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RESEARCH ARTICLE

Biocontrol Potential of Selected Actinomycete and its Metabolites against *Rhizoctonia solani.*

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ABSTRACT

The present investigation was carried out with the aim of identifying a potential actinomycete for the control of *Rhizoctonia solani* .The selected actinomycete was found to cause hyphal lysis in *Rhizoctonia solani* and inhibition of growth of mycelium by the production of toxic volatile and non-volatile metabolites and hydrolytic enzymes such as chitinase and β-1,4- endoglucanase.Solvent fractioning of exocellular metabolites present in the culture filtrate of selected actinomycete(I_9) was found to contain elements such as nitrogen(0.524%), carbon(35.87%), sulfur(0.228%) and hydrogen (7.068%). The FT-IR spectrum indicated the presence of –OH group and the GC-MS study revealed the presence of components such as hexadecanoic acid, oleic acid, phenol, benzene dicarboxylic acid, heptasiloxane, hexadecamethyl, octasiloxane and hexadecamethyl. *In vivo* study on the biocontrol potential of I_9 against *R.solani* was done using *Raphanus sativus* and showed improved growth and biochemical characteristics in *R. sativus* infected by *Rhizoctonia solani* and it could be recommented for soil treatment to manage *Rhizoctonia* root rots in the *R.. sativus.*

Key words: Actinomycetes, metabolites, Biocontrol, Chitinase, β-1,4- endo glucanase, *Rhizoctonia solani, Raphanus sativus.*

INTRODUCTION

Biological control has been widely studied as an alternative method of controlling plant diseases, since the increasing use of fungicides has caused development of pathogen resistance, problems with environmental pollution and human and animal health risks [6].They also have an important role in the degradation of polymers such as lignin, chitin, cellulose and starch [7,28] which improve the biocontrol potential against plant pathogens [14,27,35]Growth of actinomycetes and production of secondary metabolites varied with the isolate,growth condition such as carbon and nitrogen sources as well as pH, oxygen level and temperature condition [12]. *Rhizoctonia solani* is very important soil borne pathogen.

superficial, irregular, scab like black sclerotia on the infected tissue. Common symptom produced by *Rhizoctonia solani* is damping off and root rot. Actinomycetes with chitinolytic activity used to control phytopathogenic fungi [17]such as *Pythium ultimum, Rhizoctonia solani, Phytophthora capsici, Botrytis cinerea* and *Fusarium oxysporum*.Actinomycete species which was found to produce antifungal substances with high inhibitory effect against *R.solani* due to their extracellular metabolites which acted on fungal cell membrane altering its permeability [22].These effects were mainly spore germination inhibition,bursting of spores and hyphal tips and germ tube elongation[25,9].The mixture of actinomycetes and bacterial species to inhibit the pathogen and increased the induction of measurement of cotton plant[31].*Raphanus sativus*(L.) is a very popular root crop throughout India.It is grown for its fleshly edible roots which are eaten raw as salad (or) cooked. They have pleasant 'hot'taste which is due to mustard oil, produced by the breakdown of a glucoside. It is grown on all types of soils. There is every possibility for the attack of its roots by the soil-borne pathogenic fungi which may interfere with the development of the root tubers. Biocontrol of both *Rhizoctonia Solani* and *Phythium Sp* by coating radish and Pea seeds with *Trichoderma hammatum* [42].So the present investigation used to study the mechanism involved in the biocontrolof *Rhizoctonia solani*, characterize the antifungal metabolites produced by the selected actinomycete by *in-vitro* studies and to evaluate the biocontrol potential of the selected isolate against *R. solani* by *in-vivo* studies using *R. sativus.*

MATERIALS AND METHODS

Isolation and screening of actinomycetes with antagonistic acitivity against selected phytopathogens:

Actinomycetes with antagonistic activity were isolated from garden (loamy) soil using czepeckdox agar by crowded plate technique. The soil samples were serially diluted and plated. The plates were incubated at 37^0C for 5 days. The active colonies showing inhibition of nearby colonies were isolated and subcultured and maintained in Czepeckdox agar slants. The antagonistic activity of actinomycete isolates against phyto pathogens (*Alternaria, R.solani and Fusarium* sp) was tested by dual culture method using potato dextrose agar (PDA) medium.

Biochemical characterization of the selected isolate of actinomycete (I_9)

The biochemical characteristics of the isolate I_9 was studied in terms of starch hydrolysis, catalase activity, H_2S production, proteolysis activity, gelatin liquefaction, cellulolytic activity and growth on milk [32].

Mechanism of action of the isolate I_9 on *R. solani*

Antibiosis: The hyphae were aseptically removed from the fungal colony facing the antagonist (I_9) and mounted on microscopic slides using lactophenol cottonblue stain.Microscopic observation were made and photographed.

Antagonistic activity of volatile and non-volatile metabolites of isolates I_9

To find out the effect of volatile metabolites of I_9 on *R.solani*, the PDA plates were inoculated centrally with five mm culture discs of isolate I_9 and *R.solani and* the plates were kept together with adhesive tape after removing the lids, the plate containing I_9 was kept below and incubate the plate at 35 ± 2^oc [8].

The antagonistic effect of non-volatile metabolites were analysed by the supernatant obtained from the PD broth containing I_9 was added to PDA medium at concentration of 25% ,50%, 75%, 100% by adding 2% agar to the culture filtrate (pH-5) and plated. Three days old active discs (5mm dia) of *R.solani* were removed and placed at the center of the prepared PDA plates and incubated at $37^o \pm 2^o$c. After five days of incubation the percentage of inhibition was calculated [20].

Production of Hydrolytic enzymes by I_9

I) Chitinase:

Enzymatic hydrolysis of colloidal chitin was assayed following the release of N-Acetylglucosamine from colloidal chitin, chitin agar containing 0.15% chitin, 1.5% agar and 0.02% Sodium azide (NaN_3) in 50mM potassium phosphate buffer (pH-6.1). 0.25ml of crude enzyme samples were added to wells in agar medium and incubated the plates at 30± 2°c for 24hrs by clearing zone assay method [13]. Five grams of chitin powder was homogenized in 100 ml of 12m Hcl and left at 20°c for 10mins. The suspension was poured into cold water under agitation and left to settle. Precipitate was containing 1ml of 0.5% colloidal chitin, 2ml of Mcllvaine's buffer and 1ml of culture filtrate was incubated for 20 minutes at 37°c in a shaker (120 rpm) and reaction was stopped by boiling for 3 minutes. After centrifugation (2000rpm for 30minutes), 1.5ml of supernatant mixed with2ml of potassium ferricyanide reagent and heated in boiling water bath for 15minutes and the amount of N-acetylglucosamine released was estimated by absorbance of reaction mixture at 420nm by spectrometric method.

II) β –1, 4 endoglucanase

Enzyme hydrolysis of carboxy methylcellulose (CMC) was assayed by adding 1ml of sodium acetate actic acid buffer and 2ml of centrifuged culture filtrate of isolate I_9 to 4 ml of CMC and incubated in water bath for 30 minutes and 3ml of DNS reagent was added and heated for 5 minutes in boiling water bath, after colour had developed 1 ml of 40% sodium potassium tartrate was added and the tubes cooled under running tap water and absorbance was measured at 575nm in a spectrometer by dinitrosalicylic acid (DNS) method.

Solvent fractioning of exocellular metaboites present in the culture filtrate of I_9

Five days old broth culture of isolate I_9 was filtered and 100 ml filtrate was treated with organic solvents in the order of Hexane– chloroform – Diethylacetate – Ethylacetate- Butanol and the remaining aqueous layer was kept for evaporation and dired and residue further treated with Acetone -Ethanol – Methanol and the proportion of the fractions was determined and tested for antifungal activity against *R.solani* by inhibition assay method.

Frourier Transform – Infra Red analysis

The FT – IR analysis (KBr Pellet) was carried out for butanolic fraction (BM) using SHIMADZU, FT –IR 8400S.

Gas Chromatography – Mass Spectroscopic analysis

GC – MS was done for active fraction of butanolic extract residue using GC clarus 500 perkin Elmer at an injector temperature of 250°C, oven program conducted at 110°c with initial hold 2minutes to 280°c at the rate of 5°c /minute. The similarity search was done by using the library: N13T Ver.2.1. Bunge *et al.,*(2009)[12] investigated Gas Chromatography (GC) and Mass Spectrometry (MS) illustrate the splendid capacity of bacteria to produce volatile compounds .

RESULTS AND DISCUSSION

Isolation, Screening and Biochemical characterization of antagonistic actino mycetes

Nine different actinomycete species were isolated from soil and screened for the antagonistic activity against selected phytopathogens.Among the isolates I_9 showed good antagonistic activity against selected phytopathogens. Biochemical characterization was done for the isolate I_9.Which was found to be positive for all the selected biochemical tests.

Mechanism of action of the isolate I_9 on *Rhizoctonia solani*
Interaction between isolate I_9 and *Rhizoctonia solani*

Microscopic observation clearly indicated that the hyphae of *Rhizoctonia solani* were severely damaged by the presence of the actinomycetes (Fig.1).The damaged hyphae might be due to lysis of the hyphal wall (Fig.2)by the action of antibiotics and other toxic volatile and non-volatile metabolites produced by actinomycetes [39].

Antifungal activity of volatile and Non-volatile metabolites of I_9 against *R. solani*

It was observed in terms of percentage of inhibition of its mycelia growth and the percentage of inhibition was found to be 39.74% over the control (Fig.3).Inhibition might be due to the volatile metabolites produced by the actinomycete. Production of volatile metabolites which includes ethanol and cyanides as biocontrol agent [15,11,23,40,30,].Velazquez - Becerra, Crisanta (2010)[38] identified organic compound dimethylhexa decylamine from *Arthrobacter agilis* modulating bacterial growth and *Medicago sativa* morphogenesis *in vitro.*Volatile emission and biological impact of volatile compounds from *Serratia odorifera* on *Arabidopsis thaliana* was analysed by Kai, Marco(2010)[21].

The effect of concentration of culture filtrate of (I_9) on the rate of inhibition of mycelial growth of *Rhizoctonia solani* was investigated. The concentration of cultue filtrate showed preformed effects of the rate of inhibition of the growth on the concerntration of culture filtrates was found to be directly proportional. *Rhizoctonia solani* recorded 75% of reduction in growth when grown at a greater concentration (100%) of the culture filtrate significant reduction in growth might be due to the presence of more amount of inhibitory compounds in the culture filtrate (Fig-4).antifungal activity of Non- volatile metabolites such as alkyl pylons, isonitriles, polykedes, peptailbols, diketopipperazines, sesquiterpenes and steroids have been associated with biocontrol activity [18,34].

Production of hydrolytic enzymes by the isolate I_9

Hydrolytic enzymes such as chitinase and β-1,4 endoglucanase which will help in attacking the target pathogen. Clear zone formation of I_9 was due to dehydration of chitin by chitinase. Spectroscopic analysis clearly indicated that organism secreted 1.93 mg of chitinase and 0.459mg of β-1,4 endoglucanase/ml of 5 days old culture filtrate. Chitinase could cause lysis of fungal cell wall [24]. Bio control agents are known to produce β-1,3endoglucanase,β-1,4 endoglucanase, chitinase, proteases which are involved in the antagonistic activity against phytopathogenic fungi [41,36,4,5,19,16,13,33,26,1,29,37].

FT – IR and GC– Mass spectroscopic analysis

Solvent fractioning of exocellular metabolites present in the culture filtrate of I_9 was done. Antimicrobial compound recovered from the culture filtrate of *Streptomycetes* by treating twice with one volume of ethyl acetate [3].The butanolic fraction showed the presence of toxic antifungal metabolites and it was characterized. The fraction was found to be neutral with the melting point of 185^{o}c was found to contain elements such as nitrogen (0.524%), carbon (35.87%), sulfur (0.228%) and hydrogen (7.068%). The FT-IR spectrum indicated the presence of –OH group and the GC-MS study revealed the presence of components such as hexadecanoic acid, oleicacid, phenol, benzenedicarboxylic acid, heptasiloxane, hexadecamethyl, octasiloxane and hexadecamethyl(Fig.6]. *In vivo* study on the biocontrol potential of I_9 against *R.solani* was done using *Raphanus sativus*.Soil inoculation of I_9 showed improved growth and biochemical characteristics in *Raphanus sativus* infected by *Rhizoctonia solani*. (Fig.5)The results of the present study showed that the actinomycetes isolate I_9 proved to be a potential biocontrol agent for *Rhizoctonia solani*. It could be recommented for soil treatment to manage *Rhizoctonia* root rots in the *Raphanus sativus.*

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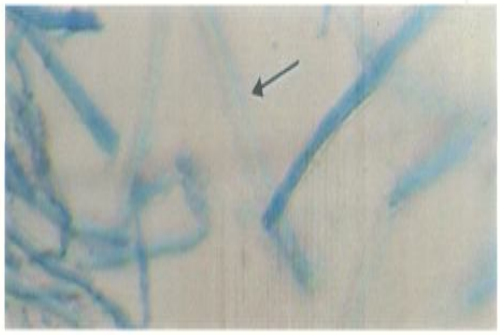

