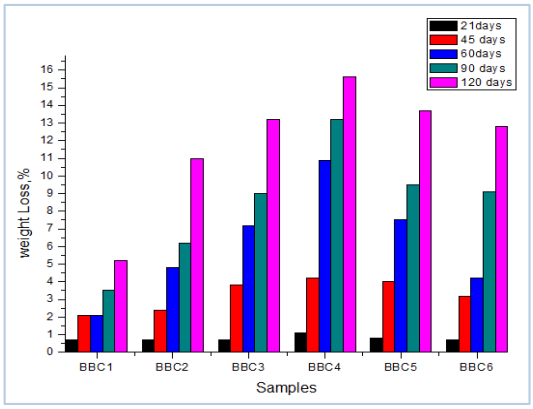


Fig. 7. Biodegradation study of Chitosan-g-PMMA/ nano-CaO (BBC-1 to BBC-6) BNCs.

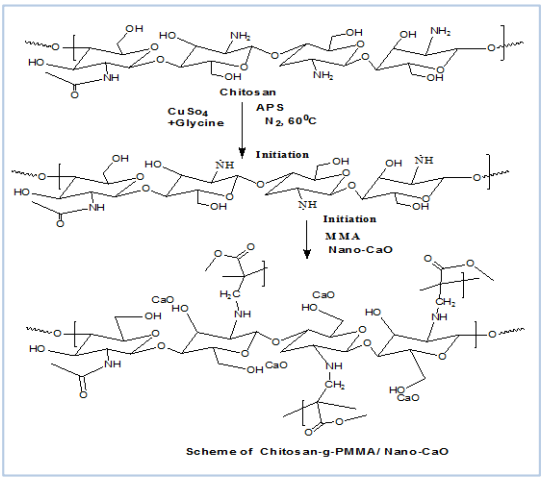


Fig. 8. Scheme-I

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RESEARCH ARTICLE

Fabrication of Cellulose Acetate Reinforced Organo Clay Ecofriendly Nanocomposites for Packaging Applications

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ABSTRACT

Ecofriendly nanocomposites have been fabricated from cellulose acetate (CA) powder with incorporation of C25A as a reinforcing material by solvent evaporation method. The consequence of Cloisite wt% on composites was evaluated by varying the composition. The morphologies of these nanocomposites were evaluated through X-ray diffraction (XRD), Scanning electron microscopy (SEM) studies. The mechanical properties of nanocomposites like hardness, tensile strength, modulus of elasticity and thermal stability showed an increasing trend with an increase of clay content from 2.5 to 10 wt %. Moreover the water retention capacity of the composites decreased considerably with increase in clay content.

Key words: Ecofriendly, Cellulose Acetate, Cloisite 25A, reinforcing material, Nano composites.

INTRODUCTION

Now a days use of polymeric materials or films for packaging purpose is a trend in the society. The companies selling various solid or liquid foods are also use plastic materials for safe storage and transportation to distant places. But due to non biodegradability nature of these covering or packaging materials, they remain as such for longer period of time and can cause numerous health as well as environmental problems. So many research is focused on manufacturing biodegradable and ecofriendly packaging materials so as to minimize the problems of environment [1] Cellulose and it's derivatives are drawing attention due to their biodegradability, those can be extensively used in making films, textile fibers, and cigarette tow. It is well known that cellulose acetate has a dimensional stability problem under high humidity and at elevated temperature. Other shortcomings are its high cost, very limited compatibility with other synthetic polymers and high processing temperature [2]. Also cellulose acetate is a

biodegradable and it's biodegradability as well as solubility highly affected by the degree of acetylation [3]. The polymer is amorphous, odorless, non-toxic, water vapor permeable, stable and soluble in acetone. It can form transparent and rigid film, but with some flexibility that supports high tension at room temperature [4] .Extensive literature has been reported on making biodegradable packaging materials using cellulose acetate with bacteriophages [5] , Cloisite 30B, triethyl citrate and thymol or cinnamaldehyde [6] , activated bentonite clay (BC) [7] , N-Amino phenyl maleimide (N-APhM) [8] , lysozyme [9], 12-Aminolauric Acid Modified Montmorillonite [10] , melt intercalation of nanoclays with plasticizer Glycerol triacetate [11], natural montmorillonite (Na-MMT) and organomodified MMT with gelatin (Ge-MMT) or chitosan (Cs-MMT) and in the presence or absence of triethyl citrate(TEC) [12] , Montmorillonite [13].

No literature reports taking C25 A as starting material for nano composites fabrication.So In the present research we have synthesized cellulose acetate modified nano clay composites after measuring the hardness of the the fabricated nano composites of various nano clay like ClousiteNa+, C30B,C25A,C10B with Cellulose Acetate. C25A reinforced nano composites shown higher hardness compared to others.So we choose C25A as our starting material for fabrication of nano composites with Cellulose Acetate.

MATERIALS AND METHODS

Materials

Cellulose acetate (Sigma Aldrich, 37.9% acetyl and Mn ~50,000 by GPC) was purified by dissolution in tetrahydrofuran (THF), precipitation in diethyl ether,followed by Soxhlet extraction with the same solvent to remove any trace of low molecular weight alcohols and water. Thereafter it was thoroughly dried under vacuum[2]. Organically modified montmorillonites (organoclays), e.g., Cloisite 30B, Cloisite Na+, Cloisite C25A, Cloisite C10A was purchased from Southern Clay Co. to use as reinforcements and dried under vacuum at 80^0C for 24 h before use. Ethanol AR 99.9% (Changshu Hungsheng Fine Chemicals Co.Ltd), Chloroform (HIMEDIA)and Acetone (Qualigen) were of reagent grade and used without further purification.

Fabrication of Cellulose Acetate / organoclays nano composites

In the first phase organoclays e.g., Cloisite 30B, Cloisite Na+, Cloisite C25A, Cloisite C10A were incorporated into cellulose acetate network by mixing 1 wt.% each with cellulose acetate solution with Acetone and Ethanol-Chloroform mixture 1:1 (v/v) were blended in a 500 mL RBF equipped with a reflux condenser at 600 rpm every time over a magnetic stirrer fitted with hot plate at 80^0 C for 8 h. Then the mixture was cooled to room temperature and films of the composites were made by evaporating the mixture solution on polished glass plates with the help of a doctor blade for a thickness of 0.08 ± 0.015mm and the hardness of the films were measured as in Table -1.

In the second phase varying composition of C25A (0.5%, 1%, 1.5%, 2% wt) were incorporated into the cellulose acetate solution of same concentration as former. The mixtures were blended in a 500 mL RBF equipped with a reflux condenser at 600 rpm every time over a magnetic stirrer fitted with hot plate at 80^0 C for 8 h. Then the mixture was cooled to room temperature and films of the composites were made by evaporating the mixture solution casted on polished glass plate with the help of a doctor blade for a thickness of 0.08 ± 0.015mm .The resulting films were dried in air for 1 hr and then washed with water to remove any residual solvents. Then the films formed were kept in water bath until further Characterizations.

Characterization Methods

Measurement of Hardness

The hardness of the composites were measured by Rockwell hardness Tester A300(HR320S),Mitutoya,Brazil at temperature(23±5)^0 C and relative humidity (50±*10*)% with specimen size 30mm x 30mm x 1mm in HRB Hardness scale.The range of force was 98.07N with minor load 60Kgf and the type of Indenter used was Ball 1/16

Measurement of Mechanical Properties

Mechanical properties of the composites were determined by tensile tester (Colorcon) specimen size was 4 x 3 cm^2 in area and 0.08 ± 0.015mm in thickness and the test speed of the machine was 5mm per minute. Experiments were performed at 22°C and 30-40% RH. Load and displacement values were recorded. Typical test parameters like maximum stress, modular of elasticity, work done, extension at break and strain at maximum load were automatically computed by software.

XRD

X–Ray diffraction (XRD) of the composites were carried out with PAN Analytical Xpert Pro Diffractometer with Co Kα radiation (30mA 40kV).The range of diffraction was 5 to 80^0 with a scanning speed of 0.020^0 per minute.

TGA

Thermo Gravimetric Analysis of the fabricated nanocomposites were carried out using a Perkin Elmer Simultaneous Thermal Analyzer (STA 6000) in the temperature ranges from 50 to 600 0C at a heating rate of 10^0/min

SEM

The surface structure of the nanocomposites were viewed through Scanning Electron Microscope (JEOL 6510 LV, Japan).The membranes were rubbed with tissue paper and then dipped in liquid nitrogen for freezing before analysis.

Contact angle

The contact angle on all the membrane surface was measured by a goniometer (DGX Digidrop, France). The contact angle of each membrane surface was calculated by taking the average of three contact angles at different positions. The membrane porosity of the films were measured by immersing the films in deionized water for 24hr and weighed after removing any traces of water with tissue paper.The films were then dried in oven at 80^{0}C for until constant weight to find the porosity by using the formula $\square = \frac{W - w}{\rho A l}$

Where W = wet film weight(g), w = dry film weight(g) , ϱ= water density(g cm^{-3}), A=film surface area(cm^2) and l= thickness of the film(mm)

RESULT AND DISCUSSION

The mechanical properties of the nanocomposites such as hardness, tensile strength and young's modulus were measured and the results are as shown in table-2.All the three parameters increased with increase in clay content upto 7.5 wt % then decreased .This may be due to the fact that beyond 7.5 wt % of clay the composite develops a brittle characteristics .So the hardnes along with other parameters abruptly fall down as clear from figure-1.Initially with insertion of nono clay into cellulose acetate structure predominantly raised the mechanical properties upto a certain limit(7.5 wt %) and then with high amount of clay , the composite properties are more lenient to clay characteristics.

The XRD data reveals that with intercalation of clay into cellulose acetate develops a crystallinity in the composites which increased with increase in the clay content as clear from figure-2. In the figure from (a) to (d) it is clear that there increase in crystallinity nature of the composites which is responsible for decrease in the mechanical properties as discussed. In figue-2 for (c) and (d) both the peaks at 2□=25^0 confirms development of crystallinity in the composites. The plane surface structure of the of the cellulose acetate (Figure-3a) was turned into rough and the roughness increased continuously as clear from the SEM images of figure-3(b) to (e) which is attributed to the fact that with intercalation of clay into cellulose acetate forms a protective layer around it, that's why the composite develops an improved mechanical properties as compared to the normal cellulose acetate.

The thermal study data of the fabricated nanocomposites were given in table-3 and graphically represented in the figure-4. All the composites starts degrading at 225^{0}C. On analysis of the tabulated data it is clear that the fabricated nano composites shown better thermal stability compared to the CA [data ref-2]. For composites (b) and (c) there is a sharp degradation at from 90 wt% to 40 wt% from 300^0 C to 400^0 C. So this 50% degradation in weigth % takes place around 100^0 C revels the more crystalline nature of the composites compared to composite (a).Composites (d) suffers a major weight loss at from 350 ^{0}C to 450 ^{0}C. From the contact angle and porosity study it is clear that with increasing clay content the water affinity of the composites decreased along with the porosity as evidenced from table-4.Which may be due to the fact that due to decreased in porosity value the water can't penetrate into the bulk of the film and hence the angle of contact decreased with increasing wt % of the clay as shown in the figure-5.

CONCLUSION

Nanocomposites of Cellulose Acetate reinforced cloisite 25A was successfully fabricated and the mechanical properties showed an improvement with increasing clay content which reveals that the composite can be with stand towards small loads. The XRD and SEM study proves the crystallinity nature of the composites. Hence proves the presence of clay in the composites and that obviously improved the properties against stress. So the stress bearing capacity of the composites has been developed with intercalation of clay into the Cellulose Acetate. The thermal study gives a picture of the thermal resistivity power of the fabricated composites of cellulose acetate with closite25A.The water contact angle and porosity examination also confirmed the hydrophobic nature of the composites. As both clay as well as Cellulose Acetate both are ecofriendly materials hence it may be find suitable for packaging applications in packaging industry.

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Table 1. Weight and hardness of fabricated nanocomposites of Cellulose Acetate / Cloisite [2/0.05(w/w)] at 80⁰ C

Sample Code	Solvent	Weight of the Nano Composite (g)	Hardness (HRB)
CA-b-Na⁺C	Acetone	1.4365	48.90
CA-b-Na⁺C	Ethanol+Chloroform	2.0048	63.31
CA-b-C25A	Acetone	2.0382	69.9
CA-b-C25A	Ethanol+Chloroform	1.9826	44.53
CA-b-C30A	Acetone	2.1373	51.06
CA-b-C30A	Ethanol+Chloroform	1.9973	61.33

Table 2. Mechanical properties of fabricated nanocomposites of Cellulose Acetate blended C25A nanocomposites in Acetone at 80⁰ C

Sample Code	wt.% of C25A	Weight of the Nano Composite (g)	Hardness (HRB)	Tensile Strength (N/mm^2)	Young's Modulus ($N-mm^2$)
CA	0	2.0	31.0	66.53	1134.23
CA-b-C25A$_1$	2.5	2.0382	69.9	87.39	1385.54
CA-b-C25A$_2$	5	2.0692	71.4	94.09	1545.32
CA-b-C25A$_3$	7.5	2.0875	75.33	100.71	1773.61
CA-b-C25A$_4$	10	2.1247	48.9	77.05	1259.83

Table 3. Thermal stability of fabricated nano composites

Sample Code	wt.% of C25A	% Weight Loss							
		200^{0}C	250^{0}C	300^{0}C	350^{0}C	400^{0}C	450^{0}C	500^{0}C	550^{0}C
CA	0	2.3	2.6	3.9	22.4	83.1	85.7	88.2	92.2
CA-b-C25A$_1$	2.5	0.7601	1.7028	3.5339	5.2397	6.6397	6.7811	7.1955	7.776
CA-b-C25A$_2$	5	0.3037	0.4348	0.7868	2.2515	6.1944	6.7114	7.1719	7.66
CA-b-C25A$_3$	7.5	0.4125	0.5544	0.8745	1.7104	6.3277	6.7611	7.2139	7.5888
CA-b-C25A$_4$	10	0.1619	0.2581	0.5971	1.0028	5.9266	6.7479	7.1816	7.5469

Table 4. Contact angle and porosity of the fabricated nanocomposites.

Sample Code	wt.% of C25A	Weight of the Nano Composite (g)		Contact angle(^{0})	porosity □
		wet	dry		
CA	0	3.531	2.0	60.53	14.07
CA-b-C25A$_1$	2.5	3.342	2.0382	57.39	13.58
CA-b-C25A$_2$	5	3.292	2.0692	54.09	12.73
CA-b-C25A$_3$	7.5	3.163	2.0875	50.71	11.20
CA-b-C25A$_4$	10	2.771	2.1247	45.05	06.73

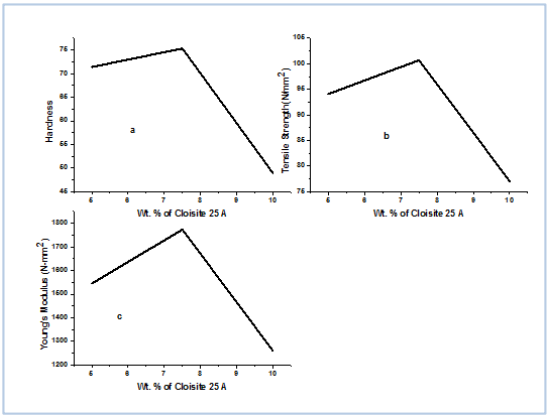

Figure 1. Comparision of (a) hardness (b)Tensile Strength (c)Young's Modulus of the composites

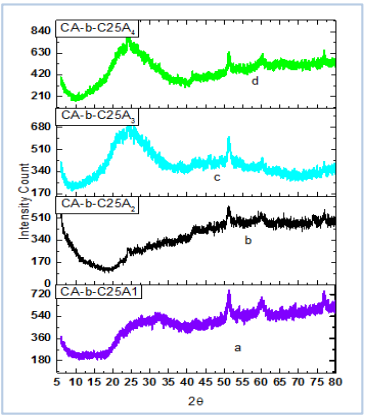

Figure 2. The XRD data of the fabricated Nanocomposites

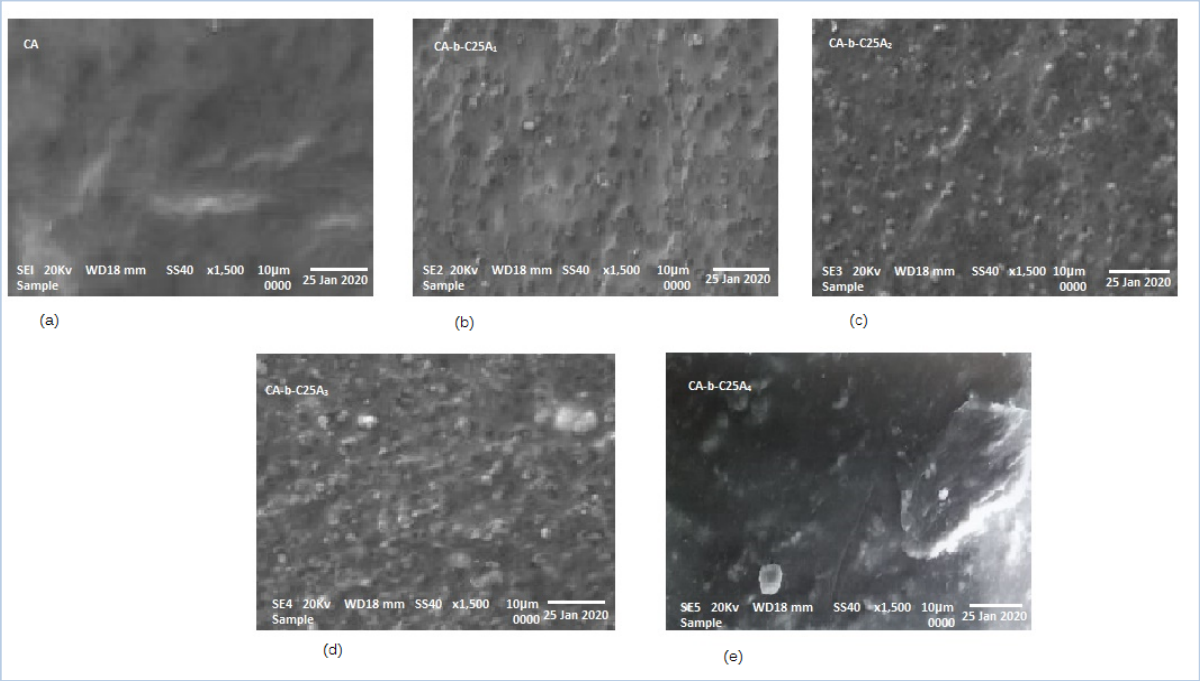

Figure 3. SEM Images of (a)CA (b) CA-b-C25A$_1$ (c) CA-b-C25A$_2$(d) CA-b-C25A$_3$(e) CA-b-C25A$_4$.