Fig.1.Damaged hyphae of *R.solani*

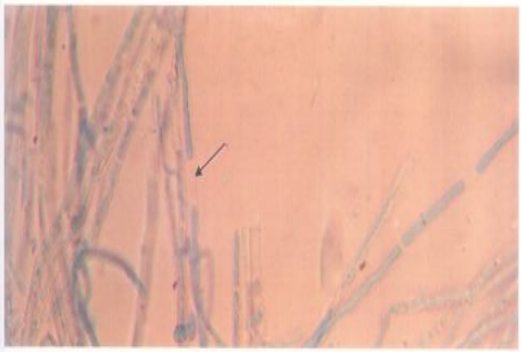

Fig.2. Hyphal lysis in *R. solani*

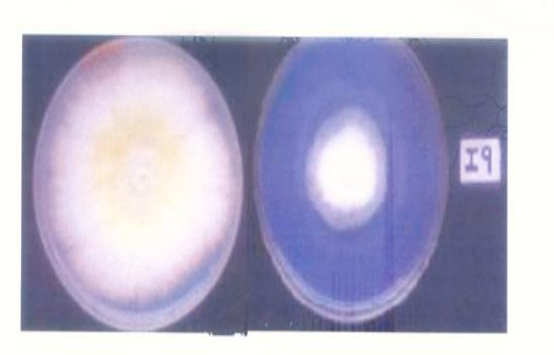

Fig.3. Antifungal activity of volatile metabolites of I_9 against *R. solani*

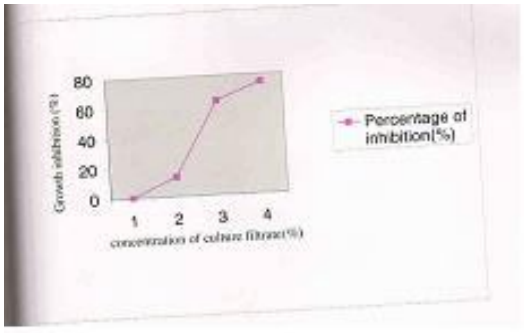

Fig .4. Effect of concentration of culture filtrate on the growth of *R.solani*

T_0-Control, T_1-Uninfected plants grown in soil infested with the selected actinomycetes (I_9).,T_2-Plants infected by *Rhizoctonia solani*, T_3, Infected plants grown in soil infested with the selected actinomycetes(I_9)

Fig.5.Effect of soil inoculation of actinomycete (I_9) on uninfected and infected plant of *R.sativus by Rhizoctonia solani*

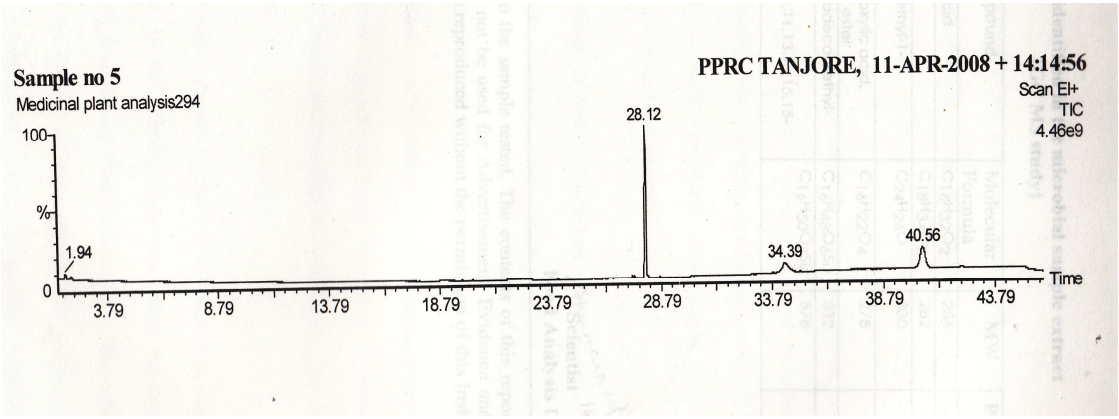

Fig.6.GC-MS spectrum indicated the presence of components.

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RESEARCH ARTICLE

Air Pollution and its Effect on Biota - A Case Study of Shivamogga City, Karnataka, India

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ABSTRACT

Air pollution is the human introduction into the atmosphere of chemicals, particulate matter or biological materials that cause harm or discomfort to humans or other living organisms or damage to the environment.The unplanned growth of cities in India has led to the problems of increasing slums, vehicular traffic and air pollution. Automobile exhaust, which also consists of all major air pollutants, is a significant source of air pollution in the urban context. The study reveals that in all the five sampling sites the concentration of SO_2 (9.15 to 16.42 μg/m^3) and NOx (18.6 to 49.0 μg/m^3) is well within the CPCB limits whereas Suspended Particulate Matter concentration, SPM (516.2 to 1005.μg/m^3) crossed the threshold limit prescribed by CPCB. The air pollution tolerance index (APTI) of 14 commonly growing plant species has been also been evaluated .High values of APTI were recorded in *Azadirachta indica*, (37.74) and *Mangifera indica*, (28.4) whereas *Tamarindus indica* (9.12) and *Terminalia cattapa* (10.71) recorded very low values of APTI.

Key words: Urban air quality, diseases, biota, APTI and CPCB (Central Pollution Control Board).

INTRODUCTION

Air pollution caused by automobiles has been described as the "disease of wealth". Sulphur dioxide, nitrogen dioxide and suspended particulate matter (SPM) are regarded as major air pollutants in India [1]. In the developing countries, air quality crisis in cities is attributed to vehicular emission which contributes to 40-80% of total air pollution [2]. Around the world, five major types of materials are released directly into the atmosphere in their unmodified forms and in sufficient quantities to pose a health risk. They are carbon monoxide, hydrocarbons,

particulates, sulfur dioxide and nitrogen compounds. This group of pollutants is known as primary air pollutants. These materials may interact with one another in the presence of an energy source to form new secondary air pollutants such as ozone and other very reactive materials. Secondary air pollutants also form reactions with natural chemicals in the atmosphere. Human health is very closely linked to environmental quality, as the etiology of most of the human diseases is being related to the status of the living environment of man. According to statistics, 25% of all preventable illnesses are caused by detrimental environmental factors. In developing countries, the air quality crisis in cities often attributes in large measures (40–80%) to vehicular emission. The improved performance of technology is presently insufficient to counteract the growth of vehicles [3]. Air Pollution Tolerance Index [APTI] suggested by [4] one of the widely used tools in India, to describe the tolerant species. Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases like carbon dioxide, oxygen and also provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level [5,6].

Study area

Shivamogga city (13°55′18″N, 75°34′12″E) is a heartland of Karnataka state, located on the banks of river Tunga. According to Shivamogga City Municipal Corporation, the city has a total area of about 50 km² (19.31 square miles) and has a population of about 4, 75,896 as per 2011 census. The climate of Shivamogga is tropical wet and dries (as per Koppen Climate Classification). This means that the winter and the early part of summer are typically dry periods. Majority of the rainfall occurs between June and early October. Shivamogga is a part of a region vernacularly known as Malnaad (land of hills) in Karnataka. Most of these hills are part of Western Ghats, a region famous for plentiful rainfall and lush greenery.The major highways NH-13, NH-206 and other State High ways pass within the city. The heavy traffic on these highways has also significantly contributed to air pollution in the city. The city has no effective means of mass transport system; therefore there is a tremendous increase in the number of two wheelers during the last five years. The problem has been compounded by the ongoing road widening work during the past one year which has led to severe health problems like asthma, hyperacidity and allergy.

MATERIALS AND METHODS

The air quality was monitored for Sulphur dioxide (SO_2), Oxides of nitrogen (NOx), and Suspended particulate matter (SPM).High volume air sampler (Envirotech APM-410-411) was used for sampling SO_2 and NOx which were absorbed in potassium tetrachloromercurate and sodium hydroxide and sodium arsinite solution respectively. Analysis of this solution was carried by West and Gaeke method and Griess –Saltzman method respectively. Suspended particulate matter was collected on pre weighed glass fiber filter (Whatman).Filter paper was again weighed and the difference in weight was used to calculate SPM in respective areas and expressed as mg/m3 of the air .The monitoring was done on 8 hourly basis for 24 hours. This research paper is the outcome of research work carried out between February 2009 to June 2009. Five different sampling sites were selected to represent different traffic intersections which include MRS, Gandhi Bazar, Aamir Ahamad Circle, Bus Stand and Mandli.Leaf samples were collected from 14 different plants commonly growing in traffic areas of Shivamogga city and the Air pollution tolerance index (APTI) was determined. The collected leaf samples were immediately brought to lab in a heatproof container. The leaf fresh weight was taken immediately upon getting to the laboratory and then samples were preserved in refrigerator for further analysis. The samples were estimated for Leaf-extract pH, relative water content, total chlorophyll and ascorbic acid following the standard methods of [7] and [8]. APTI was calculated using the formula

$$APTI = \frac{[A\ (T + P) + R]}{10}$$

Where,

A = ascorbic acid in mg/g

T = total chlorophyll in mg/g

P = pH of leaf sample

R = relative water content in mg/g.

Data related to adverse effects of air pollution on human health in five different locations of Shivamogga has been collected by the 'Questionnaire method' covering 125-130 individuals. The secondary data was also collected from various health centers, hospitals and clinics coming under the study area.

RESULTS AND DISCUSSION

The ambient air quality values with respect to three important parameters SPM (Suspended particulate matter), SO_2 (Sulphur dioxide), NOx (Oxides of nitrogen) are represented in the table 1. It is observed that at all five sampling sites the concentration of SPM varies from 516.2 to 1005.5 μg/m^3 which crossed the threshold limit prescribed by CPCB .The high concentration of SPM is mainly due to increase in the growing number of vehicles, small scale industries, demolition of buildings for widening the roads, construction of flyovers and diversions coupled with a burgeoning population. The findings are in line with [9, 10 and 11].The problem is further aggravated by the absence of proper dust control measures like grass, vegetation etc. SO_2 concentrations ranged from 9.15 to 16.42 μg/m^3 where as NO_x concentrations ranged from 18.6 to 49.0 μg/m^3. Both the concentration of SO_2 and NO_x are well within the standards of CPCB at all five sampling sites. The present study also revealed (Table-2) air pollution tolerance index (APTI) of 14 plant species. High values of APTI were recorded in *Azadirachta indica*, (37.74) and *Mangifera* indica (28.4) followed by *Eucalyptus mysoresins (*27.93), *Carica papaya*(24.62), *Ricinus communis* (22.46), *Polyalthia longifolia*(20.76), *Calotropis gigantea*(19.84), *Nerium indicum (*18.49), *Psidium guajava* (17.51), *Parthenium hysterophorus* (14.91), *Bougainvillea*(13.35), *Muntingia calabura* (11.68), *Terminalia cattapa* (10.71) and *Tamarindus indica* (9.12). A similar observation has been recorded by [12] while working on six different plant species and it was found that *Polyalthia longifolia* expressed highest APTI values .Similar observation had already been well documented [13, 14 and 15]. Table 3 reveals that exposure to indoor and outdoor air quality is different because they always change with time and diurnal pattern [16].Exposure to SPM also poses a serious risk to health. SPM includes all air borne particles in the size range of 0.5 μ to 100 μ. Urban areas exhibit both the highest level of pollution and largest target of impact on human health [17]. Diesel and Petroleum exhaust contain various substances, which are harmful to human beings [18, 19]. The data generated from the survey was analyzed to assess the allergic population and the suspected allergy causing agents.

CONCLUSION

From the results it reveals that in all the five sampling sites, the concentration of SO_2 (9.15 to 16.42 μg/m^3) and NOx (18.6 to 49.0 μg/m^3) is well within the CPCB limits. Suspended Particulate Matter SPM (516.2 to 1005.5 μg/m$^{3)}$ is the main pollutant in Shivamogga city which is above the prescribed limits of CPCB standards. This problem can be overcome by adapting advance ecofriendly transport systems and using biofuels. An attempt has also been made to study the air pollution tolerance index of selected plant species and has been evaluated and it is concluded that *Azadirachta indica*, (37.74) *Mangifera* indica, (28.4) and *Eucalyptus mysoresins (*27.93) are most tolerant species among all the fourteen plant species and most sensitive species are *Tamarindus indica* (9.12) and *Terminalia cattapa.* (10.71)

The study reveals that urban air pollutants have adverse effects on human health in Shivamogga city and many individuals residing near traffic intersections are suffering from respiratory diseases. Proper control measures may tackle this unhealthy problem of urban pollution.

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Table 1: Average concentration of SPM, SO_2 and NO_x (µg/m3) at five different sampling stations from June to October 2009

Sampling Areas	SPM	SO_2	NOx
Main receiving station (MRS)	675.5	13.0	35,61
Gandhi Bazar	697.3	10.16	38.7
Aamir Ahamad Circle	905.0	14.61	40.65
Bus stand	1005.5	16.42	49.0
Mandli	516.2	9.15	18.6

Table 2: Air pollution tolerance index (APTI) of plant species growing in Shivamogga city of Karnataka State

S. No.	PLANT SPECIES	RWC (%)	TCH (mg/g)	pH	AA(mg/g)	APTI
1	*Carica papaya*	86.04	0.79	6	23.6	24.62
2	*Calotropis gigantea*	61.22	0.76	6.5	18.9	19.84
3	*Eucalyptus mysoresins*	85.71	0.25	5.6	33.1	27.93
4	*Parthenium hysterophorus*	79.1	0.51	6.9	9.45	14.91
5	*Nerium indicum*	84.37	0.93	6.2	14.1	18.49
6	*Polyalthia longifolia*	87.8	0.24	6.1	18.9	20.76
7	*Mangifera indica*	90.9	0.51	4.6	37.8	28.4
8	*Ricinus communis*	89.7	1.24	5.9	18.9	22.46
9	*Psidium guajava*	77.14	1.25	5.7	14.1	17.51
10	*Mutangia calibra*	59.25	0.39	5.7	9.45	11.68
11	*Bougainvillea glabra*	69.56	1.17	5.6	9.45	13.35
12	*Terminalia cattapa*	83.72	0.47	4.5	4.72	10.71
13	*Azadirachta indica*	91.92	0.15	5.9	47.2	37.74
14	*Tamarindus indica*	50.9	0.07	4.2	9.45	9.12

Whereas, RWC=Relative water content, TCH=Total Chlorophyll content, AA=Ascorbic acid, APTI=Air pollution tolerance index

Table 3: Diseases due to ambient air in the city

Name of the disease	Total number of cases	Total number of persons
Asthma	30	29
Sneezing	29	19
Allergy	29	25
Hyperacidity	21	10

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RESEARCH ARTICLE

Role of Dibenzyl Disulfide (DBDS) in Increasing Sulphur Corrosion of Copper in Mineral Transformer Oil.