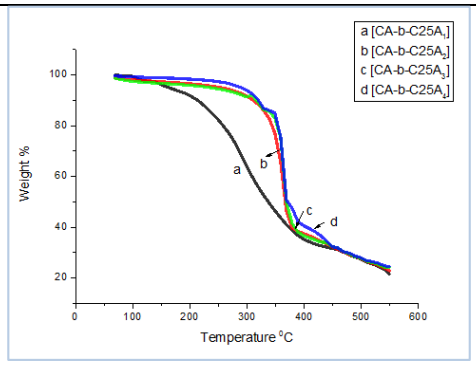

Figure 4. TG curves of the fabricated nano composites

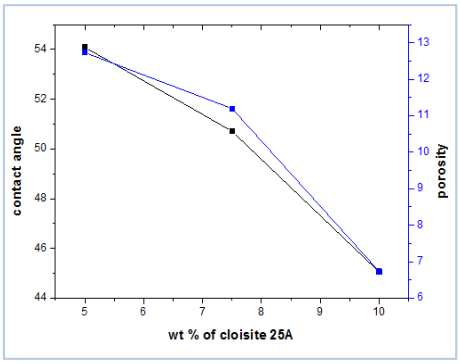

Figure 5. Comparision of wt% of cloisites with contact angle and porosity.

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RESEARCH ARTICLE

Elemental Content Present in Waste Tea Dust and its Impacts on Alteration of Soil Elemental Content

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ABSTRACT

Consumption of tea is much more popular among people. After preparation of tea, the residues are thrown into the soil as waste. As much more amount of tea dust waste have been intermixing with soil every day and changing the soil quality. So, an attempt has been taken to analyse the impact of waste tea dusts on alteration of both physico-chemical and elemental content of soil. An attempts were taken to use the waste tea dust as a bio-fertilizer. The novelty of this work is the utilization of waste as wealth in an environment friendly way. This research address the research gap by analyzing the elemental content present in tea dust. In the present study it was found that the tea dust which causes environmental pollution contains different useful elements which is major and minor element for plant nutrient. In the present study, dry tea dust powder was added to the garden soil and both initial and final physico-chemical parameters and elemental contents of soil were analyzed. It was observed that the soil which were treated with tea dust waste contained more nutrient than the garden soil. So tea dust waste can be recommended as the organic fertilizer which can fulfil the nutrient level of soil and enhance the soil fertility.

Key words: Tea dust, soil, Physico-chemical parameters, Biofertilizer, Elemental content

INTRODUCTION

Pollution of the environment are mostly influenced by consumption pattern and waste management habits of an individual (*Vaccari*et al*.,* 2019). To reach the circular economy, waste statistics should be managed (*Ana* et al., 2019). The amount of waste generated globally are estimated 7 to 10 billion tons per year (*Wilson* et al., 2015). Among which 70 % is not reused or recycled (*Tisserant* et al., 2017). Tea dust waste is one of the most important solid waste which are produced in large quantities in all the cities. The organic residue produced from any plants or animal origin has play important role in changing soil quality. The organic material affect the soil physically (*Hullugalle* etal., 1996), soil nutrient content and faunal diversity of soil (*Wade* and *Sanchez*, 1983).As we know that bio-fertilizers are the major source to enhance the growth and development of plants, So in this study elemental content of tea dust waste was analyzed and physico-chemical parameters of both garden soil and garden soil treated with tea dust waste was compared for knowing the waste composition either that is beneficial or harmful for environment.

MATERIALS AND METHODOLOGY

In this study, soil samples were collected from a garden of Centurion University of Technology and Management, BBSR, Odisha, at depths of 18 cm using soil auger. The samples were collected in a polyethylene bags and properlylabelled. Then the collected samples were taken to the laboratory and treatment was done for the preservation of soil and further analysis has been done as per standard procedure (*Saeed*and *Rafiq*, 1980). The collected samples were air dried in sun light for about twenty four hour. Then the samples were dried in an oven at 105˚C till complete dehydration. Then the sample was ground in a mortar pestle then passed through 0.5 mm nylon mesh sieve. These soil samples were again packed with the complete labelling and preserved for further analysis. Tea dustwastes were collected from different fast food shops and vending zone near Centurion University of Technology and Management, Bhubaneswar, Odisha. The physico-chemical parameters like pH, E.C., soil moisture percent (%), water holding capacity of soil and elemental content of both soil and fruit waste were analyzed using standard methods.

Statistical analysis and presentation of data

All the experiments were done in triplicates and the data presented in the figures are the means of three independent experiments. The data were analyzed statistically and standard errors of mean (SEM) were given wherever required.

RESULTS AND DISCUSSION

Physico-chemical parameters of elemental analysis of soil

The physico-chemical parameters like pH, Electrical conductivity (E.C), soil moisture content (%), water holding capacity (W.H.C), and different elements/compound contents present in garden soil and garden soil treated with tea dust were analysed.

pH

The pH values of the soil sample collected from our college garden area was found within 7.4 to 7.6. So the soil sample was considered to be basic. After addition of tea dust waste it was found to be 6.8 to 7.3 which indicates that the tea dust waste neutralizes the alkaline soil. Then after addition of tea dust waste it was found to be decreases.

Electrical conductivity (E.C)

The conductivity values in the soil samples of the area under study were found to range from0.814 mho/cm to 0.821 mho/cm. After addition of tea dust waste it was found to be 0.645mho/cm to 0.720mho/cm. Then, after addition of tea dust waste it was found to be decreases.

Soil moisture content

At pre-monsoon period moisture content were found in the range of 8.0% to 10.0% whereas at post-monsoon period it was ranged from 10.0% to 12.0%, which indicated that the moisture content present in soil of the area is suitable for crop production. After addition of tea dust waste it was found to be increases.

Water holding capacity (W.H.C)

Soil water holding capacity is a term that all farms should know to optimize crop production. When there is a difficult in the amount water in the soil, the soil profile needs to be replenished by precipitation or irrigation. In the study the soil had range between 156.53 to 158.55. After addition of tea dust waste it was found to be 160.18 to 162.15. After addition of tea dust waste it was found to be increases.

Elements/compound contents

In this study, elemental analysis of soil had been done in the basis of XRF (X-ray fluorescence). The soil was found to be contained many elements/compounds i.e given in table. There are several elements and compounds were found to be present intea dustwaste which enhanced the nutrient level of garden soil. After addition of waste, concentration of different valuable elements like P_2O_5, SO_3, Cl, K_2O, CaO and Rb_2O were found to be increased which enhanced the soil nutrient level. In a study it was reported that the application of egg shell waste enhances the soil fertility (*Biswal* et.al., 2019). In another study, it was reported the application of compost act as fertilizer on growth of onion (*Brechin* and *Mc. Donald*, 1994), on barley (*Hountin* et al., 1995). From another experiment, it was found that, the tea dust waste compost are highly desirable for plant growth (*Oladapo* et al.,2015) . The presence of some metals like copper, zinc, iron, manganese, molybdenum and nickel are essential micronutrient for plant growth (*Arzoo* and *Satapathy*, 2017). Similarly, small amount of different metals enhance the plant growth like nickel in *Macrotyloma uniflorum* (*Arzoo*et al., 2014). Compost prepared from organic waste (*Hartz* et al., 1996) increases soil organic matter on land (*Smith* et al., 1992) and provide plant nutrients in slowly available form (*Shanks* and *Gouin*, 1989). Similar results were also reported in growth of peanut crops grown with tea dust waste treated soil (*Daud* et al., 2016).

CONCLUSION

Tea dust waste can be used as biofertilizer due to presence different valuable macro and micro nutrient for plants. In the present study , it was found that the tea dust waste treated soil contains much more nutrient than the normal garden soil. So this research can say that tea dust waste can fulfil the mineral requirement for the plants growth. Overall it is an excellent organic manure for plants. It can be recommended for utilization at crop field for better growth and development of plant without causing any harm to the plant.

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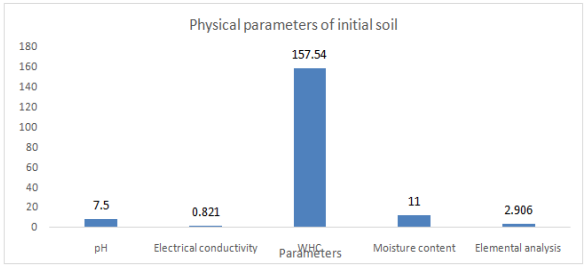
Table 1.Comparison between the elemental content present in garden soil, tea dust and garden soil mixed with tea dust

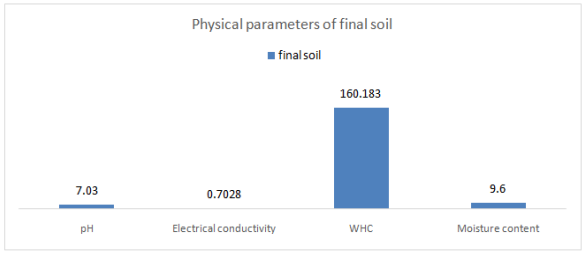
Elements/ Compound	Unit	Elemental Content		
		Garden Soil	Tea dust	Garden soil + Tea dust
Al_2O_3	%	10.712 ± 0.634	00± 00	10.291± 0.088
SiO_2	%	46.184 ± 0.602	4.691± 1.052	42.309± 0.242
P_2O_5	%	1.032 ± 0.002	8.332± 0.984	1.112± 0.024
SO_3	%	1.23 ± 0.001	8.934± 0.988	1.342± 0.034
Cl	%	2.922 ± 0.242	1.474± 1.032	1.286± 0.026
K_2O	%	1.44 ± 0.052	10.121± 1.016	2.113± 0.0118
CaO	%	0.132 ± 0.003	637.022± 0.199	1.118± 0.024
TiO_2	%	2.934 ± 0.234	2.644± 0.894	2.752± 0.028

V_2O_5	%	0.132 ± 0.001	00± 00	0.098± 0.026
ZrO_2	%	37.124 ± 0.322	00± 00	30.121± 0.022
MnO	Ppm	514.322 ± 2.621	1.092± 1.002	506.308± 0.284
Fe_2O_3	Ppm	336.232± 0.314	3.818± 0.299	332.212± 0.364
NiO	ppm	188.702± 1.582	350.434± 1.082	195.824± 0.086
Cr_2O_3	ppm	350.524± 2.291	2.122± 1.024	334.022± 0.828
CuO	ppm	74.311 ± 0.296	2.348± 1.006	70.686± 0.064
ZnO	ppm	100.212± 0.574	2.252± 1.082	98.642± 0.083
Ga_2O_3	ppm	146.904± 0.608	00± 00	140.464± 0.044
As_2O_3	ppm	31.209± 0.290	00± 00	28.008± 0.062
Rb_2O	ppm	12.102± 0.346	341.324± 0.992	121.514± 0.124
SrO	ppm	137.208± 0.635	408.031± 1.006	167.083± 0.086
Y_2O_3	ppm	131.712± 0.284	00± 00	52.001 ± 0.072
Nb_2O_5	ppm	31.024± 0.572	00± 00	29.122 ± 0.044
SnO_2	ppm	96.001± 2.091	00 ± 00	89.124 ± 0.082

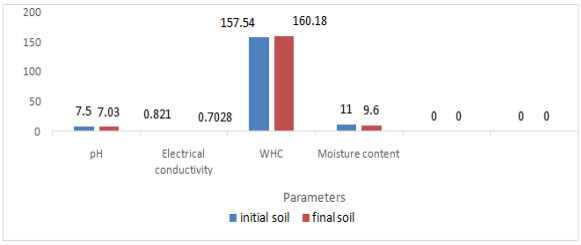
Values of five replicates ± SEM



Graph.1: Physical parameters of initial soil



Graph 2. Physical parameters of final soil.



Graph 3. Comparison between in initial soil & final soil.

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RESEARCH ARTICLE

Soil Nutrient Status of Different Farms of M.S. Swaminathan School of Agriculture, Gajapati District, Odisha, India

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ABSTRACT

The present study was conducted during the year 2018-19 at Department of Soil Science and Agricultural Chemistry, M.S. Swaminathan School of Agriculture, Gajapati, Odisha with aim to study nutrient status of different research farms. The soil samples were collected from different farms and analyzed as per standard procedure for assessing pH, EC, OC, and major nutrients like available nitrogen, phosphorous and potassium with micronutrients like zinc, copper, manganese and iron status of soils. The pH of soils varied from 6.13 to 6.80 and EC varied from 0.06 to 0.34 dS/m. The organic carbon content in farm soils were varied from 0.32 to 0.60 percentage. The available nitrogen, phosphorous and potassium ranged from 125 to 326, 31 to 141 and 185 to 571 kg/ha respectively. The available zinc, copper, manganese and iron ranged from 0.48 to 18.84, 0.56 to 3.06, 9.96 to 30.52 and 17.02 to 82.84 mg/kg respectively. The results indicate that soils were slightly acidic in reaction, non-saline and low in organic carbon. The soils were low to medium in available nitrogen and high in available phosphorous and medium to high in available potassium. The micronutrient status like available zinc was low to adequate and available copper, manganese, iron were adequate amount found in soils. The results conclude that balanced application of nutrients required for successful crop production and development of orchard in farm areas.

Key words: pH, EC, OC, micronutrient, orchard.

INTRODUCTION

The knowledge of nutrient status in soils provides an idea of the inherent capacity of soils to supply plant nutrients in adequate amount and balanced proportion. Studies were conducted by different researchers (Mishra *et al*., 2016; Bansal and Takkar 1985) to understand the presence of nutrient status in different soils and their relationship with

soil properties. Balanced use of organics, fertilizers and bio-fertilizers plays an important role to maintain soil fertility in long run. However, information in this regard for the soils of different farms of M.S. swaminathan school of agriculture, Gajapati, Odisha is scanty and therefore present investigation was undertaken to study the soil nutrient status of both macro and micronutrient in M.S.Swaminathan school of agriculture, Gajapati district, Odisha.

MATERIALS AND METHODS

The study area comes under "North Eastern-Ghat Agro Climatic Zone" with light textured soil. The situation of this farm area is situated about 50 km from coast (Sea coast). The geographical situation of this area is characterized by undulated topography with hilly terrain where the rain water is carried through hill streams & nallas in five tribal blocks. The main crops grown within the study area are rice, maize, cotton, sunflower, groundnut, mungbean and black gram. Ten numbers of surface samples collected from each farm totally 120 soil samples collected from study area and also the processed samples were analyzed for physico-chemical properties and available nutrients using operating procedure (Table 1).

RESULT AND DISCUSSION

In general, the soils were slightly acidic in reaction and pH ranged from 6.13 -6.80. The soils were sandy loam in texture with clay content starting from 13.9 to 48.6 %. Higher content of micronutrients were found in surface layers which could flow from to their regular addition through plant residues, organic manures and fertilizers.

Chemical properties of soil

The pH of surface soils of farm area ranged from 6.13 to 6.80 with mean 6.34. Most of the soils were found slightly acidic in reaction (Mitra *et al.,* 2006). All together 95.33 % of samples were acidic, 4.35 % samples were neutral and only 0.32% samples were found alkaline. The electrical conductivity value varied from 0.06 to 0.34 dS/m with mean value of 0.17. The results indicated that all the soils are normal in nature and suitable for all types of crops and for healthy plant growth due to the soils are free from salinity. Similar results were found by Padole and Mahajan (2003) in swell-shrink soils of Vidharbha region. The organic carbon content ranges from 32 to 60 % with the mean of 39 %.This values were categorized under low (below 0.5%), medium (0.5 to 0.75%) and high (>7.5). The most SOC deficient farm was soil science and agriculture chemistry followed by protected cultivation.

Macronutrient status of soils

The macronutrient nutrients status namely available nitrogen, available phosphorus and available potassium which are required in large quantity for crop production. Mostly all farm soils were low in available nitrogen content. The highest (326 kg ha^{-1}) available nitrogen content was found in plant pathology and lowest (125 kg ha^{-1}) in patikota farm **(**Fig.2)**.** All the farms of M.S. Swaminathan School of Agriculture were low in mean available nitrogen content. Similar result was also found by (Mitra *et al.,* 2002). The lowest (31 kg ha^{-1}) available phosphorus (P) content was found in Entomology farm and the highest P (141 kg ha^{-1}) was found in Bagusala farm (Fig.2). Similar finding was also reported by (Mitra *et al.,* 2002).The mean available phosphorus content was medium to adequate in all the farms. The available potassium content of all farm soils were found to be adequate. The data revealed that the highest available potassium content was observed in organic farm (571 kg ha^{-1}) and lowest (185 kg ha^{-1}) in Entomology farm (Fig 2). Similar finding was also reported by (Mitra *et al.,* 2006).

Micronutrient status of soils

The DTPA extractable Fe content in these soils varied between 17.20 to 82.24 mg/kg with a medium of 45.46 mg/kg considering the critical limit of 4.5 mg/kg for Fe (Lindsay and Norvell 1978), the soils are rated adequate in available Fe. This might ensure to low supply of oxygen in acid soil, some ferric iron oxides and hydroxides are going to be transformed to more soluble ferrous forms. It absolutely was maximum in soil science & Agriculture chemistry farm

and minimum in Entomology farm 17.20 to 82.24 mg/kg. These findings are agreement with Shukla *et al.,* 2014 in soils of Odisha. The DTPA-Mn value in farm soils of M.S.Swaminathan school of agriculture ranged between 9.96 to 30.52mg/kg with a medium value of 17.76 mg/kg considering the critical limit as 2 mg/kg. All soil samples of the farm were found to be sufficient. It might be due to rich content of this element in soil forming parent material of those local areas. Maximum content of Mn was found in horticulture farm and minimum in plant pathology farm. This type of results were found by Kumar *et al.,* (2009). The DTPA extractable Cu content in soils ranged from 0.56-3.06 mg/kg with norms of 1.67 mg/kg considering the critical limit of 0.2 mg/kg for Cu for normal plant growth (Katyal and Randha 1983). No Cu deficiency was observed in other farms. It absolutely was because of rich Cu bearing parent material of the soils. The utmost Cu was found in organic farm (3.06 mg/kg) and minimum (0.56 mg/kg.) in protected cultivation farm. The DTPA Zn result content of the soil reported in table 4. The table showed that DTPA Zn varied from 0.48-18.84 mg/kg with norms of 1.70 mg./kg. Considering the of 0.6 mg kg^{-1} (Katyal, 1985) as critical limit of DTPA-Zn. The utmost DTPA-Zn content was seen in Genetics and plant breeding farm and minimum in Horticulture farm. Similar results were found by Shukla *et al., (*2014) within the soils of Punjab and India.

Correlation of available macro & micronutrients with soil properties

To know the relationship among the available macro & micronutrients with soil properties, the results were analysed statistically. Correlation coefficients were presented in Table 5. A presence of data revealed that, there was a strong negative correlation between pH with nitrogen, phosphorous and copper and positive correlation with potassium, iron and manganese. EC showed a strong positive relationship with phosphorous, iron and copper and poor positive relationship with zinc, and potasium. Soil organic carbon showed strong positive relationship with nitrogen, phosphorous copper, zinc and reverse relation with iron, manganese.

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Table.1 Methods adopted for estimation of properties

Soil parameters	Methods adopted
Soil reaction	Jackson,1973
Electrical conductivity	Jackson,1973
Organic carbon	Walkey and Black,1934

Available nitrogen	Subbaiah and Asija,1956
Available phosphorus	Jackson,1973
Available potassium	Jackson,1973
DTPA extractable micronutrients	Lindsay and Norvell (1978).

Table 2. Basic properties of surface soil

SL No.	Name of farm	pH(1:2.5)		EC(dS/m)		SOC%	
		Range	Mean	Range	Mean	Range	Mean
1	Soil Sc. & Agril. Chem.	6.30-6.80	6.54	0.16-0.32	0.24	0.32-0.38	0.33
2	Genetics and plant breeding	6.25-6.54	6.38	0.08-0.18	0.11	0.34-0.44	0.36
3	Plant Pathology	6.13-6.21	6.17	0.07-0.14	0.11	0.36-0.46	0.42
4	Biochemistry	6.20-6.31	6.25	0.24-0.34	0.28	0.35-0.42	0.38
5	Organic Farm	6.35-6.50	6.44	0.11-0.16	0.13	0.38-0.54	0.44
6	Entomology	6.18-6.44	6.40	0.06-0.20	0.12	0.36-0.46	0.38
7	Gandahati	6.20-6.35	6.30	0.08-0.31	0.22	0.36-0.52	0.42
8	Bagusala	6.30-6.48	6.41	0.14-0.24	0.18	0.44-0.60	0.56
9	Horticulture	6.25-6.52	6.40	0.16-0.24	0.20	0.32-0.46	0.36
10	Protected cultivation	6.16-6.40	6.32	0.12-0.26	0.18	0.33-0.40	0.34
11	Patikota	6.19-6.44	6.28	0.08-0.22	0.14	0.34-0.46	0.38
12	Rasur	6.18-6.46	6.24	0.14-0.32	0.24	0.33-0.41	0.35
	Mean	**6.13-6.80**	**6.34**	**0.06-0.34**	**0.17**	**0.32-0.60**	**0.39**

Table.3 Macronutrient status of soils (kg ha^{-1}) in different farms of M.S. Swaminathan School of Agriculture, Gajapati, Odisha

SL No.	Name of farm	Available nitrogen (kg/ha)		Available phosphorus (kg/ha)		Available potassium (kg/ha)	
		Range	Mean	Range	Mean	Range	Mean
1	Soil Sc. & Agril. Chem.	140-169	146	65-110	83	360-520	491
2	Genetics and plant breeding	150-172	156	36-72	43	312-388	342
3	Plant Pathology	280-326	312	88-124	106	270-320	291
4	Biochemistry	136-210	176	64-92	74	466-550	542
5	Organic Farm	204-238	220	42-76	52	440-571	540
6	Entomology	126-142	132	31-38	34	185-230	198
7	Gandahati	190-238	212	32-56	36	392-460	435
8	Bagusala	196-244	190	110-141	132	440-510	493
9	Horticulture	144-198	176	36-74	43	260-316	291
10	Protected cultivation	126-139	128	46-68	52	336-348	342
11	Patikota	125-146	134	88-116	97	388-426	402
12	Rasur	130-188	156	99-124	108	434-496	467
	Mean	**125-326**	**178**	**31-141**	**71**	**185-571**	**403**

Table 4. Micronutrient status of soils (mgkg^{-1}) in different farms of M.S. Swaminathan School of Agriculture, Gajapati, Odisha

SL No.	Name of farm	DTPA extractable micronutrients (mgkg^{-1})							
		Zn		Cu		Mn		Fe	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	Soil Sc. & Agril. Chem.	0.80-1.80	1.30	1.36-1.77	1.42	10.36-15.60	13.90	70.12-82.24	77.24
2	Genetics and plant breeding	1.80-18.54	3.26	1.01-1.30	1.10	16.32-22.32	19.08	16.32-30.21	22.32
3	Plant Pathology	0.85-1.33	1.08	1.30-2.08	1.56	9.96-14.32	10.96	30.12-44.68	38.06
4	Biochemistry	1.66-3.68	2.92	1.36-2.08	1.44	14.02-18.32	16.20	38.28-52.64	45.74
5	Organic Farm	1.26-2.36	1.86	2.24-3.06	2.75	12.80-20.36	16.46	56.38-76.94	68.06
6	Entomology	1.20-1.56	1.36	0.68-1.20	0.96	10.36-14.36	12.34	17.20-26.80	27.20
7	Gandahati	0.78-1.22	1.04	2.38-2.88	2.64	10.32-14.28	11.48	30.32-56.68	38.64
8	Bagusala	2.10-3.44	2.52	1.80-2.56	2.22	16.20-20.68	18.47	28.32-62.32	45.99
9	Horticulture	0.48-2.66	1.56	0.98-1.24	1.10	25.36-30.52	28.30	20.35-36.14	24.18
10	Protected cultivation	0.60-1.72	0.66	0.56-0.86	0.72	24.35-29.60	26.98	35.36-46.76	39.78
11	Patikota	1.23-1.88	1.38	1.32-1.64	1.50	26.45-29.52	27.90	32.56-56.80	44.22
12	Rasur	1.36-1.52	1.46	2.28-2.78	2.50	10.04-14.77	11.06	60.38-80.30	74.14
	Mean	**0.48-18.84**	**1.70**	**0.56-3.06**	**1.65**	**9.96-30.52**	**17.76**	**17.20-82.24**	**45.46**

Table 5. Correlation between available macro & micronutrients with soil properties

Properties	pH	EC	SOC	N	P	K	Zn	Cu	Mn	Fe
pH	1									
EC	-0.007	1								
OC	0.018	-0.198	1							
N	-0.396	-0.228	0.452**	1						
P	-0.267	0.139**	0.436**	0.233	1					
K	0.130**	0.56	0.275	0.039	0.380	1				
Zn	0.125	0.027	0.267	-0.056	0.078	0.328	1			
Cu	-0.071	0.185**	0.520**	0.408	0.293	0.643	1.444	1		
Mn	0.124	-0.110	-0.151	-0.425	-0.121	-0.157	-0.007	-0.475	1	
Fe	0.199**	0.432**	-0.350	-0.017	0.446	0.723	-0.164	0.553	-0.349	1

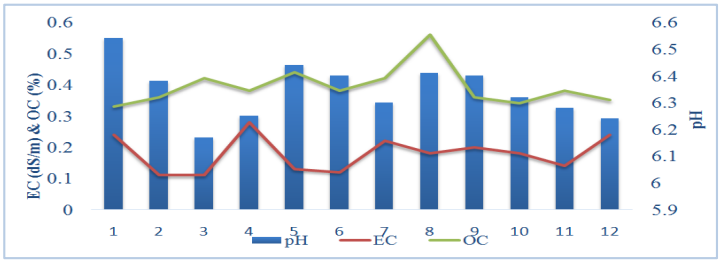

Figure 1. Status of pH, EC (dS/m), OC (%) in different farms soil of M.S. Swaminathan School of Agriculture, Gajapati, Odisha

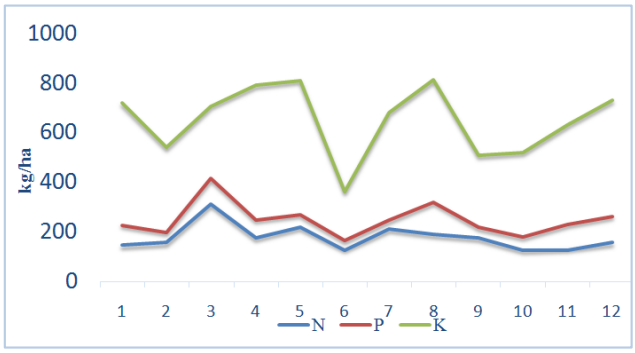

Figure 2. Macronutrient status of soil (kg ha^{-1}) in different farms of M.S. Swaminathan School of Agriculture, Gajapati, Odisha

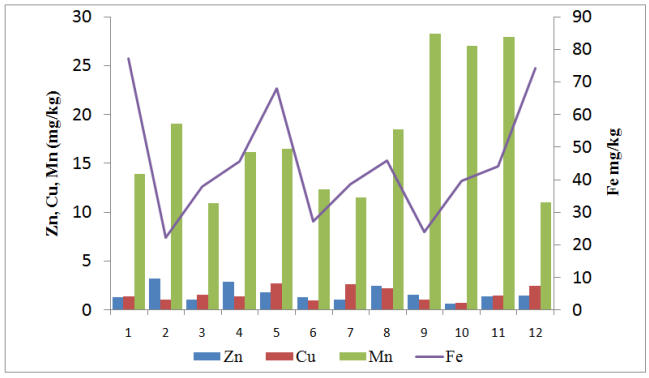

Figure 3. Micronutrient status of soils (mg kg^{-1}) in different farms of M.S. Swaminathan School of Agriculture, Gajapati, Odisha

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RESEARCH ARTICLE

Mainstreaming Biodiversity Conservation into Production Sectors in the East Godavari River Estuarine Ecosystem

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ABSTRACT

Rich in floral and faunal diversity, the East Godavari River Estuarine Ecosystem (EGREE) encompassing the Godavari mangroves (321 km^2) is the second largest area of mangroves along the east coast of India (after Sundarbans). It generartes significant ecological and economic benefits such as shoreline protection, sustaining livelihoods and carbon sink services. There are 35 species of mangroves, of which 16 are true mangroves and the rest associated mangrove species. This includes one nearly threatened species (*Ceriops decandra*) and three rare species. There are important nesting sites for migratory turtle species, notably the endangered Olive Ridley turtle, the critically endangered Leatherback turtle and Green turtle. It is an Important Bird Area (IBA) with a recorded population of 119 bird species, of which 50 are migratory. In recognition of its national and global biodiversity significance, a part of the EGREE area is gazetted as Coringa Wildlife Sanctuary (CWLS). In addition to the biodiversity significance of the area, it has also significant production potential. Anthropogenic activities like fisheries, aquaculture, manufacturing activities such as, oil and gas exploration, fertilizers, edible oil, rice products, tourism and ports are affecting the overall ecological integrity of the EGREE particularly the mangrove ecosystems in the CWLS and adjoining areas, with associated impacts on the livelihoods of local people. The paper discusses the existing institutional arrangements in the EGREE which are inadequate in addressing the biodiversity related issues from a landscape/ seascape perspective. Specific recommendations have been made keeping in mind restoration of the EGREE as the primary objective, implementing biodiversity-friendly sector plans and promotion of certain production sectors, local livelihoods and natural resources in the EGREE.

Key words: East Godavari River Estuarine Ecosystem, mangroves, production,biodiversity, conservation, livelihoods.

INTRODUCTION

India has a coastline of about 7,500 kilometers of which the mainland accounts for 5,400 kilometers, the Lakshadweep Islands account for 132 kilometers, and the Andaman & Nicobar Islands for 1,900 kilometers. The coastline is endowed with a wide range of ecosystems such as mangroves, coral reefs, sea grasses, salt marshes, sand dunes, estuaries, lagoons and natural habitats. The abundant coastal and offshore marine ecosystems include about 6,740 square kilometers of mangroves, including part of the Sundarbans, the Bhitarkanika, the Pichavaram, and the Coringa (Fig. 1), which are among the largest mangroves in the world. These habitats and ecosystems store and cycle nutrients, filter pollutants, protect shorelines from erosion and storms, play a vital role in regulating hydrological functions and modulating climate as they are a major carbon sink and oxygen source, and, in addition, sustain livelihoods of coastal communities.There are scattered efforts to investigate the ecological importance of the Godavari mangroves both as resource repository and its regulatory role in environmental disasters (Ramasubramanian, 2005; Satyanarayana, 2002; Chandra Mohan et. al. 1997; Rönnbäck et.al., 2003; Saenger and Siddiqi, 1993; Ruiz-Jaen and Aide, 2005).A series of experiments carried out by the EqTAP project have shown that mangrove forests and certain other types of coastal vegetation can effectively reduce the impact of tsunamis on coastlines (Hiraishi and Harada, 2003; Danielsen et.al, 2005).

Empirical and field based evidence is limited, but analytical models show that 30 trees per 100 m^2 in a 100m wide belt may reduce tsunami flow rate by as much as 90%. EqTAP recommend using a coastal green belt to protect homes, as it is sustainable, and much cheaper than artificial barriers. Studies in Vietnam also demonstrate the usefulness of mangrove forests in coastal protection. The value of Malaysian mangroves just for storm protection and flood control has been estimated at USD 300,000 per km of coastline, which is based on the cost of replacing the mangroves with rock walls (Ramsar Secretariat, 2001).The economic valuation of ecological services provided by mangroves as a support system for fisheries was done by Dehairs (2000). For the Godavari Estuary, this service was valued at US$ 2,700 per ha, which extrapolates to approximately US$ 90,000 annually for the entire area. Also, marine protected areas worldwide have been found to double the abundance and triple the biomass of fish (30% increases in both size and diversity of fish species in as a little as 5 years). Further, the annual economic values of mangroves, estimated by the cost of the products and services they provide, have been estimated to be USD 200,000–900,000 per ha. The mangroves of Moreton Bay, Australia, were valued in 1988 at USD 4,850 per ha based only on the catch of marketable fish (Ramsar Secretariat, 2001).

The importance of mangroves as a sink of atmospheric carbon dioxide, a major contributor to global warming, is a major area of study all over the world (Fujimoto, 1999; Pidgeon, 2009; Danone Fund for Nature. 2010). Mangroves fix greater amounts of CO_2 per unit area, than what the phytoplankton do in the tropical oceans (Kathiresan and Bingham, 2001). For example, a 20 year old plantation of mangroves stores 11.6 kg per m^2 of carbon with C burial rate of 580 g per m^2 per year (Fujimoto, 2000) and hence, mangroves provide great benefits to control global climate change by stabilizing atmospheric carbon.Table 1 gives an idea of the mangrove forest area in EGREE. The paper describes the potential and significance of leveraging biodiversity of the East Godavari river estuarine ecosystem into a range of production sectors. After the introduction, the second section gives an idea of the context in terms of geography and biodiversity. This is followed by a section on climate change. The fourth section is on the socio-economic context followed by a discussion the anthropogenic interventions in the area. The sixth section describes the existing legal and institutional mechanisms available for EGREE. The seventh section gives specific strategies (recommendations) for the protection and enhancing the production sections with respect to EGREE. The last section provides some concluding remarks.

Geographic and Biodiversity Context

The coastal region that is a focus of the proposed project, namely the East Godavari River Estuarine Ecosystem (EGREE), is located on the eastern side of the Indian peninsula, in the State of Andhra Pradesh. The long coastline of

Andhra Pradesh stretches over 973.7 kilometers (12% of India's total coastline), and covers 9 districts from Srikakulam to Nellore. A prominent feature of this coastline is its mangrove areas that extend over nearly 582 km^2 and are clustered in the estuarine areas of the Godavari River and Krishna River. The Godavari mangrove ecosystems alone constitute 321 km^2, making it the second largest area of mangroves along the east coast of India. The Godavari mangrove wetlands are located between $16^0$30-17^0N and $82^0$10-$80^0$23E in the East Godavari district of Andhra Pradesh. Apart from the Coringa Wildlife Sanctuary (CWLS), the area of the EGREE includes six Reserve Forests viz. Rathikalava, Masanitippa, Maltatippa, Balusutippa, Kothapalem and Kandikuppa. Godavari is the largest of the Indian peninsular rivers that originates in the Western Ghats, traverses a length of 1,446 km over a catchment area of 3,14,685 square kilometers, before draining into the Bay of Bengal.

The EGREE falls in the deltaic region of Godavari river system. The landscape/ seascape of the EGREE is characterized by rivers and channels, flood plains, natural levees, mangrove forests, tidal channels, tidal flats, lagoon, Kakinada bay, sand spits, mainland beaches, sand dunes and paleo sand ridges. Natural levees vary from 3 to 5 meters in width and are about 1 meter in height. This prevents free flow of tidal water in some of the mangrove areas. Kakinada bay has estuaries adjoining the lagoon which is another important geographical feature. Sediments deposited at the confluence have resulted in the formation of a number of spits. The sand spits of Kakinada bay, including Hope Island, are unique features of the area. The initial formation of a small sand spit dates back to 1864(Reddy and Prasad, 2003). The spit extended to a length of about 16 kilometers by 1968, and has grown to a length of about 17 kilometers now, with a head of about 5 kilometers and a tail of 12 kilometers.