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ABSTRACT

Transformer oil is a by-product of crude oil and hence has a *perennial* problem of corrosive and reactive sulphur. Since Power apparatus invariably use transformer oil to serve the dual purpose of insulating the transformer and maintaining thermal balance, it is very essential to adhere to accepted performance levels of transformer oil. Since transformers are subjected to overloading and fluctuations in voltage, there are locations which are traditionally called hot-spots. These regions will be in higher temperature zone and will be much above the average working temperature of 80 to 90 ° C. Hence transformer oil will undergo thermal degradation and through a series of chemical reactions, will result in formation of corrosive and reactive sulphur compounds. These compounds react with copper conductors to form copper sulphide, which is semi-conductive in nature. Due to non-linear surface and volume conductivities in paper-oil insulation, there is further increase in temperature and degradation of cellulose and transformer oil takes place. This study focuses on the role of DBDS, which is an antioxidant, in increasing copper corrosion. The effect of DBDS has been studied on typical transformer oil parameter and from the subtle variations in these parameters an attempt was made to correlate the presence of DBDS to increase in rate of copper sulphide formation.

Key words: Mineral transformer oil, Dibenzyldisulfide (DBDS), copper corrosion, total sulphur, mercaptan sulphur, paper-oil insulation.

INTRODUCTION

Transformers provide vital link between generation and transmission and also between transmission and distribution of power. Hence they are considered as very important among all electrical equipment's. Insulation system used in power transformers comprise of solid-liquid systems and solid-gas systems. In recent years, a number of failures of power transformers and reactors have taken place due to sulphur in oil causing corrosion of copper conductor resulting in formation of copper sulfide on conductors and insulating materials. The conductive copper sulfide reduces the electrical strength of the insulation system. Therefore understanding of the chemistry of copper corrosion due to sulphur on oil is of significance. Further there is sustained interest in understanding chemistry of copper corrosion for evolving techniques to mitigate this problem in huge assets like transformers.

Transformer oil is by-product of crude petroleum. Hence presence of sulphur is always expected in transformer. However a huge amount of work has been carried out on extraction of transformer oil which will be free from reactive and corrosive sulphur. The manufacturers have a tendency to add antioxidants and antiwear additives to achieve certain predetermined parameters and performance characteristics of oil. But these additives over a period of time, through a series of thermally triggered and interlined chemical reactions, lead to formation of corrosive and reactive sulphur compounds. It is interesting to observe that the main culprit in unused and used mineral insulating oils in case of majority of failed transformers [1] is dibenzyl disulfide (DBDS). It is an antioxidants or antiwear additive. Experimental results have revealed that DBDS enhances the oxidation stability [2] of oils but it also makes oils corrosive thereby attacking copper to form copper sulfide. To obtain a better understanding of the corrosive behavior of DBDS, there is need for carrying out experiments on unused and used oils by subjecting them to corrosion tests at higher temperatures. The measurement of DBDS concentration before and after the thermal shocks and physical examination of copper conductors can give idea about "corrosion potential" of DBDS. The mechanism of corrosion can also be established [3].

The focus on role of DBDS in transformer oil is not without reasons. The following issues have been studied by many researchers all over the world [4].

1. The degradation by-products of DBDS have been detected to be corrosive .
2. Many oils samples studied form failed transformers are observed to have high concentrations of corrosive sulfur and DBDS
3. Trending analysis of DBDS in transformer oil sampled from highly loaded transformers shows reduction in concentration of DBDS with time.

In this paper, the effect of DBDS under different conditions of heat ageing has been discussed. Since transformer oil analysis is carried out in a routine fashion in all substations, the method of DBDS detection has to be very simple. Hence the use of Gas Chromatography, FTIR, total sulphur and mercaptan sulphur measurements have been considered for understanding the role of DBDS in the chemical reactions leading to corrosion of copper conductors in transformers. From this study, it is expected that a simple correlation of the parameters might exist with the presence and increase in concentration of DBDS in transformers.

MATERIALS AND METHODS

Aging of transformer oil was carried out continuously at 120ºC and 140 ºC using hot air circulating oven with different concentration of DBDS (100 ppm and 150 ppm) for 170 and 70 hours. The oil samples were monitored for mercaptan sulfur, total sulphur and dissolved gas analysis.

Experimental Method

(a)Sample preparation for aging studies

Two rectangular copper strips with and without paper wrapped were dipped in two samples of transformer oil containing DBDS as an antioxidant and are subjected to thermal aging at different temperature and concentrations.

(b) Mercaptan Sulfur Analysis

Mercaptans sulphur is one form of the sulphur which is very reactive. Although it is very less in quantity in case of fresh oils, it is consumed during high temperature and its value goes down from 1 almost zero level when thermal ageing is carried out. The corrosive oils have shown random variation in Mercaptans sulphur content. Mercaptans sulphur equipment is automatic titration equipment with supporting software for measurement of Mercaptan sulphur in transformer oil. Here the sample is dissolved in an alcoholic sodium acetate and titrated potentiometrically with silver nitrate ($AgNO_3$) solution. The test is continued till it recognizes the end point (EP).

(c) Total Sulfur Analyzer

In WDXRF spectrometry, the polychromatic beam emerging from a sample surface is dispersed into its monochromatic constituents by the use of an analyzing crystal according to Bragg's Law. The wavelength for any measured line is computed from knowledge of the crystal parameters and diffraction angle. In a simultaneous WDXRF spectrometer one or more detectors are placed at the certain angle for an element and so it is possible to measure different elements.

X-Ray Fluorescence spectrometers (XRF) use high energy X-rays (or gamma rays) to excite fluorescent radiation or photons from a sample for elemental analysis. In wavelength dispersive x-ray fluorescence spectroscopy (WDXRF), photons emitted by the sample are separated or dispersed by diffraction before hitting the detector. This is accomplished by placing an analyzing crystal between the sample and the detector. Therefore, WDXRF spectrometers have better resolution than energy dispersive x-ray fluorescence spectrometers which do not contain an analyzing crystal. However, due to the increase in optical components, WDXRF spectrometers typical have a lower efficiency than EDXRF spectrometers and, hence, require a higher power x-ray tube (which can add to the cost of the instrument).

(d) Dissolved Gas Analysis

Most transformers are filled with mineral oil and it is refined from crude oil and is used because of its chemical stability and high natural dielectric strength. Being that it is a hydrocarbon material; the principal molecular makeup of transformer oil is Hydrogen and Carbon in their various forms. It is for this reason that Dissolved Gas Analysis Test (DGA) can be used to readily determine the electrical condition of the transformers [5].All transformer oils suffer electrical breakdown naturally over a period of time. However, this is a very slow process. Barring outside influences and causes, the expected life of good transformer oil will exceed 20 years. The main enemies of transformer oil are Oxygen, Water and Excessive Heat.

The incipient fault in the bulk oil equipment, generally undetected by the protection system, generates certain gases and because of the no escapes construction of oil circulation system these gases are dissolved in the oil itself. The insulating oil being under convection or circulation gets these gases dissolved homogenously. A sample of about 200 ml of this oil is generally subjected to Multi Pass Gas Extraction and Gas Chromatography to evaluate the quantum of dissolved gases, individually and collectively [6]. The gases of interest are: hydrogen, methane, ethane, ethylene, acetylene, carbon monoxide and carbon dioxide. Most commonly used method to determine the content of these

gases in oil is using a Vacuum Gas Extraction Apparatus and Gas Chromatograph. In the present study, the dissolved gas analysis was carried out using vacuum gas extraction system. All the dissolved gases were first extracted from oil by stirring it under vacuum and total gas content in percentage was measured. These extracted gases were then introduced in Gas Chromatograph for measurement of each component. A standard gas mixture having known concentration was also injected in Gas Chromatograph to obtain standard peaks.

(e)FT-IR Analysis

FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

RESULTS AND DISCUSSION

Total Sulphur and Mercaptans Sulfur of Transformer Oil

The oil samples were thermally aged and were monitored for Total sulphur and Mercaptans sulphur at regular intervals. Table 1 shows the results of mercaptan and total sulfur in oil containing 100 ppm of DBDS. It is observed there is not much of change in the total sulfur content and mercaptan sulfur. In table 2, the results of total and mercaptan sulphur are presented when 150 ppm of DBDS was present in transformer oil. There was no significant change in concentrations of total sulphur and mercaptan sulphur. In table 3, the results of DGA of transformer oil with 100 ppm of DBDS are presented. Even in this case no significant increase in concentration of different gases was noticed. In table 4, results of total sulphur of transformer oil with different concentrations of DBDS after thermal ageing at 140ºC for 48 and 96 hours are presented. There is increase in Total sulphur value with addition of DBDS to white oil even under unaged conditions. White oil does not have any additive or anti-oxidants and hence these results are indicative of the reactions taking place in the base oil.

In case of transformer oil, addition of 200 ppm of DBDS shows increase in Total sulphur from 4.084 ppm (unaged oil) to 152.529 ppm. However, with thermal ageing, total sulphur decreases to 63.246 ppm. With 2 % of DBDS is present in oil, the total sulphur increase to 4516 ppm and thermal ageing for 48 hours results in further increase to 4750 ppm. Similarly, with 3 % of DBDS in transformer oil, the total sulphur increases to 6354 ppm and thermal ageing at 140° C for 48 hours further increases the total sulphur value to 6645 ppm. Thus it is very evident that DBDS in small ppm concentration does not result in significant increase in total sulphur. But when it is present in percentage levels, it has a tremendous impact on total sulphur value. Thermal ageing aggravates the problem further.

Analysis of Transformer Oil drawn from In-Service Transformers

The results of mercaptan sulphur and total sulphur of transformer oil drawn from different transformers are also been analyzed as shown in the tables 4 and 5 below. The results show that the mercaptan sulphur values are well within the permissible limits. However, the total sulphur value is high in case of sample 4 and less than 5000 ppm in other cases. There is no correlation between high total sulphur values and copper corrosion. Hence results of DGA would be useful to arrive at definite conclusions. The DGA results are shown in table 6.

In this study no correlation between presence of H_2 or higher concentrations of CO_2 with higher total sulphur values were observed. However**,** further studies by continuous ageing and monitoring of DGA and Total sulphur are essential.

FT-IR Analysis : Fourier transform infrared spectroscopy was used to analyze the different concentration of DBDS in oil (i.e. 0.5%, 1%, 1.5%, 2%, 2.5%, 3%) and it was observed that DBDS is detected only above 1.5% as shown in figure1 , that is at 696.35cm^{-1}. The transformer oil with DBDS concentration of 2% and 3% were aged for 48 hours and the oil was analyzed using FT-IR as shown in figure2. FTIR has serious limitations when it is used in conventional method. Hence there is a need to develop alternative techniques for detection of DBDS using FTIR.

CONCLUSION

From the results presented and discussed in this paper, the following conclusions are Mercaptan sulphur values of transformer oils from in-service transformers of more than 20 years of service life has shown that mercaptan sulphur values was are within the permissible limits.Even Total sulfur values are within the permissible limits for both thermally aged and in-service oil samples.Prolonged thermal ageing at elevated temperatures of 120ºC and 140ºC for 170 and 70 hours respectively, did not affect the mercaptan sulphur values, when aged in air. This highlights the role of inert atmosphere in copper corrosion phenomenon.Mercaptan sulphur values of transformer oil samples are also within the permissible limits even after long durations of aging at 120ºC and 140ºC for 170 and 70 hours. DBDS did not show much variation even after thermal ageing at 140ºC for 48 hours when analyzed using FTIR method. This is due to limitations in the use of FTIR method. However, more work is required to adopt FTIR method to detect the reducing concentrations of DBDS.The FT-IR analysis of transformer oil with concentrations 0.5%, 1%, 1.5%, 2%, 2.5% and 3% DBDS was made and the detection limit of DBDS by FTIR is above 1.5%.DGA of aged transformer oil with DBDS of different concentration did not show much variation in concentrations of dissolved gases. Similar results were observed in case of in-service transformer oil. There are some cases in which the DGA results have shown sensitivity in terms of increase in concentrations of CO_2, H_2. This needs further study. Addition of higher concentrations of DBDS leads to increase in total sulphur values, which further increases with thermal ageing. Hence the concentration of DBDS should be minimum, so as to prevent formation of copper sulphide.