The sand spit protects the mangroves from the ocean currents and forms a sheltered coastline. In addition, accretion and natural establishment and growth of mangroves along Kakinada bay are significant and contribute to a gradual increase in the mangrove area. Kakinada bay, the sand spits and mangrove waterways of Coringa are highly dynamic. Erosion of the coastline can be seen from the Godavari river mouth to the tip of Hope Island. Elongation and enlargement of Hope Island in the north and northwest directions is also visible. There has been a shift in the sand spit towards the west, which has resulted in the loss of mangrove vegetation. Survey charts from the period 1848 to 1971 show that until 1889 the river discharged a major portion of water directly into Kakinada Bay. At present, the discharge is mainly through the mouth near Bhairavapalem on the northern side (Rao et al., 2003). The Corangi mangrove region, including the creeks and channels, is also found to be shallow near the Bay, with depths varying between 1 and 3 meters. During low tide, large areas of mud flats are exposed in Kakinada bay.

The EGREE, the abutting coastal area, and its associated open sea ecosystems, including Kakinada bay, are rich in floral and faunal diversity, and also generate other ecological and economic benefits such as shoreline protection, ecosystem based livelihoods, and carbon sink services. Mangrove forests situated in these deltaic wetlands cover an area of 32,140 hectares. In total, there are 35 species of mangroves of which 16 are true mangroves and the rest are associated mangrove species. There is one nearly threatened (IUCN) mangrove species (*Ceriops decandra*) which is not reported in other adjoining areas and there are three rare species (*Sonneratia alba*, *Scyphiphora hydrophyllacea* and *Xylocarpus moluccensis*). This is probably the only place in India where three species of Avicennia i.e. *Avicennia officinalis*, *Avicennia marina*, and *Avicennia alba* are found together in mixed forests (Rao, 2009).

Climate Change Context

The Godavari mangroves also play an important role as a carbon sink. Maintaining the extent and ecosystem functionality of the mangrove forests and preventing any further retrogression is, therefore, important as a strategy to address climate change. While mangroves play an important role in mitigating climate change, they are also threatened by climate change. To date, non-climate related anthropogenic stressors have accounted for most of the global average annual rate of mangrove loss. However, climate change-induced perturbations including relative sea level rise and change in salinity may constitute a substantial proportion of predicted future losses. Hence, attention needs to be given to augmenting the tolerance and resilience of mangroves to climate change (Eric et. al, 2008).

Climate change impacts on the mangrove ecosystems would be governed by factors such as sea-level changes, storm surges, fresh-water flows in rivers both from precipitation in their catchments as well as from snow melt in the mountains, local precipitation, salinity alterations and temperature changes that would influence evapo-transpiration. Sea-level rise would submerge the mangroves as well as increase the salinity of the wetland. This would favor mangrove plants that tolerate higher salinity. Changes in local temperature and precipitation would also influence the salinity of the mangrove wetlands and have a bearing on plant composition. It is therefore, necessary to model the specific scenarios for the various mangrove ecosystems using climate change projections, changes in freshwater and sediment flows, geomorphology, sea-level change and the land use of the coastal region. In addition, it is important to model and predict the impact of multiple stressors (climate change and other anthropogenic and natural stressors), and their compounded effects on the mangroves

The impacts of climate change on EGREE are poorly understood. However, available literature suggests that the mangrove ecosystem of the east coast of India is one of the most vulnerable regional habitats to be exposed to sea-level rise (Rao, 2009). Increasing salinity and precipitation patterns also affect distribution of salt-tolerant mangroves such as *Avicennia* spp. and *Rhizophora* spp. The seedlings of all species require very low salinity for their growth; hence, a rise in salinity could affect their survival, growth and productivity. Rising sea-level brings in salts and sulphates; diminution of rainfall reduces mudflow and nutrient influxes. Increased frequency of tropical cyclones with inundation of low-lying areas and salt-water incursion is also not ruled out. These changes might ultimately result in changed biodiversity and species migration towards land. In short, it can be presumed that the condition of the mangroves of the EGREE, which are already under considerable stress, will become further worsened due to climate change.

Socio-economic Context

Production activities such as fishing, ports and shipping, agriculture, tourism, oil and mineral exploitation contribute about 10% of the national GDP. Most of the oil and gas reserves in India lie in the coastal and shallow offshore areas. Thirty-five per cent of the coastal stretch is laden with substantial mineral and heavy metal deposits. A very significant share of India's economic infrastructure, including maritime facilities,petroleum industries, and import-based industries are located in the coastal zone; there are 197 major or minor ports and 308 large-scale industrial units in the coastal zone. Coastal fishing employs a million people full time, and the post-harvest fisheries sector employs another 1.2 million people in 3,638 fishing villages and 2,251 fish landing centers.The coastal zone of the country is under increasing stress due to industrial development, trade and commerce, tourism and resultant human population growth and migration.

The Indian Coast has 77 cities, including some of the largest and most dense urban agglomerations such as Mumbai, Kolkata, Chennai, Kochi and Visakhapatnam. With less than 0.25% of the world's coastline, India houses 63 million people or approximately 11% of global population in its low elevation coastal areas. India's coastal districts (73 out of a total of 593 districts) account for 17% of the national population, and nearly 250 million people live within 50 kilometers of the coastline. Coastal population is projected to rise to almost three quarters of the national population by 2020 (Anon, 1992). The constantly increasing anthropogenic pressures in coastal areas make coastal and marine ecosystems more vulnerable to global climatic changes, especially global warming and its consequences such as changes in rainfall patterns, storm frequency, salinity changes and sea level rise.The coastline of Andhra Pradesh too is pivotal to the State's economic development. Coastal and marine resources contribute significantly to the state's economy. GEF [1] agency funded project through UNDP was implemented in 2011-16 by the Ministry of Environment and Forests, Science and Technology department of the government of Andhra Pradesh to strengthen the policy and regulatory framework for mainstreaming biodiversity in production sectors of EGREE.

The landscape/ seascape where the project is going to be implemented is the East Godavari River Estuarine Ecosystem (EGREE). Specifically, the direct area of influence of the project, where most of the project activities will take place will be 46,450 hectares that include the Coringa Wildlife Sanctuary and the area immediately surrounding

it along with the abutting villages. The project is also expected to indirectly influence another 33,550 ha in the EGREE mostly through awareness generation, outreach and capacity development. Thus, the total area intended to be covered under the project comes to around 80,000 hectares. This includes 17,486 hectares of water body, and 32,142 hectares of mangroves, of which 21,600 hectares is within the CWLS. The coordinates for the project area are 820 8' 27" and 820 21' 50" E and 160 30' 47" and 170 0' 33" N. The entire area falls in the East Godavari District and revenue divisions of Kakinada (Mandal – Tallarevu, Karapa, Kakinda Urban, and Kakinada Rural) and Amalapuram (Mandal – I. Polavaram and Katrenikona).

The project area excludes Yanam, which is part of the Union Territory of Pondicherry. The total population of the project area is around 1 million.The main economic/ production activities in the target project area are fisheries, lime shell collection, aquaculture, salt pans, tourism, manufacturing activities (e.g., oil and natural gas, fertilizers, edible oil, rice products), and ports. In addition, there is dependency on the mangroves by local villagers. There are around 39 villages and hamlets abutting the mangroves and dependent on it for their livelihoods. Although the major occupation of the communities living in the area is fishing, other occupations include cattle rearing, agriculture, lime shell collection and production of salt. The communities are also dependent on the mangroves for fuelwood, timber and grass for house construction. The total population of the area is 79,000, with 10,261 households (MSSRF 2004).

Anthropogenic Interventions

About 6,950 traditional crafts (500 motorized and 6,450 non-motorized) are engaged in fishing activities in and around EGREE. In the East Godavari district, around 3,000 mechanized crafts are engaged in fishing, of which around 1,000 are trawlers and the others include beach landing crafts, gill-netters, liners, seines, etc. Pelagic resources are exploited using hook and lines for sharks, seer fish, tuna, etc, and boat seines for sardines, mackerels, etc. Shore seines are also operated for near shore fishery resources. Cast nets, gill nets, drag nets and trawl nets are the major fishing gear used in this region. During 1996-2006, fish landings ranged from 151,435 to 233,276 tonnes and contributed to 7.2 per cent of total fish landings of the country. Smoking, salting and sun drying of fish and shrimp are the major fish processing activities. Around Kakinada alone, fish catch has fluctuated over the last three years with an annual low of 1,925 tonnes and an annual high of 3,500 tonnes.

Aquaculture is being practiced in the EGREE since the late 1980s as an important livelihood/ economic activity. Close to the Godavari Estuarine region alone, the area of aquaculture farms increased from 2,006 hectares in 1989 to 19,239 hectares in 1999 (Andhra Pradesh Remote Sensing Centre, 1999). The increase in shrimp farming area led to an increase in shrimp production from 30,000 tones in 1990 to 102,000 tones in 1999. Lime shell collection is banned by the Supreme Court and yet there are about 50 families uprooted from their village (Balusipeta), engaged in this occupation. They live in the sea to collect the shells and are controlled by traders who advance them money so that they remain bonded to them. In the Godavari delta, about 14% of the aquaculture farms have been established on mangrove lands.The salt pan area spread is about 1,000 acres of land which is controlled by a few individuals around Coringa area. These are the erstwhile mangrove wetlands and have been in existence for more than 50 years. Salt pans attract a large number of migratory birds during winter.

The salt harvest is done 6 times a year, one cycle lasting for 30 days; employing around 500 workers throughout the year. However, the salt pans in the EGREE are not economically promising and as such are not likely to expand in future.Many medium and large-scale manufacturing units/ industries are also located in the EGREE including natural gas & oil, fertilizers, power generation, edible oil, rice products, automobile components, biodiesel, cotton yarn, Liquid Petroleum Gas Bottling, Carbon Dioxide Bottling, Iron Ore fines, Quartz Crystals, and Steel Re rolling. Most of these manufacturing units are located in and around the towns of Kakinada and Yanam and derive benefits directly or indirectly from the mangrove estuary. These activities also impact the ecological health of the mangrove ecosystems in the EGREE.Tourism is a rapidly expanding sector across India, including Andhra Pradesh, and there is need for greater capacity within this sector for managing potential adverse environmental impacts, for example,

waste and sewage disposal. Tourism development in the project area is in its initial stages and the developmental impacts of it on the Godavari mangrove ecosystem are not yet documented.

Legislative, Policy, and Institutional Mechanisms

To promote conservation and sustainable use of biodiversity, India has an extensive body of laws and policies. The most relevant policies and legislations from the project's perspective are the Biological Diversity Act of 2002, National Forest Policy of 1988, Indian Forest Act of 1927 and related state legislation, Forest (Conservation) Act of 1980, Wildlife (Protection) Act of 1972, Environmental (Protection) Act of 1986, Marine Fishing Policy of 2004, and the Joint Forest Management orders and rules promulgated by both the Government of India and the States. Other State legislation relevant to coastal and marine biodiversity includes the Andhra Pradesh Marine Fishing Regulation Act of 1994, adopted under the national Marine Fishing Regulation Act of 1978, which provides for protection, conservation and development of fisheries in Andhra Pradesh. The Act also regulates mesh size, gear and reservation of zones for different fishing sectors, and aims to protect the interest of traditional fishermen and their crafts. The Andhra Pradesh Board Norms ensure compliance with the Environmental (Protection) Act, 1986 (EPA) regarding standards for controlling water and other forms of pollution.

Given the situation wherein more ports are coming up, the establishment of an Andhra Pradesh Maritime Board is also envisaged.Further, the production sectors operating in the coastal zone are regulated by a number of laws, of which the most significant is the Coastal Regulation Zone (CRZ) Notification of 1991 and 2010, promulgated under the EPA. The 1991 notification restricts and controls development activities within a landward distance of up to 500 meters from the high tide line along India's coasts. Also under the CRZ Notification, all states are required to prepare a Coastal Zone Management Plan (CZMP) and establish a Coastal Zone Management Authority. Accordingly, the CZMP for Andhra Pradesh was developed in 1996. The CRZ Notification of 2010 has identified Coringa, East Godavari and Krishna as Critical Vulnerable Coastal Areas and stipulated that an integrated management plan shall be drawn up within a period of one year keeping in view conservation and management of the mangroves and needs of local communities. The Environmental Impact Assessment Notification of 2006 aims to protect and conserve the environment through regulation of new developments taking place by ensuring environmental compliance causing least/ negligible adverse impacts on the environment. Environment Impact Assessment (EIA) has been made mandatory for all the investment and development projects in the coasts.

The Ministry of Environment & Forests (MoEF) is the nodal agency in the administrative structure of the Central Government for planning, promoting, coordinating and overseeing implementation of India's environmental, forestry and wildlife policies and programmes. MoEF's work is guided by the set of legislative and regulatory measures aimed at the preservation, conservation and protection of the environment, as well as by the National Conservation Strategy and Policy Statement on Environment and Development, 1992; National Forest Policy, 1988; Policy Statement on Abatement of Pollution, 1992; National Environment Policy, 2006, National Biodiversity Action Plan, 2008, National Wildlife Action Plan (2002-2016) and the National Action Plan on Climate Change, 2008. The primary mandates of the Ministry are implementation of policies and programmes relating to conservation of the country's natural resources including its lakes and rivers, its biodiversity, forests and wildlife, ensuring the welfare of animals, and the prevention and abatement of pollution. While implementing these policies and programmes, the Ministry is guided by the principle of sustainable development and enhancement of human well-being.

Threats to biodiversity and ecosystem services of the EGREE

In spite of the legal, policy and institutional framework, mangrove and coastal ecosystems of Andhra Pradesh in general, and the EGREE in particular, are under increasing threat. The Godavari Delta, like many other deltaic systems in India, has been highly altered by human activity. Since 1893, mangroves in the area have been subjected

to heavy exploitation for fuel wood. Local people used the mangroves for agriculture, salt production and aquaculture. The CWLS and other areas in the Godavari Estuary Area were subjected to heavy cattle grazing, resulting in large scale depletion of mangrove forests.The mangrove ecosystem in the EGREE is still under degradation due to increasing anthropogenic pressure from rural and urban areas and its proximity to a growing industrial area. Causes for the degradation include conversion to aquaculture, pollution, eutrophication and siltation of Kakinada Bay and its rivers, anthropogenically induced river flow change and erosion, seasonal hydrological changes, and overexploitation of mangrove forests by villagers. It is estimated that 30% to 40% of the degradation of mangrove forests has taken place in the last decade due to agriculture, aquaculture and tree-felling activities, oil and pesticide pollution.The direct drivers of ecosystem degradation in the EGREE are (i) habitat destruction, (ii) excessive harvesting and consumption of coastal and marine resources, and (iii) pollution from industries, aquaculture, and urban agglomerations (Kakinada and Yanam).

Strategies to Re-establish EGREE

The Government of India is concerned about the extent and severity of coastal and marine resources degradation, and its effect on the economy at the regional, community and individual household levels, and is, therefore, requesting GEF assistance to support this project.The coastal and marine biodiversity of the EGREE, as already described under the geography and biodiversity is not only, globally significant for its biodiversity but also, in terms of climate change. Land use and land use changes are key issues in global efforts to sequester more carbon in the face of critical climate change trends. The EGREE also has national and local significance insofar as it supports human livelihoods, provides natural cycling of minerals, and acts as a potential resource for sustainable income generating activities such as ecotourism.

Coastal and marine resources provide the direct basis of subsistence for more than 40 villages/ hamlets in the immediate vicinity of the CWLS. But there is growing evidence that the EGREE's natural resources have been increasingly subjected to over-exploitation, reducing their potential to sustain the present generation, let alone meet the needs of future generations. The poor and marginalized, with no alternative options, are exploiting the natural resources to survive and the degraded resources further impoverishes these communities making survival more difficult and uncertain. It is only through judicious use of these resources and through restoring the integrity of already degraded ecosystems that rural households will be able to increase their food security and Social-economic wellbeing.

Cross-sectoral approach

The emphasis is on an integrated coastal management approach. Compared to conventional sectoral approaches, the aim is to ensure productive and healthy ecosystems by integrating all the relevant stakeholders i.e. not just the conservation sector, but also the livelihoods/ subsistence sector and other commercial production sectors. This will help to bring together knowledge and experience of the different sectors, and to reconcile different stakeholder interests and needs. Both the public and private sectors, including community based and non-governmental organizations (CBOs, NGOs) need to be engaged. There is also need for greater coordination and cooperation among government departments. Given the need to break down barriers between sectors and disciplines, the project focuses on building a cross-sectoral institutional mechanism for the integrated, sustainable management of coastal and marine resources in the EGREE so as to:

- build a platform to share knowledge and forge partnerships across sectors
- develop a common understanding of the coastal and marine biodiversity and consequences of the degradation of the natural resources
- promote the development and adoption of locally-appropriate, community-regulated sustainable resources management

- involve the productive sectors in actions to protect natural resources of the EGREE

Inter-disciplinary approach

Integration means an inter-disciplinary approach to understanding of biodiversity and ecosystem services, as well as the social, economic and political factors that contribute to their existence. This leads to identification of appropriate technical, policy, legislative and institutional interventions required to overcome the barriers and to promote conservation and sustainable use of biodiversity.

This cross-sectoral and inter-disciplinary approach will help in (i) building a common diagnosis and shared vision (ii) sharing information about past, on-going and planned development interventions; (iii) better coordinating and harmonizing existing interventions and investments; (iv) improving the design and alignment of future projects and programmes; and (v) identifying and addressing key barriers and bottlenecks to scaling up mainstreaming approaches.In the context of the target landscape, getting production sectors to factor in biodiversity conservation into their operations is going to require a significant change in thinking and practice. It is partly about giving the appropriate "push" by enshrining this thinking in the legal framework, but it is equally about drawing the sectors in to the discussion, bringing individual actors to the table, changing mind-sets, providing training and tools, and providing technical and financial "hand-holding" to demonstrate the new paradigm, in turn, absorbing some of the perceived risks in changing current practices.

A two step process is suggested: Step 1 is to begin a concrete dialogue with stakeholders through the vehicle of the Landscape-level Strategic Plan and the Sector Plans, and Step 2 is to try out specific changes in current practices. During consultations, it was felt that doing the latter without the former would antagonize the key production sector stakeholders and the project would be yet another conservation sector-led initiative that fails to obtain ownership from the production sectors.

The GEF-UNDP project (2011-2016)aims to mainstream biodiversity conservation into the production sectors of EGREE through:(1) Cross-sectoral planning in the EGREE that mainstreams biodiversity conservation considerations; (2) Enhanced capacity of sector institutions for implementing biodiversity-friendly sector plans; (3) Improved community livelihoods and sustainable natural resource use. The main aim of this project component is to mitigate the negative dependency and bring resource use to a sustainable level. In order to do this, institutional strengthening will be very important. Most of the villages surrounding the project area have formed Self Help Groups (SHGs) and other local institutions. Twenty Eco-Development Committeees (EDCs) have been constituted under the donor-funded Andhra Pradesh Forestry Project with the help of non-governmental organizations. It is anticipated that production activities in at least 80,000 ha of the EGREE mainstream biodiversity conservation objectives, in improving the conservation prospects of several globally significant species apart from contributing to the socio-economic well being of the region.

Concluding Remarks

Maintaining sustainability across the ecologically significant sites such as East Godavari River Estuary area in India is crucial from the ecological and people's livelihood perspective.Ecological sustainability would support long-term viability of globally significant biodiversity in the EGREE by mainstreaming biodiversity conservation considerations into the activities of the productions sector, strengthening conservation and management of the CWLS, as well as promoting sustainable livelihoods of local communities. In order to ensure that biodiversity mainstreaming approaches are identified, financial sustainability strategy will accompany the landscape-level Strategic Plan. The financial strategy will look at a mix of approaches such as re-alignment of existing government budgetary resources, re-alignment of existing resources earmarked under CSR programmes of large corporate institutions operating in the

area, and/ or mobilizing new resources to mainstream biodiversity conservation concerns. Capacity development is a significant factor to realise the objectives of the project. Capacity development is based on comprehensive need assessments targeting all key stakeholders that directly or indirectly impact the EGREE.To ensure that social equity is maximized, project activities targeting the livelihoods should ensure extensive stakeholder participation.

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Table 1. Mangrove forest area in EGREE

	Name of Reserve Forest	Area in hectares (within CWLS limits)	Area in hectares (outside CWLS limits)
1	Corangi	4,272	
2	Corangi Extension	18,808	
3	Bhairavapalem	1,015	
4	Rathikaluva		1,762
5	Balusitippa		1,300
6	Matlatippa		389
7	Masanitippa		546
8	Kottapalem		66
9	Kandikuppa		3,984
	Sub totals	24,095	8,047
	Total area of Mangrove Reserve Forest in EGREE		32,142

Source: Atlas of Mangrove Wetlands Of India: Part 2 Andhra Pradesh (MSSRF, 2004)

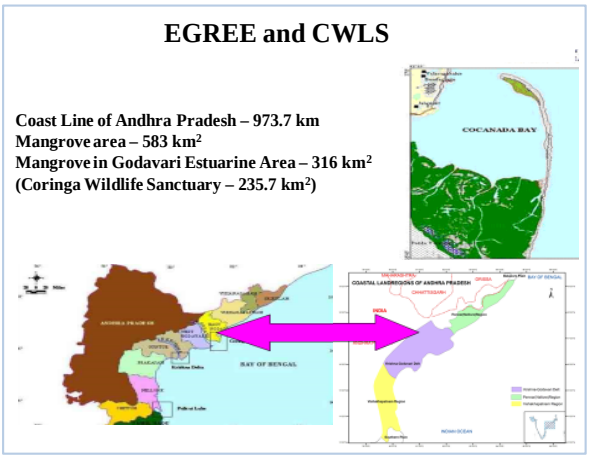

Fig. 1. Map showing the Reserve Forests of East Godavari River Estuary and Coringa WLS

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RESEARCH ARTICLE

Effects of Propolis Extract on Egg Quality Parameters and Microbial Activity of Japanese Quail Eggs

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ABSTRACT

Propolis is a resinous, dark coloured material that produced by worker honeybees which collected from plants and it has strong antibacterial and antifungal properties. The purpose of this experiment was to evaluate the use of propolis extract on egg quality parameters and microbial activity of table quail eggs (*Coturnixcoturnix Japonica*). A total of 100 fresh eggs was randomly divided into four groups (0%: group I, 5%: group II, 10%: group III, 15% group IV) and treated with 70% ethyl alcohol (group V). Consequently, the effects of storage time and shell treatments on storage time and the effects of treatments on the interior quality of eggs were determined. Bacterial activity was seen to be reduced significantly in all propolis groups. The results of the study also confirmed highly significant difference in egg weight loss, albumen-yellow indexes, and Haugh unit ($P<0.001$). While the difference in yolk index between groups was insignificant, the difference between groups with respect to albumen index, Haugh unit, albumen pH ($P<0.001$) and egg weight loss (0.001) were significant. The significance of the overall difference with regard to Haugh Units varied among groups. Albumen pH were increased with increasing storage time for each treatment. The best egg production results in terms of interior quality were obtained in egg coated with 10% and 15% propolis extract during storage. Results of the present study indicated that propolis could be an alternative hatching egg disinfectant versus a chemical disinfectant, without adverse effects on performance of quail eggs.

Keywords: Propolis, eggshell microbial activity, egg quality, storage time.

INTRODUCTION

Propolis, a product of honey having a diverse range of biological properties is characteristics to having major chemical composition including phenolic acids, phenolic acid esters, flavonoids and terpenoids such as CAPE,

artepillin C, caffeic acid, chrysin and galanginquercetin, apigenin, kaempferol, pinobanksin 5- methyl ether, pinobanksin, pinocembrin, pinobanksin 3-acetate [1]. Propolis is measurably used to prevent and cure colds, wounds and ulcers, rheumatism, sprains, heart diseases, diabetes [2-4] and dental caries because of its wide variety of biological properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, antiulcer and anti-HIV activities [4].The chemical composition and quality of propolis depends on the species, subspecies and varieties of bees affect the antibacterial activity of propolis collected from the same apiary [5].

Eggs are known to be naturally safeguard against microbial deterioration due to its biological and chemical structure with its composition [6].For determining the internal quality of commercial eggs, albumen height and albumen pH plays a vital role of which there is a decrease of height of the albumen and there is a increase of albumen pH value with an increment in storage time [7-9].During the time of laying of eggs there seems a plentiful microbes settle on the egg surface, as newly laid eggs is moist and moderately hot and the immature cuticle and few pores may be open which results in responsive to piercing by microorganisms [10-13]. Quintessential contaminators are *Salmonella*, *Pseudomonas* [14], *coliforms* and *Escherichia coli* [15]. *Yeast* and mold have also been observed as well[16]. *Lactobacillus* spp. and *Micrococcus* spp. found in ova (*Salmonella enteritidis* and host specific *Salmonella gallinarum* and *Salmonella pullorum*) are adequate to transfer to the ova by virtue of blood from the alimentary canal [17].However *Micrococcus, streptococcus* and coli-aerogenes organisms are the superior [18] *Staphylococcus aureus*, *Salmonella* spp., and *Pasteurella* spp. have also been found from the oviduct[12]. Intermittently contamination of eggs occur sub sequentially after *oviposition* and contaminators may be divided into pathogens (e.g. *Salmonella enteritidis*) or spoilage bacteria (e.g. *Aeromonas*, *Enterobacter*, *Proteus* and *Pseudomonas*).

Accordingly sanitation is vital in lucrative incubate of egg production. Fumigation, application of spray, UV light and washing with appropriate sanitizer are the assorted methods which are commonly available [19-25]. Considering the health of human, concerns for environment and demands of consumer for residue free food needs analysis of alternative low risk control methods. Taking all of these points in view an endeavour has been made in the present study to find out the practical applicability of propolis for controlling microbial activity occurring naturally on the egg shell.

MATERIALS AND METHODS

This study was conducted at the Zoology laboratory of Centurion University Technology and Management. A total of 100 eggs were obtained from 12 week old Japonica quail (*Coturnixcoturnix japonica)* which reached 85% egg production. However oviposition eggs were collected between 1000h and 900h and stored at 25°C overnight before the application of disinfectants. The eggs were randomly divided into 5 groups of 20 eggs. The following treatments were included: control without any treatment (group I), coating with 5% (group II), 10% (group III),15% (group IV) propolis extract, and coating with ethyl alcohol (group V).

Preparation of Propolis Solution

Propolis was collected from the honey bees in Odisha in 2019 and extracted according to the method suggested by Krell [26-27]. A 5% propolis solution was prepared by mixing 950 mL 70% ethanol and 50g propolis, the 10% propolis solution was 900mL 70% ethanol and 100g propolis and the !5% propolis solution was 850mL 70% ethanol and 150g of propolis. Solution were kept in a sealed container and shaken twice daily for 2 weeks.

Incubation Analyses

Eggs were incubated in a commercial incubator with dry bulb temperature of 37.5°C and 60 to 65% RH until 14d of incubation when incubation conditions were changed to 37.2° and 75% RH at the Department Of Zoology, CUTM, Odisha, India. Eggs were turned 90° once every 2 hour.

Microbiological Analyses

The 5 eggs per group were taken for microbiological analysis at 1, 7 and 14 days of incubation and immediately placed on sterile stomacher bags containing 50mL of sterile PBS (pH 7.2).A whole egg washing technique was used to recover the shell associated microorganisms for estimating the total aerobic mesophilic bacteria, coliforms, *Salmonella* ssp., *Staphylococcus* spp., and mold- *Yeast*. Serial dilution were made in PBS and then were inoculated into sterile Petri plates [28,29].Colonies were measured as log cfu/egg. Media and incubation conditions used in microbiological analysis were shown in table 1. Coliforms were determined with the most probable number technique. The incubated tubes that showed a yellow tint (acid production) and gas were considered to be positive. Suspected *Staphylococcus* ssp. Colonies were tested for coagulase activity and confirmed by other biochemical reactions.

Coating of Eggs with Coating solution and ethanol

Eggs were immersed in the 5%, 10% ,15% propolis solutions and ethanol by hand for 1 min, and this process was repeated once more after 10 mins. Following the second immersion the eggs were dried for 24hours at ambient temperature.

pH Measurement

After measurements for the albumen and yolk index parameters, the albumen was separated from the yolk. The volumes of thick and thin albumen were mixed with a spatula by hand before measuring pH value. The pH of the albumen was measured using a micro controller based pH meter.

Egg Weight Loss

Egg weight loss (EWL) during storage time was calculated by individual measurement of initial and weekly weight changes in each group as follows:

$EWL(\%) = (W_o - W_1/W_0) \times 100$

W_0= Initial Egg weight

W_1= Egg weight during measurement day

Albumen index

Eggs from each treatment group were broken on a flat surface where the height of the albumen was measured in millimetres, halfway between the yolk and edge of the inner thick albumen, using a standard tripod micrometer. Measurements were taken from three points on the inner thick albumen at the intervals of 10 mm from each other. Measurement of albumen width was to 0.1 mm using a steel Vernier caliper. The albumen index (AI) was calculated as follows [35]

AI (%)= [Albumen Height/(Albumen Length+ Albumen Width)/2)]×100

Haugh unit

Haugh unit (HU) values were calculated using the following formula [36]

$HU = 100 \text{ Log } (H+7.57 - 1.7 \times W^{0.37})$

Where H is the albumen height (millimetres) and W is the egg weight (grams).

Yolk index

The yolk index (YI) was calculated as follows [37]

YI (%) =(Yolk Height/Yolk Width)×100

Data Analysis

In this study, the combined effect of propolis and storage time and microbiological activity on internal egg qualities was evaluated. The data was subjected to Past 3 software with treatments and storage time as the main effects. When the main effects were significant at P<0.05, difference between means were tested using Duncan's multiple range test.

RESULTS AND DISCUSSION

Albumen pH

To evaluate the changes in albumen pH at the time of storage, it was vital to measure the initial albumen pH immediately just after the oviposition. Albumen height and Albumen pH determines the albumin quality. During the time of storage initial albumen levels in each group increased, but this increase in the control group and the groups coated with ethyl alcohol was higher than the increase in the other treatment groups coated with different concentrations of propolis. The initial albumen pH level of the control group was 8.18 and that of the group coated with ethyl alcohol 8.70. These values increased to 9.43 at the end of fifth week (Table 2).

In this study the albumen pH values measured after the fifth week was lesser to the pH value of quail eggs reported by Baylan who found it to be 9.52 at 20°C after 30 d of storage, and by Aktan, who reported a pH of 9.78 after 7 d of storage. Some researchers Silversides, Li-Chan and French have been reported that the starting value of albumen pH for chicken egg was between 7.6 and 7.9. In the present experiment the basic albumen pH in quail egg was 8.81(Table 7). This value is similar to that of previous studies, in which the initial albumen pH of quail egg was determined to be between 8.56 and 9.17 [8,38,39]. These results are in agreement with previous studies conducted by Silversides and Scott[8], Samli et al[40], Caner[41], Akyurek and Okur[42], Aktan[43], Baylan et al[44], Hristakieva et al[45], and Jin et al[46], who found initial increase of albumen pH level depending on storage time.

Egg Weight Loss

Factors such as egg surface coating with different materials and storage time affects the internal egg quality characteristics. The egg weight loss of each treatment group increased significantly with storage time (P< 0.001) (Table 3) (Table 7). The egg weight loss of groups 6.07, 5.63,4.9, 4.45 and 5.71 respectively. The highest egg weight loss values were obtained from the control group and samples coated with ethyl alcohol. While the egg weight loss in the first week was 1.06%, it reached 5.35% after the fifth week. In the present experiment egg weight loss differed significantly between all treatment groups (P<0.001) (Table 8). The highest average egg weight loss was 3.46% in group I, followed by group V (3.33%).These results are in agreement with those of Romao et al.,[53] who reported egg weight losses of 2.72 and 2.39% during 2 wk of storage at 20°C in meat and laying type quails, respectively. Lacin et al.,[54] also reported similar egg weight loss (2.56% during 2 wk of storage). In this study the determined egg weight loss following 2 wk of storage was 2.01% which was similar to the results given previously. In this study weight loss in uncoated eggs was found to be significantly higher than that found in coated eggs(p<0.001). The samples with 15% propolis coating had significantly lower weight loss compared to the other two propolis concentrations. This finding is in agreement with the results reported by Agyun and Sert [27]. These authors reported 1.74, 1.75, and 1.37% and 2.86, 2.55 and 2.28% weight loss over 7 and 14 d of storage respectively for quail hatching eggs coated with 5,10 and 15% propolis. Coating eggs using various concentrations of propolis may prolong their shelf life by preventing weight loss by limiting the loss of humidity and carbon dioxide through the shell.

Albumen index

Some undesirable physicochemical changes in egg structure arose with increased storage times [40,47]. The albumen index significantly decreased with increasing storage time in all treatment groups (P<0.001). The highest decrease in the albumen index after storage times compared with initial albumen index after storage times compared with the initial albumen index values was in group V, followed by group II, I, III, and IV. This result agreed with those of previous studies[9,48,46,49], which reported that the albumen index decreased with increasing storage time. The

effect of storage time on the albumen index was significant (P<0.001) (Table 8). In the present study the albumen index differed significantly among the treatment groups. The highest albumen index values were obtained groups II(9.35%), III(9.25%), and IV(9.25%) which were coated with propolis extract. The albumen index values of the control group and the group coated with ethyl alcohol were 8.41 and 8.61% respectively.

Haugh unit

The Haugh unit (HU) is related to albumen quality and measured as a function of the inner thick albumen height and egg weight. Weekly changes in HU values of all treatment groups during storage time are given in table 6. The basic HU value (95.28) decreased with increasing storage time. At the end of the storage time (week 5), the HU value was found to be 81.30. The differences between the weeks were statistically significant (P<0.001) Table VII. The HU values during 5 wk of storage were higher in group IV (86.45) than group I(85.1). The decreasing HU values with increasing storage time were supported by previous studies [46,48] conducted with chicken and coturnix eggs. At the end of storage, the highest HU values were determined in group III and IV. This result is in agreement with those of Allenoni and Antunes [50], Caner[41], and Copur et al [48]., i.e., studies that found a higher HU value in coated eggs compared to uncoated eggs.

Yolk index

During the storage the yolk index (YI) decreases through the diffusion of water from the albumen to the yolk, resulting in changes in the vitellin membrane and in liquefaction of the yolk [8,9]. The YI decreased with increasing age of the hen eggs[51], though this trend was not confirmed in Japanese quail eggs, and the YI of quail eggs was higher than that of chicken eggs[51]. The difference in YI among treatment groups were insignificant (P>0.05) (Table 8). The determined basic YI value (48.10%) was approximately similar to the values obtained by Zita et al., who reported results of 49.11% for 9-wk-olg quail, 47.66% for 13-week-old quail and 50.81% for 37 wk-old quail. The basic YI decreased significantly with increasing storage time. The basic YI values decreased from 48.10 to 34.02, 31.33, 27.56, 29.87 and 26.45% for 1, 2,3,4, and 5-wk storage time respectively. These results are in agreement with those of previous studies [9,41,52]which found a significant decrease in YI with increasing storage time in various poultry eggs.

Microbiological activity

Application of different disinfection solutions significantly (P< 0.001) affected total aerobic mesophilic bacteria values. The microbial count at incubation periods of 1 days decreased from 3.35 to 1.21 log cfu/egg with the P15 propolis treatment. The decreased rate of microbial loads continued parallel with progressive incubation durations. The lowest and the highest aerobic mesophilic bacterial counts of all storage periods were determined in the P15 treatment and in the alcohol control treatment respectively. Significant (P<0.01) differences were also found in values of coliforms bacteria , which were entitled as a fecal contamination indicator in foods, among study groups with respect to the type of disinfectants. The lowest coliform count (1.01 log cfu/egg) was obtained from the P15 treatment. The colony count of Salmonella ssp. was reduced (P<0.01) by disinfection treatments.