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Table 1: **Results of aged oil samples with DBDS concentration of 100 ppm at 120ºC**

Mercaptan sulfur (ppm)			Total sulfur (ppm)	
Aging (no. of hrs)	**Oil with copper strip**	**Oil with paper wrapped copper strip**	**Oil with copper strip**	**Oil with paper wrapped copper strip**
10	0.16	0.13	30.1675	31.0225
25	Low	0.12	28.984	29.4795
46	0.17	0.13	29.4945	31.4795
79	0.09	0.1	32.874	34.099
105	0.11	0.06	33.565	32.892
170	0.12	0.14	32.634	32.735

Table 2: Oil with 150 ppm DBDS aged for 70 hours at 120ºC

Aging (no. of hrs)	Mercaptan sulfur (ppm)	Total sulfur (ppm)
50	0.08	46.287
70	0.07	50.952

Table 3: DGA of aged oil samples with DBDS concentration of 100 ppm

Dissolved Gas Analysis										
Oil with copper strip (Concentration on ppm)										
Aging (hrs.)	**Gas content µl/L**	**CH_4**	**C_2H_6**	**C_2H_4**	**C_2H_2**	**H_2**	**O_2**	**N_2**	**CO**	**CO_2**
10	119252	1	ND	3	ND	ND	34171	81970	ND	499
26	145429	1	ND	ND	ND	ND	33097	83407	ND	359
46	113435	ND	ND	ND	ND	ND	28649	78613	ND	508
79	125069	ND	ND	ND	ND	ND	30192	72906	ND	522
105	119252	ND	ND	ND	ND	ND	29057	71569	ND	465
170	125069	ND	ND	ND	ND	ND	30769	72477	ND	410
Oil with paper wrapped copper strip										
10	116343	3	2	18	ND	ND	34057	78946	ND	777
26	95983	1	ND	ND	ND	ND	20664	54703	ND	167
46	116343	ND	ND	ND	ND	ND	28649	78613	ND	456
79	125069	1	ND	ND	ND	ND	26926	71279	ND	217
105	122160	ND	ND	ND	ND	ND	29878	70766	ND	394
170	122160	ND	ND	ND	ND	ND	30083	70429	ND	473

Table 4: Total sulphur of different concentration of DBDS in transformer oil and white oil after ageing at 140ºC

Identification	No. of hrs.	Total sulfur ppm
White oil	Unaged	13.211
200 ppm DBDS	Unaged	60.668
200 ppm DBDS +copper	90	92.215
Transformer oil	Unaged	4.084
200 ppm DBDS + copper	Unaged	152.529
200 ppm DBDS +copper	90	63.246
2% DBDS	Unaged	4516.516
2% DBDS	48	4750.516
3% DBDS	Unaged	6354.080
3% DBDS	48	6645.352

Table 5 : In-service oil samples analyzed for mercaptan sulphur and total sulphur

Sl. No	power rating	Year of commissioning	Mercaptan sulfur (ppm)	Total sulfur (ppm)
1	8 MVA	1993	0.89	4220.485
2	8 MVA	1961	0.19	1561.479
3	8MVA	1998	0.13	1760.786
4	5MVA	1989	0.11	10004.540
5	8MVA	1993	0.13	1820.930
6	5MVA	1997	0.26	2716.495
7	8MVA	1993	0.07	1820.930
8	8MVA	1997	0.10	1456.058
9	5MVA	2000	0.08	344.554
10	12.5MVA	2008	0.07	281.407
11	5MVA	1996	0.67	1944.050

Table 6 : In-service oil samples analyzed for mercaptan and total sulphur

Sl. No.	Power rating	Date / year of commissioning	Mercaptan sulphur (ppm)	Total sulphur (ppm)
1	167MVA	4/6/2005	0.205	430.865
2	12.5MVA	1998	0.1	2277.874
3	63/70MVA	5/1/2005	0.06	414.764
4	10.5MVA	3/5/2010	Invalid	17.387
5	10.5MVA	9/7/2010	0.245	24.205
6	315KVA	3/5/2010	0.07	32.961
7	315KVA	9/7/2010	0.115	18.389
8	7.3MVA	1972	0.225	824.064
9	7.3MVA	1978	0.38	1155.672
10	305KVA	1982	0.08	987.456
12	83.3MVA	24/11/11	0.07	294.4125
13	83.3 MVA	25/9/1986	0.07	505.1645
14	83.3 MVA	10/7/1987	0.09	851.076
15	83.3 MVA	10/7/1987	0.05	163.9835
16	105 MVA	26/8/08	0.12	211.786
17	86.6 MVA	9/6/08	0.07	317.464
18	20 MVA	2002	0.08	2809.2535
19	70 MVA	4/1/1998	0.86	3393.0715

Table 6: In-service oil samples analyzed for dissolved gas analysis

Sl. No	Power rating	Gas content	CH_4	C_2H_6	C_2H_4	C_2H_2	H_2	O_2	N_2	CO_2	CO
		µl/L	Values in ppm								
1	167MVA	114103	2	ND	1	ND	ND	19093	71868	1292	ND
2	12.5MVA	105017	493	103	1110	764	ND	19085	64268	2385	ND
3	305KVA	111340	3	1	3	ND	ND	30519	75618	4023	ND
4	63/70MVA	110852	2	ND	5	ND	ND	26115	63186	1064	ND
5	10.5MVA	114270	1	ND	1	ND	ND	25847	64852	1374	ND
6	10.5MVA	114270	1	ND	1	ND	ND	25676	61629	1310	ND
7	315KVA	114270	2	ND	1	ND	ND	24617	66138	1181	ND
8	315KVA	102400	3	1	7	ND	ND	21891	63973	2462	ND
9	7.3MVA	107618	30	32	15	ND	ND	27355	64338	10138	ND
10	7.3MVA	113435	15	27	184	17	59	27349	66712	2183	ND
11	83.3 MVA	105017	493	103	1110	764	ND	19085	64268	2385	ND
12	83.3 MVA	111340	3	1	3	ND	ND	30519	75618	4023	ND
13	83.3 MVA	110852	2	ND	5	ND	ND	26115	63186	1064	ND
14	83.3 MVA	114270	1	ND	1	ND	ND	25847	64852	1374	ND
15	105 MVA	114270	1	ND	1	ND	ND	25676	61629	1310	ND
16	86.6 MVA	114270	2	ND	1	ND	ND	24617	66138	1181	ND
17	20 MVA	102400	3	1	7	ND	ND	21891	63973	2462	ND
18	70 MVA	107618	30	32	15	ND	ND	27355	64338	10138	ND
19	12.5 MVA	113435	15	27	184	17	59	27349	66712	2183	ND

Transformer oil

White oil

0.5%

1%

1.5%

2%

2.5%

3%

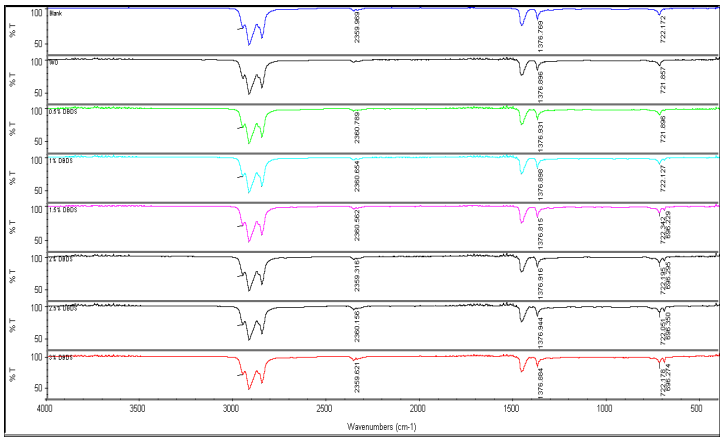

Figure 1: Spectrum of transformer oil containing different concentration of DBDS

2%

3%

2% aged

3% aged

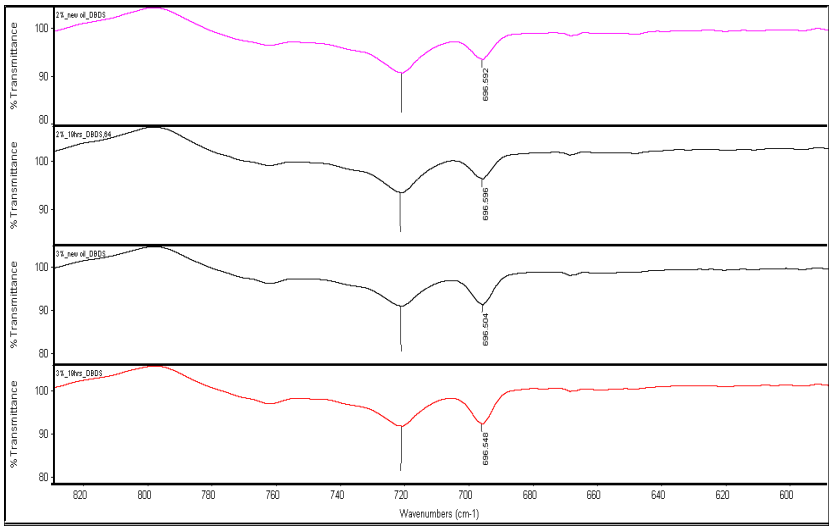

Figure 2: Spectrum of aged transformer oil with different concentration of DBDS

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REVIEW ARTICLE

Effects of Urbanization on Functional Diversity of Species- a Review

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ABSTRACT

Urbanization effects bird community structure and composition and increases species abundance but decreases species richness. When natural habitats get replaced by human dominant systems as a result of urbanization, it leads to species extinction and biotic homogenization. In urban areas native tree species harbour more birds than exotic tree species. Granivore species are less affected by urbanization followed by aerial insectivores and ground foraging insectivores. Corvids and raptors are well suited to urban environments but few birds are highly sensitive to urbanization and are slowly disappearing from our environments. Although numerous studies have been carried out to study the community-level responses of bird species to human-induced habitat alterations, lot more research needs to be done. Conservation measures should focus on protecting the habitats of urban birds by incorporating ecological perspective into urban landscape planning. This paper reviews the effect of urbanization on bird species and various factors that govern richness and abundance of bird species in the urban.

Keywords: Urban ecosystems, extinction, heterogeneous, ecotone, species, guild.

INTRODUCTION

Urbanization involves alterations and modifications of landscapes in a natural ecosystem often leading to changes in species composition [1]. Urban areas are clusters of fragmented city centres occupying high population and witnessing rapid urban development with its fringes occupied by less populated and less developed areas [2]. The pressure of human dominating the ecosystems has lead to over consumption of resources [3] with the world's urban population steadily raising from the current 49% to 61% by 2030 [4]. This will further modify natural areas into highly-modified and altered heterogeneous landscapes where species composition and diversity vary greatly [5,6]. How does urban ecosystems differ from other natural ecosystems is that, in these human occupy a dominant role in

the ecological pyramid [7].As urban ecosystems are expanding to adjacent landscapes, more and more areas would be a part of conversion process effecting ecosystem functioning and processes [8].

Of the recently updated IUCN report the extinction of species is thousand times higher than that previously documented in 1997. Among the varied causes of species endangerment and extinction (urbanization, agriculture and interactions with non-native species) urbanization ranks highest [9].Urbanization is said to effect geomorphological and hydrological processes in a natural ecosystem, disrupting the cycling of nutrients, water and sediments. These modifications in the physical environment results in the emergence of unique and taxonomically variant communities of organisms, occupying varied matrix in the urban settings [10,11].

How natural are urban ecosystems?

Urban ecosystems are natural ecosystems like any other ecosystems in nature with low stability and different dynamics are often characterized with more native species and varied species composition.Urban ecosystems function in a combination of biological and physical patches that are characterized with rich spatial and temporal heterogeneity [12]. Urban ecosystems were regarded as closed self-regulating forms in nature but recent studies prove they function as multi-equilibria systems, open, dynamic and unpredictable where variation and external disturbance forms an important characteristic [13]. When natural habitats get replaced by human dominant systems as a result of urbanization, it leads to species extinction and biotic homogenization [14,15,16] representing threat to biodiversity at varied scales.

Birds along an Urbanization Gradient

Birds serve as good ecological indicators as they are highly sensitive to environmental degradation and are easily observable [17]. Urban areas are often characterized by abundant food resources and low mortality [18]. Along an urbanization gradient, urban habitats are composed of less species diversity and are often dominated by the abundance of few non-native species [19,20,21]. In areas with moderate level of urbanization, often species diversity is found to be higher especially along the sub-urban fringes [22,23]. Urban fringes provide suitable habitats for many native and non native species, but diversity in the urban matrix declines as urbanization gradient increases [24]. In city centres, bird species composition is more varied in different habitats and in lesser urbanized areas, species composition is most similar [25]. A very important attribute of the landscapes between urban and natural habitats are ecotones or habitat edges. Ecotones are ecologically active zones [26] and provide unique environment with varied landscapes and higher structural complexity of vegetation for various functional aspects of birds [27,28,29,30].

Fragments play a crucial role in the functioning of an urban ecosystem and its natural entities. Often fragments operate as ''ecological traps'' as they attract wide diversity of birds [31] by providing suitable nesting and breeding habitats for birds and may also function as a detrimental factor as birds are often exposed to predation and competition along these fragmented edges. [32]. Conserving structurally complex foliage of native species often contributes to higher species richness and abundance of migratory passerines in forested [33] and greener cover landscapes along an urbanization gradient.

Effect of structural complexity of vegetation on species richness

The relation between vegetation complexity and bird species richness, species community composition and diversity has been well studied and documented [34,35]. Vegetation stages a crucial role in determining bird community composition [36,37]. Often native vegetation is positively correlated with native bird richness and exotic species is associated with the richness of exotic bird community [38]. Bird community composition is often altered in response to exotic plant species [39] and few urban species takes advantage as it shields them from predators [40]. Bird species richness and diversity is correlated with various attributes of vegetation such as foliage height, species diversity, foliage volume and percent vegetation cover [41,42]. In an urban and sub urban landscape, species richness and community composition of birds is often governed by attributes like tree foliage cover and tree height. Urbanized centres which occupy more native vegetation retain higher species than those areas with non native species [43].Composition of plant species in the urban environment differs from that of less disturbed areas [44] as urban plants are ruderals, tolerant to disturbance and synanthropic, habituated to human dwellings [45] or hemerobes, found in areas of human impact [46]. Hence urban fauna fall under "Naturalized Urban Plant Community" [47] which includes species that are cosmopolitan, that spread extensively and colonizes urban areas all over the world [48].

Does guild partioning effect species composition?

Some common species with a broader territorial range flourish well in urban settings as they are gregarious and are not affected by external disturbances [49]. This has well studied and has been defined as a trait that would facilitate and spread the invasion of exotic species into natural environments [50].Various studies have been carried out to study the community-level responses of bird species to human-induced habitat alterations. Species less sensitive take advantage of the altered habitats like abundant food resources, better climatic conditions and less predation resulting in higher abundance.Species sensitive to habitat alterations are adversely affected and decline and may also face extinction (Marzluff 2001). Urbanization often favour species that are granivores followed by aerial insectivores and ground foraging insectivores and residents birds over migratory species [52,53,54]. Omnivorous species are well adapted to urban environments than species from other foraging guilds (granivores, frugivores and insectivores) as the presence or absence of these species are limited by resource availability [55,56,57]. Several studies related to guild partioning have found that nestlings in urban habitats have a lower body mass than the nestlings in natural habitat due to lower and reduced quality of food intake [58,59,60] and also due to contamination of food and water by toxic materials [61,62].

Effect of patches on distribution of species

Bird communities are influenced by patch factors and this has been well documented [63]. Bird diversity and community assemblages are often governed by the kind of land use patterns and the kind of landscape surrounding a patch. Urban structures often obstruct movement of birds along corridors [64,65]. Birds that have a narrow home range often occupy small patches as they require less space for foraging and for other behavioural activities and have low diversity than larger patches [66]. Isolated patches usually have less species diversity than connected patches, as isolation restricts bird movement due to lack of corridors and influences the occurrence or non-occurrence of a species [67,68]. Patches with higher canopy heterogeneity, would sustain rich bird diversity than patches with lower foliage cover [69]. Often fragmented patches leads to biotic homogenization and may decrease regional bird diversity increasing local diversity [70,71].

Is resource matching an important gradient in bird diversity?

The main factor that governs abundance of species in urban and rural landscape is resource-matching (72,73). Variation of resources in urban often support those species that are competitively superior and less sensitive to environmental changes which enable them to select better sights than individuals that are inferior, highly sensitive and select low-quality sites (74,75). Few grassland birds and forest species are sensitive to vegetative attributes like shrub, herb, litter cover, foliage height and diversity and total foliage volume (76). Hence they select areas that are potentially viable for them for nesting and breeding. Urban areas provide better habitats for corvids and raptors as abundant resources in and around human settlements help them to minimize their energy expenditure (77,78).

Urbanization and avian survivorship

Avian survivorship in urban areas depends upon their sensitivity and adaptability to urban resources [79] predation rate [80 and 81], nest parasitism [82] and competition. The other factors vulnerable to avian survivorship in urban areas are risk of collision with man-made objects [83], changes in the prey-predator assemblage [84], food resources availability [85,86] and disease [87].

Construction of roads is often associated with urbanization. Building of roads can result in fragmentation of a habitat, isolating populations of species, polluting the environment with increased noise and automobile exhausts. Often birds get attracted to roads along fragmented habitat corridors, resulting in direct collision with automobiles [88,89] and resulting in mortality. Raptors are better suited to urban environments as they less persecuted than rural environments and have an adequate [90] supply of food. Raptors with a broader home range in the urban are less affected by habitat reduction than with species occupying narrow home range [91].Vulture population world over increased at the onset of urbanization but steadily declined as waste and garbage grounds stopped invading cities [92].Few studies have pointed out an decrease in the egg size of the urban nesting birds like the song thrushes and the Black Billed Magpie but not evident among Starlings [93]. But species from the corvidae family usually have a higher reproductive success rate as they are well adapted to human habitations [94] and nest predation is less. Collision of birds with power lines in the urban also causes death or injury to birds [95].In many studies urbanization is often associated with human visits to urban parks and such visits would low reproductive capacity in birds [96] decreases hatching success [97] and increases predation as these birds are often noted by the visitors. Studies also relate to different species of birds habituating to disturbance and resulting in various behavioural changes [98]. Usually human activities negatively affect avian populations and their communities [99]. Several recent studies provide insight into birds modifying their songs in the urban and responding adaptively to noisy urban environments [100,101].

Role of conservation

Native species play a very important role in maintaing diversity and an adaptive management effort needs to be implemented to restore and conserve native species in urbanized environments [102]. Studying urban dynamics and its functional aspects at landscape level would provide a better insight in developing designs for effective management practices [103]. Recent studies put forward the role of gardens in supporting avian populations of national conservation concern [104,105,106,107,108]. Varied kinds of birds visiting our gardens would evoke interest in nature and beautiful life forms around us. This would inspire the conservationists [109,110] and naturalists of the future. When a step is put forward to preserve trees in the urban this would in turn minimise the cost of constructing and maintaining green areas [111]. Community-based projects which fosters to the needs of society and develops an appreciation for the nature should be carried out [112]. City planners and urban foresters should incorporate more ecological perspective into urban landscape planning for conserving and protecting urban birds.

CONCLUSION

Urban bird studies have indicated that urban environments are less species diverse than rural habitats but have higher species abundance.Some studies have highlighted a peak in species richness at intermediate levels of urbanization.Some studies have pointed out how rapid and continuous urban development poses threat to those species that are sensitive to various environmental stressors.More studies on urbanization and its effect on species would provide an insight into population level responses of birds to various demographic parameters in varied landscapes. Recent research indicates urbanization changes animal behavior morphology population dynamics and community structure. Urbanization tends to select omnivorous, granivorous and cavity nesting species. Urban areas that tend to retain more native vegetation are found to be more species rich. The primary functional aspect for avian conservation is to understand the various ecological services urban areas provide and to justify how important it is to protect the species inhabiting these landscapes for maintaining a healthy environment. Urban areas and the ecological phenomena functioning in urban landscapes are unique and provide good study designs to researchers, conservationists and city planners.

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RESEARCH ARTICLE

Effects of Vermicompost on Plants

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ABSTRACT

Earthworm *Eudrilus eugeniae* was maintained using cow dung manure. Compost was collected at regular intervals for testing the germination and growth of some selected plants. Different concentrations of compost were used to test the germination and growth of *Amaranthus polygonoides*, *Chenopodium album*, *Amaranthus spinosus*, *Vigna radiate*, *Cajanus cajan*. The growth of the plant in terms of leaves, stem and root production were noticed. The results showed that 50% and 75% concentrations were found suitable for germination and growth of the above plants. The utility value of compost was discussed with reference to previous reports.

Key words: *Eudrilus eugeniae*, germination, compost, concentrations

INTRODUCTION

Vermicomposting is the usage of earthworms to convert vegetable waste to a 100% natural plant fertilizer. The use of worm farms for vermicomposting is becoming a favorite way of converting waste to a valuable product while also growing more worms to increase the capacity of the worm farms. The most significant benefit is that the nutrients in earthworm compost are very easily absorbed by the roots of plants. Unlike chemical fertilizers, vermicompost is not easily flushed from the soil because of the worm mucus that it contains [1]. Plants have longer to obtain the nutrients and get the maximum benefit.

As the compost is passing through the body of the worms it is enriched with bacteria and microbes. As the compost works on the plants and they become healthier the need for pesticides is reduced. The chemical fertilizers might increase plant yields but they do nothing for plant health. Continued use of chemical fertilizers inevitably leads to a breakdown in the soil. Ammonia and salts build up which attack the plants making them less able to withstand disease [2]. Among the hormones that earthworm compost contains are hormones that help plants to grow.

Germination of seeds is encouraged, the growth of the plant is stronger and the crop yield improved [3]. This natural support for the plants is not available with chemical fertilizers. The distribution of the compost through the soil also helps to encourage healthy root growth.

The germination test of some vegetable plants seeds were studied using the compost in different percentage with control. In plant test, some of vegetable plants growth parameters (Root weight, Stem weight, Mass production or Leaves weight) were analyzed in different compost from different wastes were analyzed.

MATERIALS AND METHODS

Germination test

Germination test were carried out in the seeds of five vegetable plants such as *Amaranthus polygonoides* (Sirukeerai), *Chenopodium album* (Palakeerai), *Amaranthus spinosus* (Thandankeerai), *Vigna radiate* (Greengram) and *Cajanus cajan* (Redgram) with compost in different concentration (25%, 50%, 75% and 100%). Control was maintained in which vermicompost was not provided.

Plant growth test

In order to test the effect of vermicompost on the growth of the plants, some vegetable plants were chosen and trails were performed. The following plants were selected to study the growth parameters: *Amaranthus polygonoides*, *Chenopodium album*, *Amaranthus spinosus*, *Vigna radiate* and *Cajanus cajan*. The growth parameters such as root weight stem weight, Mass production or Leaves weigh were noticed.

RESULTS AND DISCUSSION

Plant germination tests

Being rich in macro and micro nutrients, the vermicompost has been found ideal organic manure enhancing biomass production of a number of crops [1]. The higher germination rate was found 89% in *Amaranthus polygonoides*, 96% in *Chenopodium album*, 77% in *Amaranthus spinosus*, 97% in *Vigna radiate* and 91% in *Cajanus cajan* in 50% of vermicompost.

Vermicomposts consistently promote biological activity which can cause plants to germinate, flower and grow and yield better than in commercial container media, independent of nutrient availability [2]. The superior growth was found *Amaranthus polygonoides*, *Chenopodium album*, *Amaranthus spinosus*, *Vigna radiate* and *Cajanus cajan* in the concentration of 75% of vermicompost. Substitution of small amounts of vermicomposts into soil has resulted in significant increase in the germination and growth of marigolds tomatoes and peppers, in greenhouse trials, when all necessary nutrients are available of 5-30% into the medium [2].

Vermicompost contains plant growth regulators and other plant growth influencing materials produced by microorganisms [3]. Krishnamoorthy and Vajrabhiah [4] reported the production of cytokinins and auxins in organic wastes that were processed by earthworms. Vermicompost also contains large amounts of humic substances [5] and some of the effects of these substances on plant growth regulators [6].

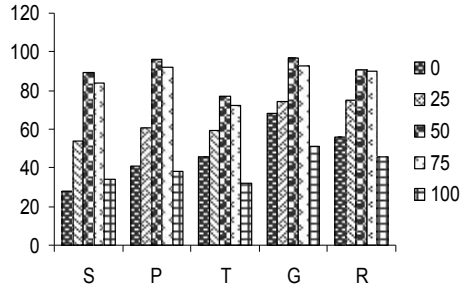

Fig 1: Germination of seeds in different concentration of Vermicompost

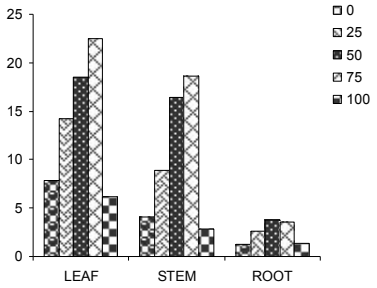

Fig 2: Growth of *Amaranthus polygonoides* (Sirukeerai) in different concentration of Vermicompost

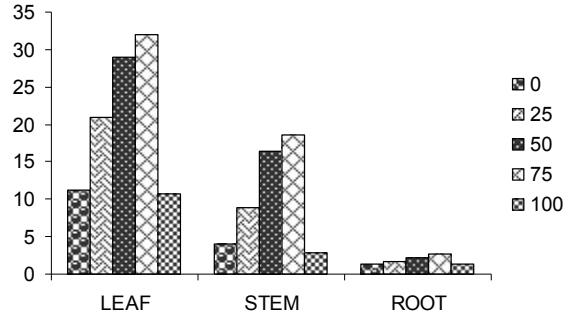

Fig 3: Growth of *Chenopodium album* (Palakeerai) in different concentration of Vermicompost

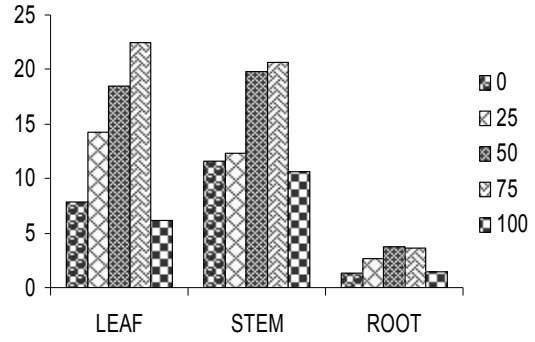

Fig 4: Growth of *Amaranthus spinosus* (Thandankeerai) in different concentration of Vermicompost

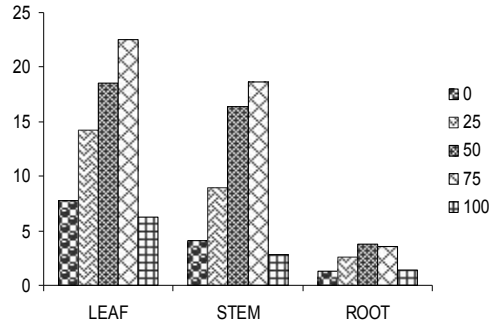

Fig 5: Growth of *Vigna radiate* (Greengram) in different concentration of Vermicompost

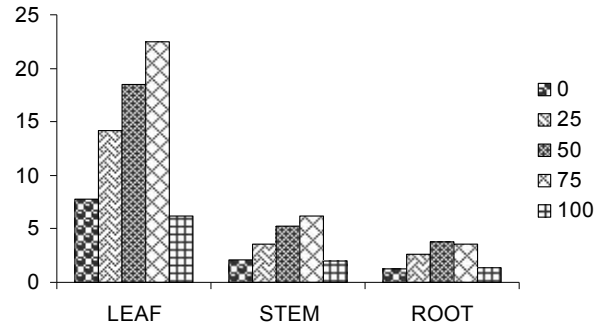

Fig 6: Growth of *Cajanus cajan* (Redgram) in different concentration of Vermicompost

CONCLUSION

Concentrations of compost were used to examination the germination and growth of plants. The outcome showed that 50% and 75% concentrations were found suitable for germination and growth of the plants. Such substitutions would be economically desirable since acceleration of growth of bedding plants is a requisite of agriculture area.

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RESEARCH ARTICLE

Screening Potential Phytoagents for Insulin-Like Effect in MSG-Induced Diabetic Albino Mice, *Mus musculus*.