Application of propolis in all incubation periods diminished the *Salmonella* count. *Salmonella* values of the alcohol treated group were higher than the other treatment groups. *Salmonella* counts at the beginning and at the end of the incubation period were observed to be 2.1 and 3.0 log cfu/egg, respectively. *Staphylococcus* ssp. counts in all incubation periods decreased with the higher propolis concentrations. Although the inhibition rate at the beginning of incubation for P5 treatment was 40%, it increased to 51% in the P15 treatment. Mold-yeast counts at the beginning of incubation were 4.2 and 3.8 log cfu/egg respectively. This count decreased with increasing propolis concentration and it reached 2.1 log cfu/egg in P15. The difference between P10 and P15 treatments were not significant at the end of incubation. This result agrees with the findings of [55], who showed that propolis has antifungal activity.

Future scope

Although propolis is an alternative to hatching egg, although it is a disinfectant versus a chemical disinfectant, without adverse effects. Alternative area for this research is to avoid microbial contamination through propolis other than harmful chemicals.

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Table 1.Media and incubation conditions used in microbiological analysis

MICROORGANISMS	MEDIUM	TEMPERATURE (°c)	TIME (D)	REFERENCE
Total aerobic *Mesophilic* bacteria	Plate count agar(5% NaCl were added)	37	2	30
Mold- yeast	Potato dextrose agar(pH 3.5; acidified with 10% tartaric acid)	20	5	31
Coliform bacteria	Macconkey's broth	37	2	32
Staphylococcus ssp.	Baird Parker agar(supplemented with 5% egg yolk-tellurite)	37	1	33
Salmonella ssp.	Selenite cystine broth and bismuth sulphide agar	37	1	34

Table 2. Effect of propolis coating on albumen ph during 5 wk of storage

GROUP	WEEK0	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
I	8.18 ± 0.1^{d}	8.9 ± 0.01^{c}	$9.21\pm0.02^{a,b}$	$9.31\pm0.01^{a,b}$	9.12 ± 0.01^{b}	9.41 ± 0.01^{a}	65.89	0.000
II	9.02 ± 0.5^{c}	8.8 ± 0.05^{c}	$9.19\pm0.05^{a,b}$	$9.27\pm0.02^{a,b}$	9.11 ± 0.01^{b}	9.30 ± 0.02^{a}	102.201	0.000
III	9.06 ± 0.08^{d}	8.87 ± 0.01^{c}	9.27 ± 0.02^{b}	9.32 ± 0.01^{a}	9.10 ± 0.02^{c}	9.27 ± 0.01^{b}	221.86	0.000
IV	8.9 ± 0.04^{d}	9.1 ± 0.05^{c}	9.30 ± 0.01^{a}	9.31 ± 0.01^{a}	9.09 ± 0.01^{c}	9.26 ± 0.01^{b}	67.52	0.000
V	8.7 ± 0.07^{c}	9.02 ± 0.01^{d}	9.21 ± 0.04^{c}	9.25 ± 0.01^{c}	9.13 ± 0.01^{c}	9.43 ± 0.02^{a}	169.278	0.0000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different ($p<0.001$)

Table 3.effect of propolis coating on egg weight loss during 5wk of storage (%)

GROUP	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
I	1.25±0.01^{a}	2.21±0.12^{a}	3.54±0.24^{b}	4.43±0.54^{b}	6.07±0.04^{c}	14.53	0.000
II	1.13±0.05^{d}	2.20±0.13^{c}	3.01±0.01^{c}	4.28±0.02^{b}	5.63±0.45^{a}	45.56	0.000
III	1.07±0.08^{d}	1.94±0.12^{a,c}	2.65±0.04^{c}	3.9±0.54^{b}	4.9±0.33^{a}	23.85	0.000
IV	1.04±0.01^{d}	1.7±0.10^{c}	2.37±0.01^{c}	3.38±0.04^{b}	4.45±0.01^{a}	29.78	0.000
V	1.28±0.01^{d}	2.43±0.11^{c}	3.21±0.21^{c}	4.25±0.12^{b}	5.71±0.41^{a}	34.122	0.000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Table 4. Effect of propolis coating on value of albumen index during 5 wk of storage (%)

GROUP	WEEK0	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
I	13.14±0.44^{a}	10.30±0.17^{b}	7.14±0.01^{c}	7.10±0.02^{c,d}	6.35±0.19^{d,e}	5.45±0.02^{e}	57.55	0.000
II	14.18±0.35^{a}	10.62±0.17^{b}	8.08±0.19^{c}	7.43±0.02^{d}	7.13±0.20^{d}	6.24±0.24^{e}	89.56	0.000
III	14.33±0.29^{a}	10.30±0.32^{b}	8.59±0.15^{c}	7.77±0.04^{c,d}	8.02±0.14^{c,d}	7.19±0.14^{d}	62.22	0.000
IV	14.19±0.55^{a}	9.83±0.22^{b}	9.01±0.01b,^{c}	8.02±0.15^{c,d}	7.65±0.54^{c,d}	7.28±0.45^{d}	41.56	0.000
V	14.04±0.37^{a}	10.01±0.32^{b}	8.03±0.22^{c}	7.22±0.45^{c,d}	6.47±0.17^{d,e}	6.02±0.15^{c}	78.52	0.000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Table 5.effect of propolis coating on haugh units during 5 wk of storage

GROUP	WEEK0	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
I	91.82±0.69^{a}	88.47±0.45^{b}	82.14±0.71^{c}	82.16±0.62^{c}	79.45±0.09^{d}	79.85±0.02^{d}	43.50	0.000
II	94.50±0.48^{a}	88.27±0.42^{b}	86.04±0.49^{c}	82.06±0.53^{d}	82.86±0.70^{d,e}	80.14±0.70^{c}	63.215	0.000
III	95.18±0.36^{a}	88.58±0.77^{b}	83.60±0.60^{c}	83.60±0.43^{d}	84.07±0.65^{c,d}	83.15±0.57^{d}	45.253	0.000
IV	94.73±1.22^{a,b}	88.20±0.50^{b}	84.23±0.53^{c}	84.22±0.53^{c,d}	83.45±0.55^{c,d}	83.01±0.21^{d}	30.21	0.000
V	94.86±0.48^{a}	88.66±0.48^{b}	81.53±0.46^{c}	81.53±0.46^{d}	80.06±0.46^{d}	80.01±0.37^{d}	101.45	0.000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Table 6. Effect of propolis coating on yolk index during 5 wk of storage (%)

GROUP	WEEK0	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
I	47.01±1.02^{a}	39.54±0.40^{b}	31.30±0.65^{c}	31.21±0.45^{c}	27.61±0.02^{d}	25.45±0.01^{e}	130.261	0.000
II	47.81±0.56^{a}	39.69±0.30^{b}	34.80±0.45^{c}	31.64±0.25^{d}	31.89±0.01^{d}	26.00±0.55^{e}	140.25	0.000
III	48.56±0.12^{a}	40.05±0.12^{b}	35.23±0.85^{c}	32.45±0.12^{d}	28.45±0.20^{e}	26.01±0.12^{e}	132.20	0.000
IV	48.68±0.62^{a}	39.18±0.22^{b}	35.12±0.54^{c}	30.18±0.01^{d}	27.32±0.12^{e}	26.54±0.45^{e}	164.52	0.000
V	48.42±0.45^{a}	40.25±0.45^{b}	33.75±0.12^{c}	31.25±0.02^{d}	28.27±0.42^{e}	26.34±0.27^{f}	201.5	0.000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Table 7. Mean weekly changes in initial egg quality for all treatment

PROPERTIES	WEEK0	WEEK1	WEEK2	WEEK3	WEEK4	WEEK5	F	P
EGG WEIGHT LOSS(g)		1.06±0.03^{e}	1.18±0.05^{d}	3.00±0.01^{c}	4.01±0.75^{b}	5.35±0.69^{a}	121.45	0.000
YOLK INDEX(%)	48.10±0.24^{a}	39.64±0.12^{b}	34.02±0.12^{c}	31.33±0.04^{d}	29.87±0.65^{e}	26.45±0.29^{f}	725.526	0.000
ALBUMEN INDEX(%)	14.01±0.01^{a}	9.33±0.12^{b}	7.45±0.05^{c}	7.49±0.01^{d}	7.05±0.45^{d}	6.32±0.14^{c}	300.448	0.000
HAUGH UNIT	94.28±0.45^{a}	88.50±0.17^{b}	84.61±0.22^{c}	82.10±0.21^{d}	82.01±0.35^{d,e}	81.30±0.85^{e}	221.216	0.000
ALBUMEN pH	8.81±0.25^{e}	8.74±0.02^{d}	9.19±0.45^{b}	9.11±0.78^{a,b}	9.1±0.02^{c}	9.2±0.01^{a}	204.545	0.000

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Table 8. Means of interior egg quality at the end of the experiment on the different treatment groups

PROPERTIES	GROUPI	GROUPII	GROUPIII	GROUPIV	GROUPV	F	P
EGG WEIGHT LOSS(g)	3.46±0.15^{a}	3.17±0.02^{a,b}	2.77±0.02^{a,b}	2.57±0.45^{b}	3.33±0.65^{a}	3.074	0.001
YOLK INDEX(%)	37.75±0.52	35.34±0.01	35.59±0.96	34.12±0.78	34.64±0.21	0.659	0.536
ALBUMEN INDEX(%)	8.41±0.15^{b}	9.35±0.02^{a,b}	9.25±0.78^{a}	9.25±0.68^{a}	8.61±0.68^{a,b}	2.456	0.027
HAUGH UNIT	85.11±0.41^{c}	86.56±0.01^{a,b}	86.59±0.45^{a}	86.45±0.38^{a}	84.24±0.46^{b,c}	5.061	0.001
ALBUMEN pH	9.01±0.02^{b}	9.09±0.02^{a}	9.09±0.05^{a}	9.11±0.75^{a}	9.08±0.96^{a}	4.658	0.001

I: Control group, II: 5% propolis, III: 10% propolis, IV:15%propolis,V:alcohol

$^{a-e}$ means with different superscripts within rows are significantly different (p<0.001)

Fig 1. Production of yellow tint confirm presence of Coliform bacteria

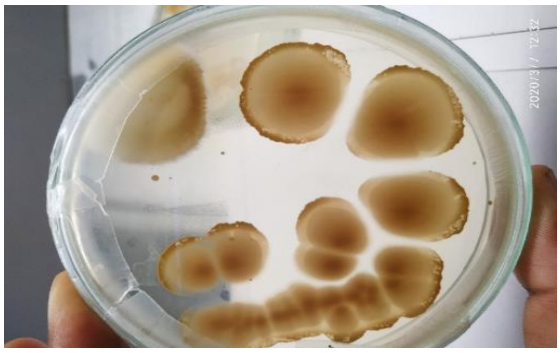

Fig 2. Mold- Yeast

Fig 3. *Salmonella* ssp. detected by coagulase activity

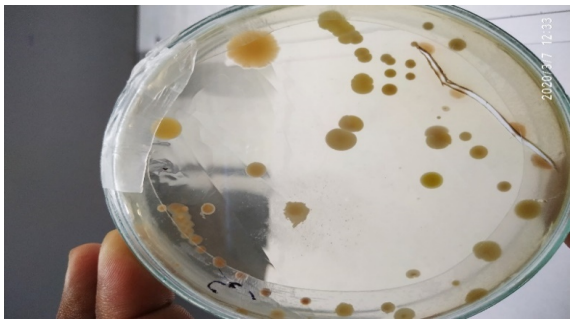

Fig 4. *Staphylococcus* ssp

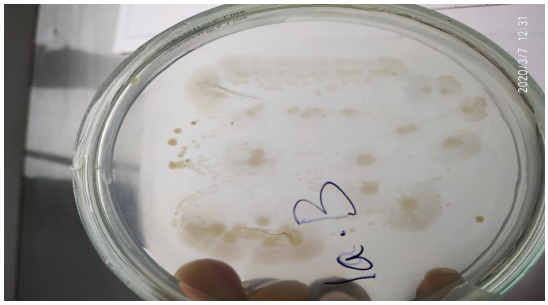

Fig 5. Total aerobic mesophilic bateria

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SPECIAL ARTICLE

AVIR - Anti Virus India Research COVID-19: Drug Discovery Safe Sources

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SPECIAL ARTICLE

INTRODUCTION

The disease COVID-19 is caused by the virus SARS-CoV-2 [1] and is a RNA particle (virus). It is extremely infectious. Prognosis being seriously grave. At presentation and until discharge/demise fever be 37.5°C (99-100^0 F; i.e. low pyrexia); acute Lymphocytopenia (< 1500 cells mm^3); cum Leukopenia; mild Thrombocytopenia (< 150,000 mm^3); Rhabdomyolysis; bilateral patchy shadowing (ground glass type) with prominent heavy-stringy exudates. Yet, sans any damage to the underlying pulmonary matrix as are noted in Tuberculosis; etc. Pneumonia; pulmonary crisis; acutely down-turned thoracic cage mechanics; Right ventricle failure; acute kidney failure; acute heart failure i.e., a cascade is the reported order. Other less notable symptoms being WBC distribution width; high C-Reactive protein; Chloride ion deficiency; etc. Lungs bilateral fibrosis, saline infusion leads to heightened pleurisy & lobe shearing. Gravity route of saline infusion fails as system does not take. Infusion site edema & hematoma. Patient can neither remain supine nor alpine due to enlarging flooding. Severe angina pectoris - yet any analgesic is contra. Steroids also contra indicated yet severe acute COPD symptoms presents. The clinical observation mostly being drawn upon from NEJM & Elsevier [2] while the investigation based averments are taken from Wei-jie Guan, et.al [3]. Architecture, scale, surface morphometry, long & active life from NIH & NASA-JH [4,5]. Lactokalamia; hyponatremia; rigor; K^+ depletion; cerebro spinal involvement are not reported; even vascular complications being conspicuous by absence. These part there has been HIV (anti-RNA virus) vaccine failure [6] and serious side effects with anti-SARS therapy [7]. There being NO drug that is specific these presents a new natural source(s) and chemical moietie(s) for consideration by the stake holders. 1st time report.

Chemical moieties

While the other folinic moieties [10] are RNA specific Ellagic acid is more Table 1. However, it is excessively prone to self-reorganisation and self-reformation. Hence, the acidic (low pKa value) large hydroxyl folins have a sterling role as beside members (complementing - synergic) for they directly contribute Ellagic moieties post any form of hydrolysis in the gastric; peri-gastric and mid gut phases (also re-form back to parent architecture subject to fluid availability). Hence ad-libitum potable water is advised. The Ground glass phenomena (possibly) are degradation products (polymeric protinase & filamentous) of limited proteo-lysis of the WBCs & erythrocytes, some pulmonary cells, the stringy mucus & oxidative stress of the inhalations. The constituent members of such degraded material have a very large cross section and large mass and low specific gravity which is why they may be prefer sheath formation (stratified at top). All this is marked by phagocytosis failure and consequent systemic down turn of the WBC production as Response Pathology. Member No. 2 in either Table have a singularly salutary role in re-ennobling re-phagocytosis ability & process. Sans Rhabdomyolysis kidney failure in particular is due to response pathology (with Rhabdomyolysis renal failure is due induced; reversible). Citrate buffer will however up-regulate viriemia. Diarrhoea is also response pathology as the system attempts auto purging due to virions crowding the gut and adversely drawing upon the Akkermansia muciniphila (punicaligins are effective here too) [11]. The COVID-19 glossary is also relied upon [12].

In place of salt or sweet, harsh and bitter members be preferred. Neem oil on gums. Rain is acutely deleterious for Large\small virus particles (in the out-pandemic limiting natural barrier of meso & cynoptic scale). And rains can be induced in places of pandemics [13,14]. Hydro carbons specially Jatropha oil inhalation i.e., large lamp burning in clinical settings shall additionally help (the large Corona is intensely Nitrogen (N) dependant and hence targets the alveolar envira as choice destination). Natural hydrocarbons shall additionally down-turn virion mitosis. NO_2 is also an anti-dote. NO_2 cannot be generated in the pulmonary parynchema hence posits as the choice destination for SARS & CoV-2 spp.

BACKGROUND

Drug Source & Moiety Identification - Clinico-Field Observations

Herein we present our cue as to what was the natural or the field or working envira based observations that led us to such discoveries and subsequent inventions. Table 2.

Food

Hippocrates of Kos, Greece, (DoB 460A.D.) said "Let food be thy medicine and medicine be thy food", i.e., 'functional food' be our guiding light [19a – 19c]. In situations viz., SARS\COVID-19 food is as vital as clinical intervention. Intubation being the sole route a mild warm soup/fine slimy pushable be considered having the following constituents. All organic farming products. Wild *Solanum virginianum*; Green plantain with the green skin (for abundant phosphates along with enzymes & oils); *Mahua longifolia;* Yam (Dioscorea); Pumpkin; & dates. Quantity @ 50ml 2 times per day with bitter water ad libitum (preferably heavy water H_2O_2) admixed with neem leaf extract 5% v/v.

Other Items

The COVID-19 spp., are nitrogen (N) dependant and hence crowd the lungs wherein air mass sans O2 is abundant = ground glass phenomena as process end. Beedis (chewing of Kendu leafs/ tobacco leafs) and nicotine vectored as warm fluid shall act as prophylactic. Initial/chance finding and subsequent literature review indicates a chequered history of use & reportage [20-28] Table 3.

CONCLUSION

AVIR is original find. Is RNA specific. Arose out of 2 decades + long on foot community assistance. Historically known candidates APIs and are safe sources/starting materials. Numerous formulations are possible (mono or poly moiety). No patent shall be applied for as the above presents are part of Social contract. Open for stake holders to avail patents if that serves better. Nothing happened overnight.

DECLARATION

No grants; No donors. Nothing. Transpirations are from long period purely social service.

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Table 1. Emergency internal medicine possibility at anthropomorph wt.60Kg

Sl No	Chemical Moiety	Component	Modus	Purpose
1	Ellagic Acid	~60-100mg/dose	Oral & Nasal	Anti corona; 12hrs blood life. *Ultra high repeat loading doses causes centro lobular necrosis* (liver)
2	Punicalin-s full group (Hydroxylic/carboxylic)	~do	Oral & Nasal	Anti virus broad spectrum; 48hrs blood life. Very safe.
	Adjuvant members			
3	4-Acetamedophenol	250mg	Nasal & IV	Anti-inflammatory & transcription rate reduction
4a	Ampicillin	<300mg/dose	Nasal	Target carrier & OM permeabiliser
4b	Ciprofloxacin	Do. 4a is better. For it is more toxic to capcids.		
5	Penicillin	<250mg/dose	Nasal	Do; Vital organ protection from response pathology ketons
	While ampicillin at sub-clinical potency is Virion specific. Cipro is better for the lung field members.			