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ABSTRACT

Eleven potential herbal antidiabetic agents were screened to study their insulin-like effect in managing diabetes which was induced by MSG administration in albino mouse, *Mus musculus*. The results revealed that the highest potential was exhibited by *Spirulina platensis*. Other agents such as *Gymnema sylvestre, Momordica charantia, Coccinia indica,, Eugenia jambolana, Cassia auriculata, Anona squamosa, Catharanthus roseus, Aegle marmelos, Ocimum sanctum, Pterocarpus marsupium* did not show any significant effect. The study confirms the specific nature of the MSG diabetes.

Keywords: Herbal extract, MSG diabetes, Blood glucose, Obesity

INTRODUCTION

Diabetes epidemiology has had a profound impact on diabetes research, care and prevention in the last two decades. Diabetes and its complications pose a major threat to future public health resources throughout the world. Based on a complication of studies from different parts of the world, the World Health Organization (WHO) has projected that the maximum increase in diabetes would occur in India. Recent population based studies have revealed the prevalence of Type II diabetes in different parts of India [1]. There are numerous risk factors for Type II diabetes. They are aging, diet, obesity, family history etc. Central obesity is the vital risk factor which is known to predispose individuals to insulin resistance. It is reported that approximately 55% of patients diagnosed with Type II diabetes are obese.

One of the obesity-inducing factors is reported to be the food flavoring agent, monosodium glutamate (MSG), which is used in plenty in food industries. Glutamate is found naturally in protein containing foods, fermented (or) hydrolyzed protein products etc [2]. It is also commercialized by Ajinomoto Company Japan, under the name AJINOMOTO "essence of taste". It stimulates glutamate receptors located in taste buds and enhances the taste of

food [3]. MSG is now used experimentally to induce obesity and type II diabetes[4]. It is noticed that glutamate is a neuro transmitter in the brain. It exists in the extra cellular fluid in small concentration about 8 to 12µl. When the concentration rises above this level, the neurons begin to fire abnormally. At higher concentration, the cells undergo a specialized process of delayed cell death known as excito-toxicity. A neonatal (or) young adult administration of MSG induces obesity in mice [5].

World Health Organization has suggested the evaluation of the plants as effective therapeutic agents, especially in areas where we lack safe modern drugs. There are about 800 plants with antidiabetic potential as per the ethno-botanical information reports [6]. Though it may not be possible to list all of them, some of the potential examples are *C.auriculata, E.jambolana, Salacia reticulata, A.marmelos, M.charantia, Azadirachta indica, G.sylvestre, Achyranthes aspera, Artemisia herba alba, Opuntia fuliginosa, O.sanctum, P.marsupium, Panax ginseng, Ficus hispida, C. roseus and Nelumbo nucifera* [7]. The biologically active compounds of many of the plant agents have been isolated. However, there are no reports on the effective phytotherapeutical agents for MSG diabetes**.** This study was carried out with the objective of screening few plants with established antidiabetic potential for their short and long term hypoglycemic potential in MSG diabetic condition.

MATERIALS AND METHODS

Animal care

The inbred strain of albino mice, *Mus musculus* was used for this study. They were obtained from Tamil university, India. Fifty two adult mice (60 days old) of either sex weighing about 30-33g were selected for the study. The animals were housed in colony cage at an ambient temperature of 25±2°C with 12hr light: 12hr dark cycle. The animals were fed *ad libitum* on commercially available pelleted diet (Sai Durga Feeds, Bangalore, India). The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by the Animal Ethical Committee was followed regarding injection and other treatment of experiment (Reg.No.437/01/c/CPCSEA).

Chemicals

MSG was obtained from local market in Trichy, India under the name Ajinomoto. Glucose oxidase kit was (manufactured by Coral Clinical Systems, Bldg. 'D', Plot No. M-46, Phase-III B, Verna Ind. Estate, Verna, Goa-403 722, India) purchased from Ponmani & co, India.

Induction of obesity and associated diabetes

To induce diabetes, MSG was administered intraperitonially at multiple (10 doses) dosage of 6.5mg/g of mice causing obesity [8]. This dosage was already fixed in our laboratory. MSG induced hyperglycemia (having fasting blood sugar level more than 190mg/dl) after 60 days of administration. These animals were used for the experiment.

Plant Material

Eleven plants used were *G.sylvestre, M.charantia, C.indica, E.jambolana, C.auriculata, A.squamosa, C.roseus, A.marmelos, O.sanctum, P.marsupium* and *S. platensis.* They were collected from forest area of Perambalur District, Tamilnadu, India. The species were identified and authenticated by the department of Botany, Holy Cross College and voucher specimen deposited.

Preparation of extracts

G.sylvestre

Dried powdered leaf extract of *G.sylvestre* at the dosage of 0.03mg/g body weight /animal in water was used [9].

M.charantia

About 10g of seedless fruit was blended with 7ml of 95% ethanol. It was left at room temperature with occasional shaking for 48 hrs and filtered.

C.indica

10g of leaves was soaked in 30ml of 60% ethanol for 4 days and it was filtered [10].

E.jambolana

Seeds were dried in incubator for 2 days at 40°C and were crushed in electric grinder. It was powdered with 100% methanol in 1:5 ratio and incubated at 37°C for 36 hrs. After incubation it was filtered [11].

C.auriculata

250g of flowers were subjected to continuous hot extraction with water at 60°C for 6 hr in 1:3 ratios.

A. squamosa

Dried leaf powder was cold macerated with distilled water and boiled till it was reduced to half of its original volume. It was cooled and filtered [12].

C.roseus

50g of fresh leaves (White variety) were crushed in stainless steel mortar and squeezed by means of a fine cloth to separate the juice.

A.marmelos

5g of dry leaf powder was extracted with 75% ethanol in 1:3 ratios [13].

O.sanctum

10g of dry seeds were crushed, cold macerated in 75% ethanol and filtered [14].

P.marsupium:

100g of the heartwood powder was boiled with 300ml of water for 20min. Filtrate was used [15].

S. platensis

Commercially available *S.platensis* powder from EID parry was used. 100mg was dissolved with 2.5ml of water.

Experimental procedure

The extracts were administered to study the short-term insulin-like effect in the MSG diabetic mice. The blood sugar was measured by glucose oxidase method for five consiquitive hours. The control was also measured side by side. From the results, the extracts with some impact were chosen and were administered daily to diabetic mice for 10 days. The fasting sugar levels were estimated on the 10^{th} day. The statistical analyses were carried out using the package, SPSS for windows [16].

RESULTS AND DISCUSSION

Serum glucose level

Serum glucose level was used as the vital parameter in screening. To study the short term effect of phytotherapeutic agents, serum glucose of control, diabetic and the extracts-treated groups were measured at an interval of 1hr from initial to 5th hr. In table I, the potential phytotherapeutic agents were identified by observing the results of diabetic treated group. *S.platensis* reduced the blood sugar to 118, 89, 79mg/dl respectively at III, IV, and V hours, which were on par with the control. *C.auriculata and P.marsupium* decreased glucose level to 160mg/dl and 165mg/dl respectively. Table 2 presents the fasting blood sugar on 0 and 10th day of the administration of the selected extracts which were subjected to Duncan's Post Hoc multiple comparison test. In all extract fed groups, a reduction in blood sugar was observed. In particular, *S.platensis* fed group exhibited reduced sugar level from 208mg/dl to 92.66mg/dl which formed homogenous subset with the control.

The results of this study have revealed the potential hypoglycemic agent for MSG diabetes. Of the tested plants, *G. sylvestre, M.charantia, C.indica, A.marmelos, O.sanctum, E.jambolana, C.auriculata, A.squamosa, C.roseus, P.marsupium and S.platensis*, only *S.platensis* showed the potentiality for short term glucose homeostasis. Many researchers have reported the antidiabetic potential of the above plants. Aqueous extract of *P.marsupium* which is rich in poly phenolic compounds like marsupin, pterosupin, and pterostilbene. It caused an increase in insulin release through stimulation in beta cell regeneration in STZ induced diabetic rats [17].

Treatment with *C.roseus* leaf extract in alloxan induces diabetes which enhances tissue response to glucose, increases synthesis and release of insulin from beta cells. The effect of leaf extract was mainly due to the active principle like catharanthin, leurosine, lochnerine, tetrahydroalstonin, vindoline and vindolinine [18]. Supplementation of the specific dose of LHII and FIIC which are isolated from seed of *E.jambolana* results in a significant correction of fasting blood glucose level with respect to STZ-induced diabetic group [19].

Pectin isolated from the fruit and the aqueous leaf extract of *C.indica* has got good hypoglycemic and hypolipidemic effect in experimental diabetes induced with Alloxan [20]. Aegelin, lupeol, eugenol and marmesenin isolated from *A.marmelos* to alloxan induced diabetes noted antidiabetic, antidislipidemic, anti-inflammatory, antioxidant and hepatoprotective activity. The long term administration of gymnemic acid from *G.sylvestre* to STZ induced diabetic animal increases hypoglycemic condition by preventing the destruction of beta cells. It also increases the activity of enzymes responsible for glucose uptake, utilization and inhibits peripheral utilization of glucose by somatotrophin and corticotrophin [21]. In diet induced hypercholesterolaemia, saponins, triterpenes and flavonoids of *O.sanctum* controls antidiabetic effect [22]. The alcoholic extract of *M.charantia* enriched with charantin, steroidal saponins and momordicin showed the ability to enhance cells uptake of glucose to promote insulin release and to potentiate the effect of insulin in STZ induced diabetic rats [23].

C.auriculata flowers possess antihyperlipidaemic effect in addition to antidiabetic activity in STZ diabetic rats. This was mainly due to the active compound phthalateDi (2-ethyl) hexylphthalate [24]. The *A.squamosa* aqueous extract supplementation is useful to control the blood glucose level, improves the plasma insulin, lipid metabolism and is beneficial in preventing diabetic complications from lipid peroxidation and antioxidant systems in STZ- induced diabetic rats [25].

Thus, the phyto compounds of the tested plants act in a protective manner preventing beta cells from destruction by nullifying the effect of a toxic exposure. This ability to offer protection to beta cells may be of particular benefit to newly diagnosed type 1 diabetics. In the condition of experimental diabetes induced by STZ and alloxan, the chosen plants showed insulin mimic action which may lower blood sugar. The long term administration of plant extracts

reverses the damage to the beta cells and actually repopulates the islets [26]. In MSG induced diabetes, the potential tested plants do not induce hypoglycemic activity. *S.platensis* is the only plant which possess antidiabetic activity in short term studies. However, administration for 10 days has resulted in a significant hypoglycemic effect. The impact is the most significant with *S.platensis* [27]. *S.platensis* contains beta-carotene, tocopherals and phycocyanin, has the ability to scavenge free radicals, decreases nitrite production, suppresses inducible nitric oxide synthase (iNOS) expression and inhibit liver microsomal lipid peroxidation [28]. This study proves the high potential of *S.platensis* in the management of MSG-induced diabetes.

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Table 1. Blood sugar level of MSG-diabetic mice at the first five hours after the administration of the chosen anti diabetic plants.

Groups	Fasting (mg/dl)	I hour (mg/dl)	II hour (mg/dl)	III hour (mg/dl)	IV hour (mg/dl)	V hour (mg/dl)
Control	90±.816	87±.816	89±.816	85±1.82	87±1.82	90±.816
MSG	212±1.70	215±1.41	209±2.16	211±2.16	213±2.58	212±1.63
G.sylvestre	205±1.63	198±2.62	185±5.71	176±6.48	177±4.32	189±.816
M.charantia	210±4.16	196±3.74	191±1.82	185±5.71	213±2.16	215±1.41
C.indica	209±2.94	184±2.70	178±1.82	175±1.82	165±1.82	175±1.41
E.jambolana	206±.816	264±1.82	195±2.16	184±.816	156±1.82	180±2.58
C.auriculata	200±1.82	213±1.82	183±1.29	167±1.41	167±1.41	160±1.82
A.squamosa	220±4.08	210±1.63	180±1.63	165±4.08	158±1.63	171±1.29
C.roseus	197±2.06	187±3.91	180±3.65	171±3.65	185±3.65	190±3.65
A.marmelos	230±4.08	195±1.82	170±1.82	140±1.82	162±1.82	179±2.58
O.sanctum	215±1.82	207±1.63	190±4.76	170±1.82	189±2.70	200±.81
P.marsupium	199±1.63	177±2.94	159±2.58	159±2.16	133±2.58	165±4.54
S.platensis	200±2.16*	164±1.82*	123±2.94*	118±3.36*	89±3.41**	79±1.82*

Significant at *P value < 0.05 compared with control, Insignificant at **P value > 0.05 compared with control

Table 2. Duncan's post hoc multiple comparison test to show homogenecity in blood sugar level among experimental groups of swiss albino mice *mus musculus* on 10 days duration.

Duration of days	Group of animals	Subset for alpha = 0.05		
		1	2	3
0	Control	89.6667		
	MSG		210.6667	
	C.auriculata			214.6667
	P.marsupium		208.3333	
	S.platensis		208.0000	
10	Control	88.6667		
	MSG			212.0000
	C.auriculata		119.3333	
	P.marsupium		122.6667	
	S.platensis	92.6667		

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RESEARCH ARTICLE

Post Tsunamic Ambient Gamma Survey of Pondicherry Coastal Ecosystem (East Coast of India).

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ABSTRACT

The scintillometric survey was made over a period twelve months from April-2010 to March 2011 in seven stations at interval of five Kilometer approximately. The ambient gamma radiation ranged from 3.03±0.49 μRh-1 to 5.04±0.39 μRh^{-1}with the mean value of 3.79± 0.70 μRh^{-1}. The survey made away from shore to the living area of the fishing community at varying distance up to 50 meter. Among seven stations four stations were registered elevated level of gamma at 50 meter. The dose transfer to the coastal population is 5.446nSvh^{-1}.The annual average effective dose was calculated as 0.047 mSv.

Keywords: Ambient gamma, Tsunami, radiation dose, Seasonal change

INTRODUCTION

Tsunami is series of huge waves caused by the displacement of large amount of water due to the earthquake at the bottom of the ocean due to the natural disasters. The recent Tsunami [1] affected many countries of Indian Ocean region, include India. In India east coast are worsely affected from Kanniyakumari (TamilNadu) to Andraprathesh. It causes the elevation of few physiological changes in ground water of the affected area. [2]. It also caused significant geomorphologic changes along the coastline, such as eroding sand beaches and enlarging water channels [3].The marine is a source of various elements including radioactive ores, whereas post Tsunamic profile of water, sediment and biota were analyzed by various researches, but no data were found with reference to radioactivity. Radiation is one of the major parameter as for as environments is concern. Unlike meteorologically induced waves Tsunamis are capable of affecting deep shelf and ocean floor and hence they transport sizable amount of sediment towards land and finally when they cross the shore sediment on coast, it also alters the geomorphic features considerably [4]. Hence the present surveys were made to understand the impact of Tsunami and add base line data for post Tsunamic activity. Such investigations can also be useful for assessment of public dose rates, the performance of epidemiological studies, and keeping reference-data records to ascertain possible changes in the environmental radioactivity duo to nuclear, industrial, and other human activities. The ambient gamma radiation level also varied in

time according to the local meteorological conditions which affected the concentration of radon daughters [5]Present study is an attempt to survey the ambient gamma radiation and changes, dose transfer, and annual average effective dose to the coastal populations of Tsunami affected areas the Pondicherry.

Study Area

Pondicherry is one of the Union Territories of India located at 162 Km south of Chennai, the capital of Talmilnadu and 22 kms north of Cuddalore, the capital of South Arcot District of Tamilnadu (Fig. 1). Pondicherry is surrounded by Bay of Bengal on East, and on the other sides is the South Arcot District of Tamilnadu. Pondicherry is located at 11°56'N latitude and 79°53'E longitude.

Among its diverse attractions is a coastline of 32 km, palm-fringed beaches, backwaters, fishing villages, beach resorts, the Sri Aurobindo Ashram, the international city of Auroville. Pondicherry coast is one of the significant ecosystems, hence 39 species of fishes, 15 species of decapod Crustaceans, 15 species of molluscs including 9 species of gastropods [6]. There are several fishing sites and an estuary; open sandy shores are located on the coast. Based on this sampling stations were identified

MATERIALS AND METHODS

Scintillometer

The ECIL Scintillometer, Type SM 141E is used to measure terrestrial gamma radiation levels. It is a rugged, light weight and portable scintillometer designed for radiometric, geophysical and environmental reconnaissance surveys. The Microcontroller based design employs accurate measurement of radiation levels and the large crystal volume employ reliable statistics. The radiation levels are displayed on the 2X16 LCD module having antiglare and backlight facilities, which facilitates better visibility under direct sunlight and even in low light condition. The use of the Scintillation detector renders the SM 141E highly sensitive as compared to the survey meters featuring GM detectors.

RESULTS AND DISCUSSION

The terrestrial gamma radiation levels in Pondicherry coast were measured and the results presented in Table 1. The gamma radiation level in Pondicherry coast ranged from 3.18±0.36 μR/hto 5.04 ± 0.39 . Among these seven stations Kalapet (S1) registered the high mean value 5.04 μR/h. Measurement and gamma radiation at varying distances from each sampling station are revealed a specific trend. At Kalapet (S1) gradually increased from 5.04μR/h to 57μR/h with increasing distance away from the shore. High value was registered (57 μR/h) at the 50m distance. Aurovile (S2), Aurovile registered the value ranged from 3.39 μR/h to 19.8 μR/h increasing distance from 5 – 50m away from the shore 19.8 μR/h registered the value at 50m. Veerampattinam (S3) shows increasing trend 3.03μR/h to 8.1μR/h. The value of S3 is comparatively low when compare to other stations (S1, S2, and S5). The minimum value registered at 5m and the high value registered at 30m distance whereas the 50m, value is 7.1μR/h. Aryankuppam (S5), also registered the increasing value ranged from 4.1μR/h to 9.4μR/h. The value 9.4μR/h registered at 50m, and we got 12.4 μR/h. whereas the S4, S6 and S7 is a residential area of the fishing community, Hence the trend shows increasing distance increase the value, especially at 50m (Fig 2). The mean value of terrestrial gamma radiation level is 23.27μR/h at 50m. The annual effective doses of the Pondicherry coastal ecosystem were calculated from equivalent dose rates multiplied to time and the occupancy factors of 0.2 for environment. The average annual equivalent dose of Pondicherry due to gammabackground radiation at 50m is: 23.27 × 0.2 = 5.446 nSvh-1 and annual equivalent dose is: 5.446 × 24 × 365 × 10-6 = 0.047 mSvy-1. To estimate annual effective dose, the conversion coefficient must be taken into account from the absorbed dose in air to the effective dose. Gammaradiation is less absorbed in children and infants resulting in a higher dose conversion coefficient (adults: 0.7, children: 0.8 and infants: 0.9). Then the annual average effective dose for adults would be: 0.047 × 0.7 = 0.049 mSvy-1 [7,8].

In the present study in Gamma radiation levels in Pondicherry coastal region are measured. The values indicate specific patterns. In Pondicherry coastal environment the gamma radiation level fluctuated from 3.1μR/h (Veerampattinam) to 5.1μR/h (Kalapet). The Kalapet registered the high value among four stations. The increasing distance increases the value, especially at 50m. At the 50m distance, of Kalapet registered the maximum value (57μR/h). The Veerampattinam registered the lower value (3.1 μR/h) at station while in the increasing distance, at 50m registered the higher value but it is comparatively low (7.1 μR/h) among four station. In general gamma radiation values registered in the present study are fall on the world range (28 – 120 μR/h [9] and Gulf of Mannar coastal region 10 -45μR/h. [10] But it is low in the radioactivity survey in coastal region and Kanniyakumari district, Tamilnadu, carried out by Raju and Singh [11] reported a high background level ranged from 200 – 1600μR/h . The gamma level of Pondicherry coast is less than that of Gulf of Mannar and Kerala coast (100 – 3000 μR/h) as reported by Pillai and Kamath [12]. The dose transfer to the coastal population is 5.446 nGyh-1 which is lower than the world wide mean value 44 nGyh-1.

CONCLUSION

Post Tsunamic change does not influence the gamma radiation of this ecosystem. The dose transfer to the population is lower than world mean value (44 $nGyh^{-1}$)

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Table 1: Monthly data of the ambient gamma of Pondicherry coastal ecosystem

Months	S1	S2	S3	S4	S5	S6	S7
January	4.7	3.1	2.5	3.4	2.8	3.7	4.2
February	4.9	3.6	3.4	3.9	3.2	3.5	4.0
March	5.6	3.9	4.2	4.2	3.4	3.2	5.3
April	5.7	3	2.4	4.5	3.0	4.3	5.4
May	5	3.3	2.9	4.9	3.5	4.7	4.2
June	4.9	3.5	3.1	3.9	2.7	3.6	4.1
July	4.4	3.7	3.2	5.2	4.1	3.8	3.7
August	5.4	2.9	2.8	5.4	3.3	3.1	3.6
September	5	3.8	2.9	4.1	3.1	3.4	3.7
October	5.4	3.7	3.1	4.2	3.0	3.6	3.8
November	4.8	3.2	2.5	3.4	3.1	3.0	3.9
December	4.7	3.0	3.4	3.9	3.0	3.3	4.2
Range	4.7 – 5.7	3.0 – 3.9	2.5 – 4.2	3.4 -5.4	2.8 -4.1	3.0 – 4.7	3.7 – 5.4
Mean ± SD	5.04±0.39	3.39±0.35	3.03±0.49	4.25±0.64	3.18±0.36	3.6±0.49	4.1±0.58

Table 2: Ambient gamma survey at different distance away from Shore

Distances	S1	S2	S3	S4	S5	S6	S7
shore	5.04	3.0	3.03	4.25	3.18	3.6	4.1
5	5.8	3.5	3.2	RA	4.0	RA	4.0
10	6.4	4.2	3.4	RA	3.7	RA	VG
20	6.5	4.7	4.9	RA	4.4	RA	VG
30	10.1	15.0	8.1	RA	6.4	RA	VG
50	57.0	19.8	7.1	RA	9.4	RA	VG

RA- Residential Area VG – Vegetative Growth

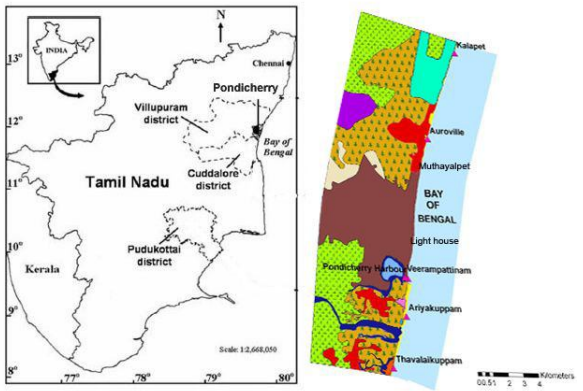

Fig.1.Showing the study Area and the Sampling stations (S1-S7)

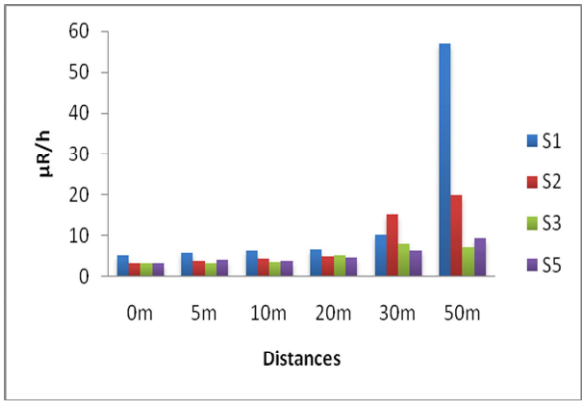

Fig.2. Ambient Gamma at different intervals away from shore

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RESEARCH ARTICLE

Efficacy of *Allium sativum* L.Extract as Insecticides against the House Fly (*Musca domestica* L.)

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ABSTRACT

Crude extract of *Allium sativum* have been screened for their larvicidal activities against *Musca domestica*. Three instars larvae of housefly were treated with the different concentrations by dipping method for 24 and 48 hrs. The LC_{50} values of the extract of *Allium sativum* were found to be 30.67, 34.67 and 38.33 ppm in 24 hrs and 23, 26.33 and 29.67 ppm in 48 hrs on 1st, 2nd and 3rd instars respectively. The data indicate that the *Allium sativum* fruit extracts can be applied as an optional point source control of house fly.

Keywords: *Allium sativum*, instars, *Musca domestica*, crude extract

INTRODUCTION

The housefly, *Musca domestica* L., is one of the most common insects, closely associated with human settlements, food and utensils. Flies feed and breed on decaying matter, human waste and food; and are therefore considered to be mechanical vectors of pathogens such as bacteria, protozoa and viruses. The housefly is categorized by the U.S. Food and Drug Administration as an important contributing factor in the dissemination of various infectious food-borne diseases such as cholera, shigellosis and salmonellosis [1].

There is a growing need for effective and biodegradable pest-control compounds. Nineteen new major pesticides were introduced from 1961 to 1970, eight from 1971 to 1980 and only three from 1981 to 1985 [2]. Due to increasing resistance of the vectors causing diseases of importance in public health, to chemical insecticides, is necessary the searching for alternative control methods, as the use of *A.sativum* extracts with insecticide activity, owing to its capacity of biodegradation and no environmental damage [3].

In this study, *Allium sativum* was used to study the effect of insecticidal activity against first, second and third instar larva of *Musca domestica*.

MATERIALS AND METHODS

Exctract of *Allium sativum* was prepared by grinding without adding water. Different concentrations 20, 40, 60, 80 and 100 ppm were made with water. Twenty number of 1st, 2nd and 3rd instar larvae of *M. domestica* were selected separately for each set of treatment. Five numbers of glass beakers of 250 ml capacity were taken and labeled for different concentrations in addition to one for control. Larvae were treated by dipping method as explained Begum et al. [4]. Each experiment was conducted in triplicates along with the control group. Mortality of larvae followed by the exposure was recorded after 24hrs up to 48hrs. LC_{50} was calculated using Karber's method [4].

RESULTS AND DISCUSSION

The results presented in (Fig no: 1 and 2) exhibit the toxicity of extract of *Allium sativum* against 1st, 2nd and 3rd instar larvae of *M. domestica* larvae, in 24 and 48hrs respectively. The treatment of three instars of *M. domestica* larvae with different concentrations of the extracts exhibited relatively lower percent mortality after shorter duration (24hrs) than that at longer duration (48hrs). The extract was found to be quite effective against *M. domestica* larvae as 100% mortality was observed at 100 ppm in all the three instars The LC_{50} of 1st instar was 30.67ppm at 24hrs short duration and 23ppm at 48hrs long duration (Tab no: 1). The 1st instar was sensitive than 2nd instar and the 2nd instar was sensitive compare than 3rd instar larvae. The longer duration exhibited high mortality percentage in lower concentration. In this study it was proved. The LC_{50} of 2nd instar was 34.67 ppm at short duration and 26.33ppm at long duration. The LC_{50} of 3rd instar was 38.33 ppm and 29.67 ppm at 24hrs and 48 hrs respectively. Campare to control all the stages shows significant mortality percentage against *Allium sativum* extract. Ande, [5] studied the biological activity in *Peganum harmala* seed extract and leaf extract of *Acalypha indica, Carica papaya, Santalum album* and *Calotropis gigantica* against the larval stages of the housefly *Musca domestica*. Successful or complete adult emergence rates were consistently lower with all plant based diets than with control experiment at leaf concentration.