6	Ethanol pure/desiccated	3-5ml/hourly	Foss; Nasal; Res Tract; bronchus; etc.	Capillary, Airways dilator, expectorant; OM permeabiliser-efficient uptake; Virion debacaliser; vehicle; exudates lysis.
7	Prochlorperazine maleate	2mg every 3rd hr.	sublingual	Thwart RV acute faltering & subsequent induced and pseudo issues.
8a	Allyl Benzens/Eguenol, Loaded in Clove	In mcg	Sublingual. As in 6	Potent Viricide. Prophylactic & Therapeutic.
8b	Nicotine (all processed & semi processed viz. Zardaa; Zafraanee; etc.)	1-2mg	Atomised inhalation with Ethanol & gurgling with 2H2O. 5ml + 5ml	Prophylactic & Therapeutic. Full pulmonary parynchema access & efficacy. Potent Viricide.
8c	1 Bidee / cigarette per day shall be prophylactic. 1-2mg Nicotine inhalation shall be prophylactic			Theory
Alert	**1 -** Large vol parentrals will heighten pleurisies and hematoma at infusion point = endless complications & intractable. **2 -** Iodine & iodised moieties will strongly upregulate primary & secondary pathologies. Corona virus is I dependant. Therefore Bromine derivatives as orals & fluoride derivatives as inhalation or sub-lingual (iodine hyper synthesis containment).			
Room pressure 60-100 hPA [8,9] less than that of the outer natural + Forced inhalation of atomised moieties during the initial 6 critical hrs. Prefer dry O_2 for induced breathing (in place of hydrated O_2). Hydroxylic members auto-interact with starch - degenerate. Hence weak acidic solutions be preferred as the holding medium. Constituent 1 & 2 are a excellent couple in caption domain. While 1-6 form a squad = MDT *multi drug therapy*. Ethanol inflicts acute insult to the virions.				

Table - 2

Sl. No	Inventor's cue – inspiration for items as in Table-1
1	**Ellagic Acid :** Author-2 was using the natural best to combat drug resistant malaria (pf & pv) 1997, whence he chanced upon the common relief accruing to clinically distinctive cases of viremia. Gallagic group; etc.
2	**Ellagitanins :** -do- anthocynins; polyphenols; etc. Role of Para magnetism Ref- 15
3	**4 – Acetamedophenol :** During the same period it was noted the paracetamol up-regulated relief. Later established that paracetamol sub-clinical assists drug positioning (vectoring & delivery), etc. Affinity for all other types of anti-inflammatories; Versatile.
4	**ANTIBIOTICS : Ampicilin :** contains Sulphur which is well indicated in pulmonary fibrosis. Has a adamantin type terminal (shield) makes it stable & useful & also ligand purpose with other moieties in sub clinical. **Penicillin :** -Do. Whole body equal efficacy, including synovial fluids and cartilage. Ampicilin has more affinity for the pulmonary parynchema. **Ciprofloxacin** : contains Flurine azide which is (very) potent viricide and is also well indicated in pulmonary fibrosis. **NOTE** : Some anti-malarials have engineered adamantin rings; bolus-SOS application cases often come back with arthritis of the small joints (poly arthritis); wt loss. Is anti-fibber. Hence sub-clinical MDT; longer therapy period.
5	**Ethanol :** Has extreme OM penetration ability. Discharge character. Necrosis potential. Ionic & thermal shock. Disrupter of bio-chemic signalling (para magnetism specially).

6	**Prochlorperazine maleate :** Clinical wisdom. Observation. Intervention practices based.
7	**Nicotine :** Author -2 toured country side numerously & variedly on foot and in public transport, stay put with rural-remote – disconnected people. Observed that bidee (Indian native cigarettes) makers roll the bidee in hand (central Odisa). Zafranee patti. Non seem to suffer from upper respiratory tract infections and or chance/opportunistic viruses including CMVs and or seasonal. Rampant in neighbouring villages and or in residences a bit afar. Anti-viral therapy + bide and or pikaa (N Andhra) smoker relieves viral symptoms precipitously; also larynx toner. Anti-cholonergic; anti-steroid (seems to wash down effect on steroid dependence). Nicotine & or anthocynin gurgling shall also clear the fossa of the settled virions.
8	**Iodine :** Clinical wisdom. Observation. Intervention practices based. Cholinerigc. Up-regulates malignancy; bacterimia; malariasis; etc; pro fibrosis. Ref. 16 *Omaria Story*
9	**Room pressure 60-100 Hpa less :** Mid night fatalities post cyclone pass and discomfiture during atmospheric high pressure (gravity waves); Medical Meteorology, See Ref.. 13 & 14.
By disrupting paramagnetism pathways selectively viremia can be arrested and or swept aside precipitously.	
>2 decades of attentive observation. Clinical; field; cultural; societal; heritage...etc. Nothing overnight.	
Note : {1} ation wide burning of oil lamps/hydro carbons shall repel the moisture and because clear sky AND WHEREAS sub-continent wide bristi yoga (fire ritual~rain drill) shall because copious rain (see ref 13). {2} Ellagic acid & Ellagitanins are solely loaded in the rind of the ayurvedic Punica. Commencing with ***Charak Samhita*** (Albino Formulary Compendium) Ay texts mentions indo ayurvedic punica variedly.	

Table 3. Indicative Histeriography of Punica and or its Moieties Research for Drug

Sl No	Year	Conveniently extrited details. Select.	Remarks
1	1996	Pomegranates could help in battle against AIDS. Reuters News Media, Inc. March 10 1996, http://www.aegis.com/news/re/1996/RE960310.html	Inaccessible. Not available.
2	1998	Jassim SAA, Denyer SP, Stewart GSAB: Antiviral or antifungal composition comprising an extract of pomegranate rind or other plants and method of use. US Patent . 5,840,308	November, 1998.
3	2000	Why a pomegranate? *British Medical Journal* 2000; 321 doi: https://doi.org/10.1136/bmj.321.7269.1153	04-11-2000
4	2000	*news.bbc.co.uk/2/hi/south_asia/988316.stm* Tuesday, 24 October, 2000, 12:16 GMT 13:16 UK India claims herbal malaria cure	BBC News about Author's work in the remote of rural india – using indo Ayurved Punica.
5	2004	Bhattacharya D, Punica Granatum's Dermis indicates Prophylaxis against Malaria & wide spectrum anti-viral property in Human use. American Journal of Tropical Medicine & Hygiene , Oct. 2004, Abstract No.968, 171(4), 288.*m* http://www.ajtmh.org/content/71/4_suppl/225.full.pdf+html	Submitted 30-6-2004. Dermis use. Pan Global – 1ST
6	2004	Neurath, A.R., Strick, N., Li, Y. & Asim K Debnath, *Punica granatum* (Pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide. *BMC Infect Dis,* 4, 41. https://doi.org/10.1186/1471-2334-4-41	Submitted 8-7-2. Fruit juice use.

7 shaded boxes – Author involved.	2010	Dell'Agli et al. Elagitannins of the fruit rind of pomegranate *(Punica granatum)* antagonize *in vitro* the host inflammatory response mechanisms involved in the onset of malaria, *Malaria Journal*, 9:208	This mss. cited Sl No 5. Thereafter a surge in interest for Punica is noted world-wide, mostly members - Chinese Academy of Sciences. india - Nil (look downs & feigns).
8	2012 & 2017	Punica has also been evaluated as having efficacy for human load bearing joints and for prostate cancer; among others. Versatile	Ref No. 17 & 18 (also see ref 22-28).
www : Swift & topically search indicates an interesting histology about the scholarly efforts using the Punica fruit & its dermis only. There are not many. Numerous studies commences post Sl. No.7.			
CONCLUSION : Indian ayurvedic Punica is quite different on pan global basis. It is entirely medicinal.			

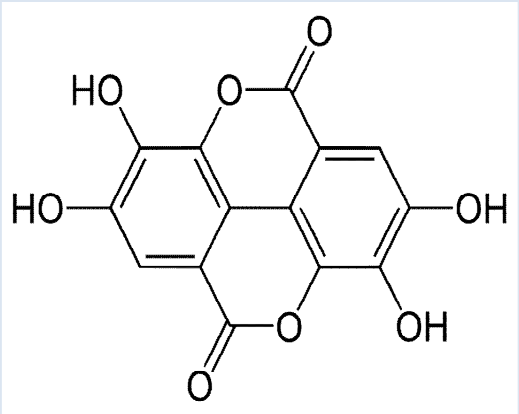

Fig.1. Ellagic acid. Small Dalton.

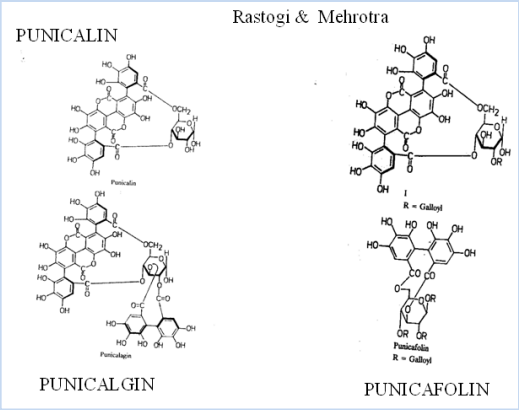

Fig.2. The punicafolins. Large moieties-1000-1100 Daltons.

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RESEARCH ARTICLE

Trustworthiness of Vulnerable VMs with Increasing Number of Attacks in Cloud Environment

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ABSTRACT

This paper outlines the modeling dynamics of single population i.e. Exposed Virtual Machines (EVM) and dynamics of both populations i.e. Exposed Virtual Machine (EVM) and Infectious Virtual Machine (IVM) in the cloud environment. The proposed work will also explain the utility of cloud Virtual Machines (VM) and the impact of Anti Malicious Software (AMS) with its efficiency in the cloud environment so as to increase the trustworthiness.

Keywords: Cloud computing; Cloud Virtualization Security; Predator-Prey model; Exposed Virtual machine; Infectious Virtual machine.

INTRODUCTION

A node in the cloud environment stores data and information and gives the user a platform to use the application in the form of services. This creates a condition for intrusions or attacks in the cloud based applications. An attack is defined as an external force by which the nodes existing in one state get transferred into other.[4] Based on the attacks in the cloud environment, the nodes are classified into different types.Vulnerable/exposed nodes are the nodes those can be exploited by the malicious attacks. Some vulnerable nodes on which attacks are carried out but still they cannot help in propagation of infection are called attacked nodes. When some nodes are infected and help in propagation of infection, they are called infectious nodes. These nodes are of the most hazardous category. The recovered nodes from the infectious category and having no infections are called as non-infectious nodes. Therefore, it is better to predict infectious nodes in the network. It can be predicted from the attack history or from the vulnerability analysis of the network which requires a considerable efforts and use of resources.[5] [6]

The trustworthiness in a cloud based network is a concept that encompasses not only security but also safety, survivability of its nodes and other properties that guarantee that a network will behave as expected [5][6]. A network is worthy and trusted when number of infectious nodes are minimized. Therefore, a prediction method can help to fix the suspected domain to check the infectious nodes. It can be achieved by the help of Predator-Prey Model. Predator-Prey model [6] is established for computing the the Exposed Virtual machine (EVM) and Infectious Virtual machine (IVM) to check the trustworthiness in the cloud based network. Our proposed model is to make the cloud computing architecture perfect and to build a more comprehensive network. Two of the possible cases for the established model for modelling of dynamics of single population i.e. Exposed Virtual machine (EVM) and modelling of dynamics of both populations i.e. Exposed Virtual machine (EVM) and Infectious Virtual machine (IVM) in the cloud are discussed.

The Predator-Prey Model can be understood by considering the growth of rabbits in the presence of foxes in the jungle. If there is an infinite food supply, rabbits would live happily and experience exponential growth.[4] On the other hand, if foxes are left with no prey to eat, they would die faster than they can produce, and would experience exponential population decline. A similar analogy to the Predator-Prey Model can be used to predict the growth of malicious attacks in the cloud based Networks. It will basically ensure the degree of the security of virtual machines in a cloud environment which helps the cloud service providers to take quick decisions about up gradation of the counter attack measurements. In order to simulate these dynamics, mathematical models need to be developed [5][6]. The population dynamics of predator-prey interactions can be modeled using the Lotka–Volterra equations, which is based on differential equations. These provide a mathematical model for the cycling of predator and prey populations.

NOMENCLATURES

Parameters and Explanations

N =The maximum number of Virtual machines (VM) present in the cloud environment.
N_S = The total number of non-vulnerable VM (un-exposed VM)
N_0= The initial no. of VM that vulnerable to attack i.e. the prey
N_A= The number of attacked VM in the cloud
P = The number of infectious VM actually searching i.e. the predator
A = The coefficient of attack i.e. N_A per *P*
K = The maximum number of attacks that can be made per *P* during the period N_0 are vulnerable
α =Coefficient of intraspecific competition
β =Per-capita rate of predation of the predator.
γ =Death rate of predator.
Δ =The product of the per-capita rate of predation and the rate of conversing vulnerable VM into infectious VM
μ_1 $=\gamma/\delta$
μ_2 $= \alpha/\beta$
dP/dt=Growth rate of infectious VM in cloud i.e. Predator
dN_0/dt =Growth rate of vulnerable VM in cloud i.e. Prey

MODELLING OF DYNAMICS OF SINGLE POPULATION (EVM)

Mathematical models and logic suggest that a coupled system of predator and prey should cycle: predators increase when prey are abundant, prey are driven to low numbers by predation, the predators decline, and the prey recover, ad infinitum. Lotka-Volterra Model is a model that simulates predator-prey interactions. Predator-Prey Model is one of the interesting dynamics in the biological world which describes the interactions between species [1-3]. In the ecological environment, predation explains a biological interaction where a predator forages on its prey. The

predator-prey relationship is substantial in maintaining the equilibrium between various animal species. With these concepts we have used a similar analogy to use the Predator-Prey Model in cloud environment, which can predict the growth of a malicious attack. It is basically used to understand the basic concept of Prey-Predator dynamics using the established Mathematical model of Lotka-Volterra Equations, [22-25] i.e., how predators affect prey populations, and vice-versa. Also it will analyze the population pattern variation of EVM and IVM i.e. predator and prey, by changing critical parameters like initial population of either Prey and/or Predator in a cloud based environments [5][6]. So the steps for the Predator-Prey model are

i. EVM rises to a constant number of amounts per unit of time as new nodes are added to the network. In other words, there are no other factors limiting EVM population growth apart from predation.[15]
ii. Each IVM infects a constant proportion of the EVM population per unit of time; In other words, doubling the EVM population will double the number infected per IVM, regardless of how big the EVM population is [16].
iii. IVM reproduction is directly proportional to EVM consumed; another way of expressing this is that a certain number of EVM consumed results in new IVM s.
iv. A constant proportion of the IVM population dies per unit of time. In other words, the IVM death rate (approaching to non-recoverable state) is independent of the recoverable process as there are other means like hardware failure or power failure.
v. N_0 is the number of Prey i.e. exposed VM in cloud to the malicious attacks.
vi. *P* is the number of some Predator i.e. infected VM in cloud which is ready to spread the infection to other healthy VM.
vii. dp/dt and dN_0/dt represents the growth rate of two populations i.e. infected VM and exposed VM respectively.

In a cloud based network EVM can be reproduced by adding new nodes and the IVM can be recovered by the help of Anti Malicious Software (AMS). Another aspect is here the predation which affects the EVM populations based upon the attacks as shown in figure [5]. In this situation the exposed virtual machines grow exponentially as shown in Figure .2.

MODELING OF DYNAMICS OF BOTH POPULATIONS (EVM AND IVM)

In a cloud based network EVM can be reproduced by adding new nodes and the IVM can be recovered by the help of Anti Malicious Software (AMS). Another aspect is here the predation which affects the EVM populations as shown in figure.3. This reflects the assumption that the IVM reproduction is proportional to rate of predation on the EVM. In this case the EVM and the IVM compartments cycle, with the EVM population crashing as the IVM population increasing.[6] To understand the basic concept of Prey-Predator dynamics using the established Mathematical model of Lakota-volterra Equations, i.e. how predators affect prey populations, and vice-versa and to analyze the population pattern variation, by changing Critical parameters like initial population of either Prey and/or Predator(EVM and/or IVM)[15-16]. The results of one of the many permutations of the experiments are graphed below based on the parameters of predator prey model.

EVM rises to a constant number of amounts per unit of time as new nodes are added to the network. In other words, there are no other factors limiting EVM population growth apart from predation with prey population as shown by figure 4.Each IVM infects a constant proportion of the EVM population per unit of time; In other words, doubling the EVM population will double the number infected per IVM, regardless of how big the EVM population is, as shown by figure .5. IVM reproduction is directly proportional to EVM consumed; another way of expressing this is that a certain number of EVM consumed results in new IVM. A constant proportion of the IVM population dies per unit of time. In other words, the IVM death rate (approaching to non-recoverable state) is independent of the recoverable process as there are other means like hardware failure or power failure, as shown by figure 6.
This reflects the assumption that the IVM reproduction is proportional to rate of predation on the EVM as depicted by figure 7. In this case the EVM and the IVM completes the cycle, with the EVM population crashing as the IVM

population increasing, followed by a crash in the IVM population. We have picked up some random results from the table and have plotted in graphs. It is clear from the graph that both populations (Predator and Prey) show cyclical behavior, and that the predator population generally tracked the peaks in the prey population. However, before concluding that the experimental results truly support the predictions made by the Lakota-volterra model, there are some information about this experiment that need to be considered. Analyzing in details with the parameters Initial Predator Population, Initial Prey Population, Predator Death Rate, Prey Birth Rate from the tables and graphs following conclusions have been made. If the Initial Predator Population changes, remaining Initial Prey Population, Predator Death Rate, Prey Birth Rate as constant in three cases we get IVM reproduction is proportional to rate of predation on the EVM as shown in Figures 8 to 10.

If the Initial Predator Population changes, remaining Initial Prey Population to a high value of 1080 and Predator Death Rate, Prey Birth Rate as constant in three cases we get IVM reproduction is initially more and gradually with the use of AMS it is proportional to rate of predation on the EVM as shown in Figures 11 to 13. If the Initial Predator Population changes, remaining Initial Prey Population to even more high value i.e. 2000 and considering Predator Death Rate, Prey Birth Rate as constant in three cases we get IVM reproduction is initially more as no. of EVM increased and gradually with the use of AMS, it is proportional to the rate of predation on the EVM. As shown in figures from14 to 16. If the Initial Predator Population, Predator Death Rate, Prey Birth Rate are changing and initial Prey Population remains constant in three cases, then we get IVM reproduction based upon the values of either Predator Death Rate or Prey Birth Rate. If the values are reduced by half, then initially predator populations are more and gradually with the use of AMS it will be under control as shown in Figures 17 to 19.

If the Initial Predator Population, Predator Death Rate, Prey Birth Rate are changing and Initial Prey Population remain constant in three cases we get IVM reproduction based upon the values of either Predator Death Rate or Prey Birth Rate.[6] If the values of predator death rate will increase and the birth rate of prey is reduced by half then predator populations is dominant over the prey populations and if the values of predator death rate will increase with the birth rate of prey in same proportions then IVM reproduction is proportional to rate of predation on the EVM as shown in Figures 20 to 22.

CONCLUSION

This paper describes the simulation and prediction of EVM and IVM using a new model based on the Lakota-volterra equation known as Predator-Prey Model considering the Initial Predator Population, Predator Death Rate and Prey Birth Rate. This analysis also explains the dynamics of single population i.e. Exposed Virtual Machines (EVM) and dynamics of both populations i.e. Exposed Virtual machine (EVM) and Infectious Virtual machine (IVM) in the cloud environment which will basically be used to check the trustworthiness in the cloud virtualization environment

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Table 1. EVM & IVM Parameters

Sl.no	Initial Predator Population	Initial Prey Population	Predator Death Rate	Prey Birth Rate	Predator Effectiveness In %
1	5	200	100	100	50
2	8	200	100	100	50
3	10	200	100	100	50

Table 2 EVM & IVM Parameters

Sl.no	Initial Predator Population	Initial Prey Population	Predator Death Rate	Prey Birth Rate	Predator Effectiveness In %
1	5	1080	100	100	50
2	8	1080	100	100	50
3	10	1080	100	100	50

Table 3 EVM & IVM Parameters

Sl. No.	Initial Predator Population	Initial Prey Population	Predator Death Rate	Prey Birth Rate	Predator Effectiveness In %
1	5	2000	100	100	50
2	8	2000	100	100	50
3	10	2000	100	100	50

Table 4 EVM& IVM Parameters

Sl. No.	Initial Predator Population	Initial Prey Population	Predator Death Rate	Prey Birth Rate	Predator Effectiveness In %
1	5	200	150	100	50
2	5	200	100	150	50
3	5	200	50	50	50

Table 5 EVM & IVM Parameters

Sl.no	Initial Predator Population	Initial Prey Population	Predator Death Rate	Prey Birth Rate	Predator Effectiveness In %
1	5	200	150	50	50
2	5	200	150	150	50
3	10	200	50	50	50

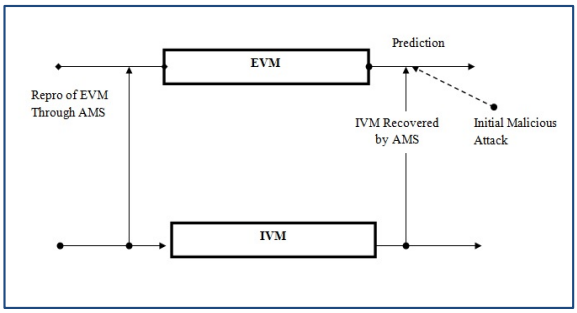

Figure .1 Model Diagram of Dynamics of Single Population i.e. EVM

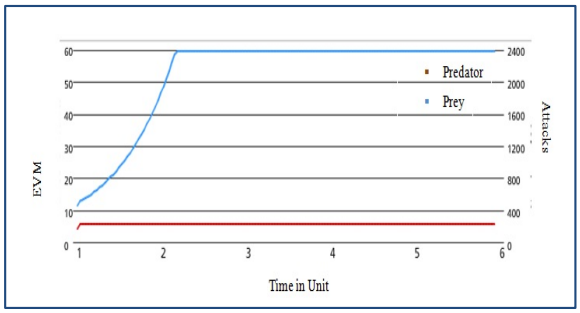

Figure .2 EVM Growth with Time and Attacks

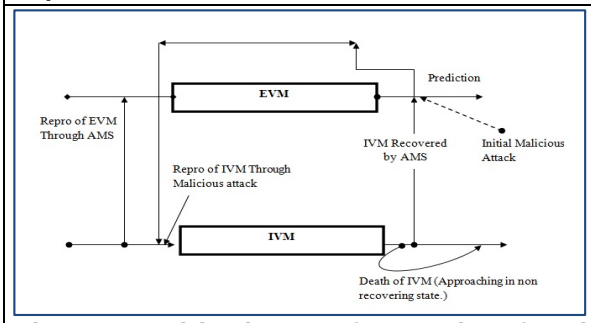

Figure 3 Model Diagram of Dynamics of Both Populations i.e. EVM and IVM

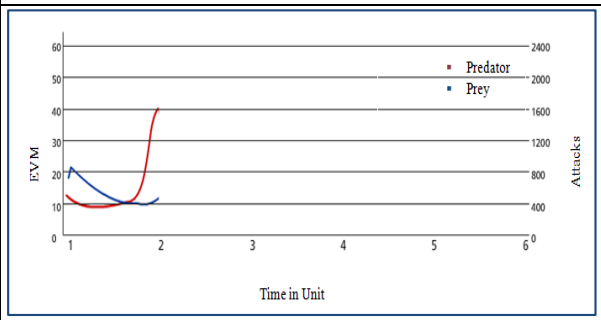

Figure .4 EVM Population Growths

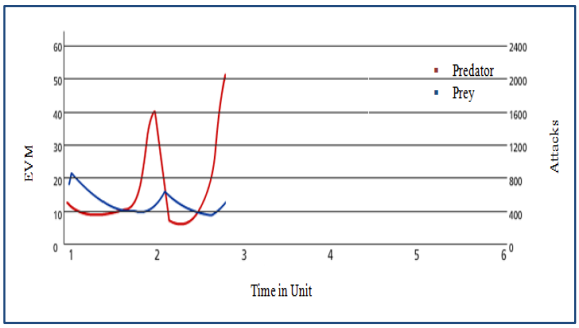

Figure 5 EVM & IVM Population Growth

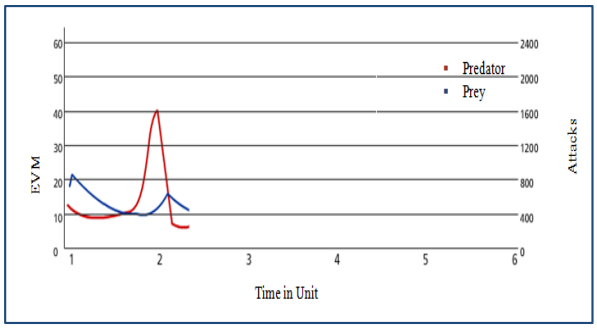

Figure .6 EVM& IVM Population Growth

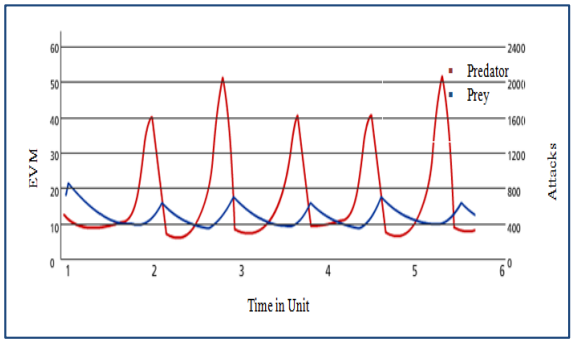

Figure: .7 EVM& IVM Population Growth

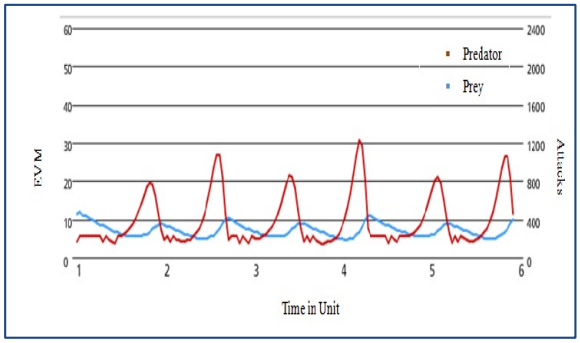

Figure 8 EVM& IVM Population Growth

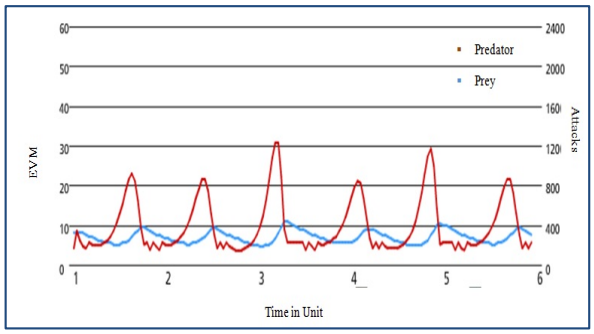

Figure 9 EVM& IVM Population Growth

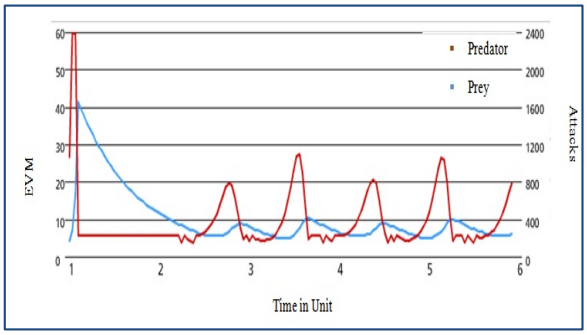

Figure 10 EVM& IVM Population Growth

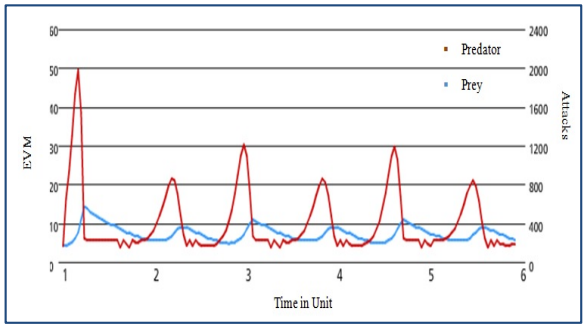

Figure 11 EVM& IVM Population Growth

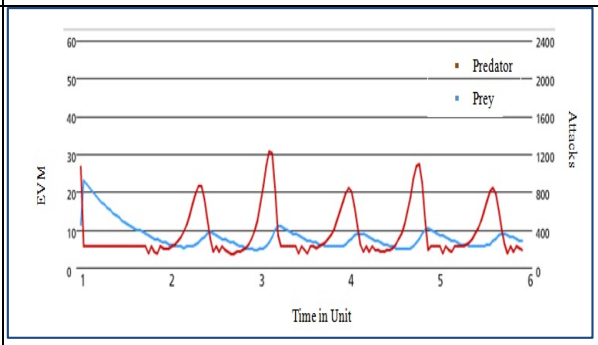

Figure 12 EVM& IVM Population Growth

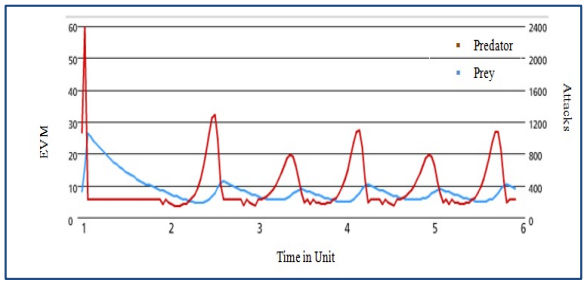

Figure 13 EVM& IVM Population Growth

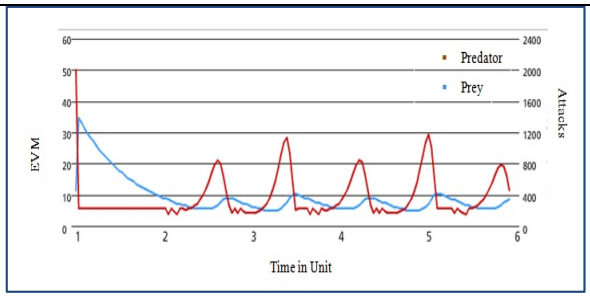

Figure 14 EVM& IVM Population Growth

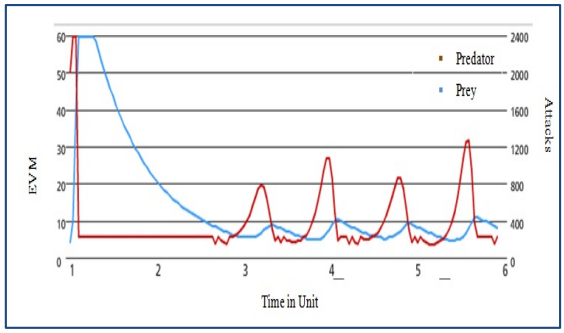

Figure 15 EVM& IVM Population Growth

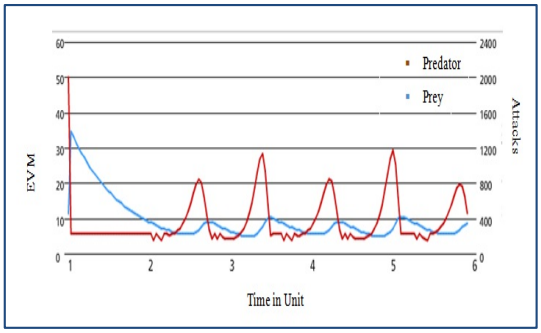

Figure 16 EVM& IVM Population Growth

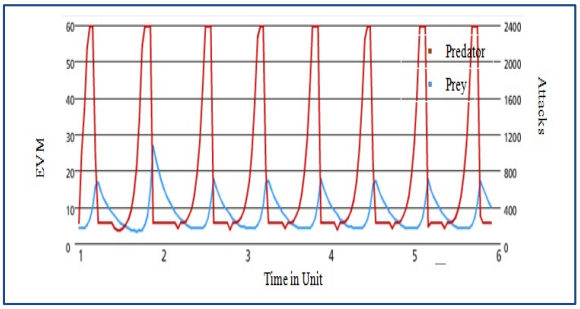

Figure 17 EVM & IVM Population Growth

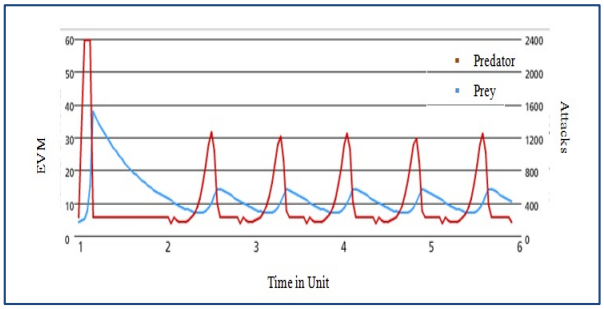

Figure 18 EVM & IVM Population Growth

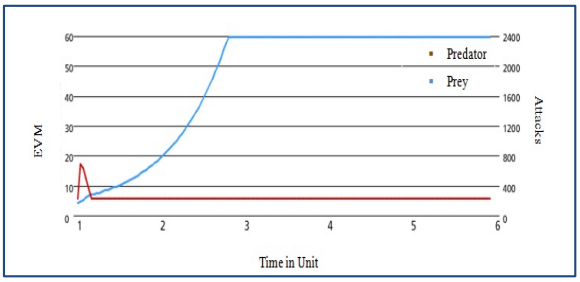

Figure 19 EVM & IVM Population Growth

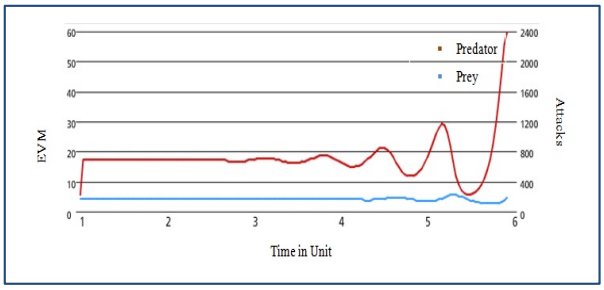

Figure 20 EVM & IVM Population Growth

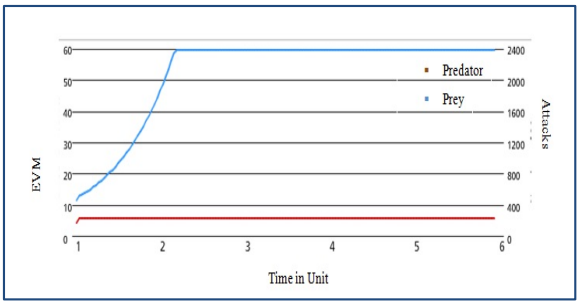

Figure 21 EVM & IVM Population Growth

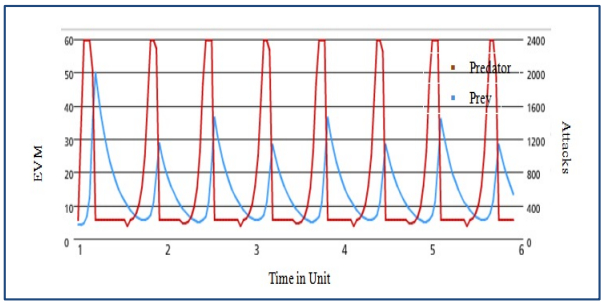

Figure 22 EVM & IVM Population Growth