Many plants have been reported about their potential insecticidal actions on larvae of house flies via crude extracts or extracted active compounds [6]. *Calotropis gigantica* gave the highest larval retention activity and it increased with leaf extract concentration, *Acalypha indica* and *Carica papaya* plants lost their larvae retention activity with increased leaf extract concentration [7]. At a higher dose, however, *P. harmala* showed larvicidal activity, thus suggesting that the plant material loses its desirable activities with increasing concentration. *Acalypha indica* showed similar but milder activities [5].

CONCLUSION

Research findings are generally in accordance with the present report and we can conclude that *Allium sativum* products may be used as population controlling agents for *Musca domestica* as they are cheaper and biodegradable, producing minimal pollution. Moreover, slow resistance develops in insects against *Allium sativum*.

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Table 1: LC_{50} (ppm) of larvae on *Allium sativum* extract at different concentrations

Instars	Duration Hrs.	LC_{50} (ppm)
I	24	30.67
II		34.67
III		38.33
I	48	23.00
II		26.33
III		29.67

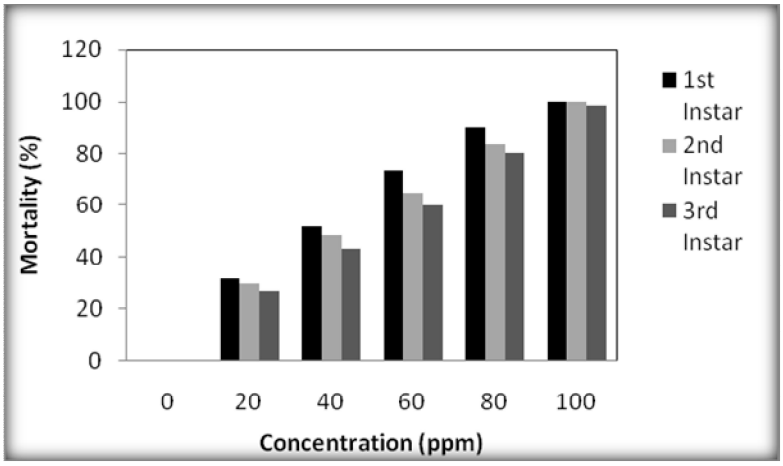

Fig no 1: Mortality of larvae on *Allium sativum* extract at different concentrations for 24hrs

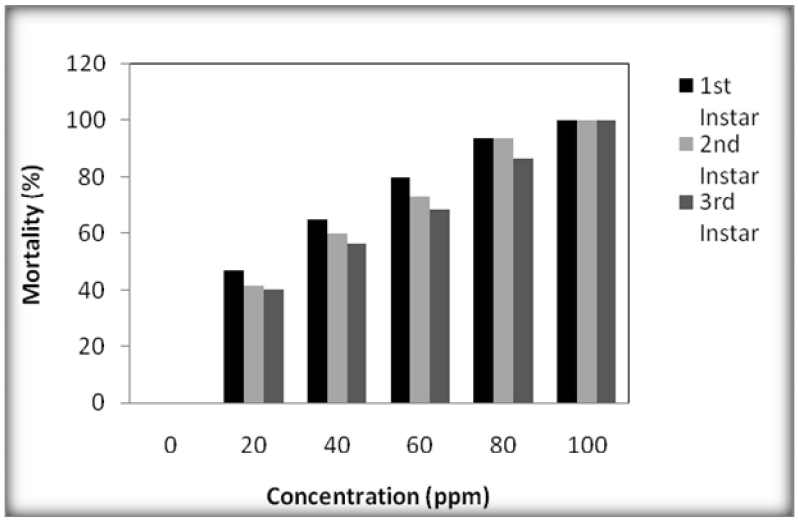

Fig no 2: Mortality of larvae on *Allium sativum* extract at different concentrations for 48hrs

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RESEARCH ARTICLE

Changes in Soil Chemical Properties Influenced by Reclamation and *Casuarina equisetifolia* L. Plantation in Sodic Soil of Karur District, TamilNadu, India.

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ABSTRACT

Continuous irrigation with poor quality agricultural drainage water resulted in alkalinization of soil at Moorthipalayam village of Karur District. Field experiment was carried out in a representative land for reclamation and planting suitable pulpwood tree species in 2007-08. The soil was sandy loam in texture and had high in pH (8.21) and EC (2.56 dS m^{-1}), low in organic carbon (0.28 %), nitrogen (131 kg ha^{-1}) and potassium (273 kg ha^{-1}) and medium in phosphorus (8.95 kg ha^{-1}) with an exchangeable sodium percentage (ESP) of 39.65. An integrated reclamation approach to the land was executed through chemical and phytoremediation methods and raising ameliorative tree species. The soil was first amended with gypsum @ 50 per cent of normal gypsum requirement and the field was leached out completely. Green manure crop viz., *Sesbania rostrata* was raised in the field and incorporated into the soil at flowering stage. *Casuarina equsetifolia* L. (Casuarinaceae) consisting 87 clones and 3 seedling materials produced from IFGTB were planted in the field on September 2008. Soil samples were taken at four stages viz. after gypsum application, green manure incorporation, one year after *Casuarina* planting and two years after *Casuarina* planting. The soil analytical results indicated that gypsum application reduced the ESP from 39.65 to 25.45. Further reduction in ESP and electrical conductivity (EC) of the soil was observed after the green manure incorporation besides increasing the organic carbon content. *Casuarina* plantation in the amended soil helped in increasing the soil organic carbon and available macronutrients.

Keywords: Sodic soil, reclamation, gypsum, green manure, *Casuarina* plantation and soil chemical properties.

INTRODUCTION

Alkalinizing of agricultural lands resulted from continuous irrigation of poor quality agricultural drainage water was found at Moorthipalayam village in Karur District of Tamil Nadu. Crop productivity started declining and the ground water table increased besides salt accumulation in the cultivated land. The high salt content and / or high pH cause changes in solubility, availability and efficiency of plant nutrients in the poor quality water irrigated soils. Further, in salt affected soils, the soil biology, including the population of microbes and enzymatic activities, which are known to play a greater role in nutrient availability and crop growth, are adversely affected largely due to the salinity on osmatic and ionic stress induced by the salts [5]. Sustainable crop production in the areas under long term irrigation with poor quality water depends on the scientific management of the soil. Generally two types of approaches for rehabilitating these areas were followed. The first one is improving soil properties through suitable chemical amendments or otherwise by growing efficient genotypes of crops/trees which could tolerate the existing saline/sodic stress conditions. The second is through bio remediation process using locally available organics for sustainable crop production. In any case the main objective is to reduce soil exchangeable sodium content to a level that permits conditions for plant growth. Taking all of this into consideration, the aim of the present study was to evaluate the positive changes in soil chemical properties in a reclaimed soil under *Casuarina* plantation at Karur region.

MATERIALS AND METHODS

Characterization of agricultural drainage water

The main consideration for classification of irrigation waters are EC, chloride and bicarbonate contents. The drainage water collected from Moorthipalayam village was analyzed for chemical composition. The chemical composition of agricultural drainage water is given in table 1.

Experimental area and soil characterization

A representative field with agricultural drainage water irrigation at Moorthipalayam village was selected for reclamation and planting of salt tolerant crop. Initial soil profile analysis was carried out in the field. Soil samples at the depths of 0-15 cm, 16-30 cm and 31-45 cm were taken and analyzed for their physical, chemical and ion exchange properties. Based on the results of profile analysis the soil was characterized. The soil profile analysis report is given in table 2. Gypsum requirement to neutralize the soil also estimated in the laboratory.

Amendments used in this study

Agricultural grade gypsum was procured from the Trivangore Fertilizers Limited and applied in the field. The green manure *Sesbania rostrata* was sown in the field and incorporated in the soil. The *Casuarina* leaf fall also considered as an amendment. The samples of gypsum, green manure at incorporation stage and *Casuarina* needles were analyzed for their chemical composition and presented in table 3.

Reclamation process

The land was ploughed deep with disc plough followed by criss-cross tyne ploughing to bring the soil into fine tilth. Just before the last ploughing, gypsum @ 50 percent of the normal gypsum requirement (3 t ha^{-1}) was applied during August 2008. The field was irrigated by flooding the water, puddled for through mixing of gypsum and soil than drained after 48 hours. After draining the irrigation water completely and allowed the soil to dry for 3 days. Then the seeds of the green manure crop viz., *Sesbania rostrata* were sown at the seed rate of 25 kg $ha^{-1}$and ploughed. After 45 days after sowing attaining i.e. at flowering stage, the green manure biomass was incorporated to the soil by using rotavator during October 2008.

***Casuarina* planting**

After gypsum application and green manure incorporation in the soil, the field was leveled once again and 1 cubic foot pits were made with a spacing of 3M × 1.5M. *Casuarina* planting materials consisting 87 clones screened from IFGTB and 3 seed materials were planting in the reclaimed land and necessary intercultural operations were carried out.

Soil sample collection and analysis

The soil samples (0-15 cm) collected at after gypsum application, 30 days after green manure incorporation into the soil, one year after *Casuarina* planting and two years after *Casuarina* planting were air-dried, powdered and passed through 2 mm sieve, labeled and stored in cloth bags for various analysis. The soil samples were analyzed for pH, EC, Organic carbon, available NPK, Exchangeable Sodium Percentage (ESP) and Cation Exchange Capacity (CEC) as per the standard procedures given by Jackson (1973)[7].

RESULTS AND DISCUSSION

All the amendments introduced in the soil positively influenced the soil properties at all the stages of soil sampling. Reduction in the soil pH, electrical conductivity and exchangeable sodium percentages was observed during reclamation and *Casuarina* planting. Except sodium, the concentrations of exchangeable cations were improved in the study site. The nutritional status as well as the cation exchange capacity of the soil was improved considerably with the addition of gypsum application followed by green manure incorporation and *Casuarina* plantation.

Soil pH

The pH of the initial surface soil was 8.21 as the irrigation waters used in these sites are rich in bicarbonate and chloride. Due to the application of gypsum, green manure incorporation and followed by *Casuarina* plantation, the pH reduced to a tune of 7.93. The favorable reduction in the soil pH in all the study locations could be attributed to the prolonged decomposition of added green manure as well as the leaf litter fall of *Casuarina* plantation. During the process of decomposition, a significant quantity of CO_2 is liberated and the decomposed product contains appreciable amounts of organic acids [1]. Similar findings were reported by Wen Qixiao[14] and Yu Tianren (1988) and Udayasoorian et al. (2009)[13]**.**

Soil Electrical Conductivity

Increased sodium content of the soil always result in dispersion of finer soil particles leads to surface hardening and low permeability. Under such condition, the leaching of excess salts in the soil is almost impossible. This condition often reflects in increased electric conductivity of the soil. In the present study also, the initial soil analysis indicated the high EC value of 2.56 dS m^{-1}due to the sodium rich condition. The application of gypsum and green manure incorporation in the soil followed plantation of *Casuarina* reduced the EC of the soil over a period of two years. After the reclamation process, the EC of the soils reduced to 2.18 dS m^{-1}. The reduction was due to the combined application of organic (green manure) and inorganic (gypsum) ameliorants, which caused the soluble salts to leach into deeper layers of soil profile. Gypsum application also helped in improving the soil physical structure resulted in enhanced leaching of the salts [6]. On the other hand, *Casuarina* plantation further reduced the salt content of the soil by registering 2.15 dS m^{-}1. *Casuarina* leaf litter fall improved the leaching of salts by way of production of organic acids, sequestration salts and thus reduced the soil EC [11].

Soil organic carbon

The organic carbon status of the soil is improved considerably after the reclamation process and *Casuarina* plantation. At the end of second year *Casuarina* plantation, the organic carbon content of the soil was registered as 0.42 percent as compared to initial content of 0.28 per cent. The reduced pH and EC of the soil helped for better proliferation of soil microbes and in turn enhanced the decomposition of added green manure and *Casuarina* leaf litter. The organics used in the study carrying significant carbon, might have hastened the microbial decomposition and subsequently build up of the organic carbon status in the soil [12].

Soil available nutrients

Nitrogen

The increased N availability might be due to the increased decomposition of applied organic amendments under favorable soil environment like reduction in salinity and sodicity and due to reduced volatilization, leaching and denitrifying losses [3]. Duhan *et al*. (2005) [4] suggested that the organic manure treated soils act as harbor of numerous microorganisms that are associated with fast decomposition and mineralization of organic materials in the soil and thus the availability of N greatly increased.

Phosphorus

Generally, the phosphorus content of the added inputs is the most important factor for phosphorus mineralization in the soil. The applied inorganic amendments constituted appreciable quantum of P. Under favorable soil environment conditions, the P solubilizing microbes multiply at a faster rate. The phosphorus solubilizing bacteria have the capacity to produce acetic, citric and lactic acids with which the insoluble phosphates get solubilized and the P availability becomes higher [10].

Potassium

The improved N availability observed in the present study with the various organic amendments might bring about more K into soil solution by the way of exchange reaction. The NH_4^+ ion having greater replacing power and preferential absorption characteristics over K^+ would have lead to constant release of exchangeable K from the soil lattice into the soil solution and contributed to the increased NH_4OAc- K content of the soil [15]. The differences among the bio-resources treatments in increasing the K availability could be ascribed to the variation in the amounts of total K added through bio-resources, their rate and amount of mineralization.

Exchangeable Sodium percentage (ESP)

The soil Exchangeable Sodium Percentage in this study was decreased with application of amendments and *Casuarina* plantation. Initially, the ESP was 39.65 and decreased with the progress of soil amelioration and *Casuarina* plantation. At the end of second year, the ESP was recorded as 22.18 with a reduction of 44 percent over the initial period. This reduction phenomenon suggests that the exchangeable cations (Ca, Mg and K) were increased in the soil due to the combined application of organic and inorganic amendments. The sparingly used soluble gypsum in the study contains 19.5 per cent Ca, 3.05 per cent magnesium and 15.29 per cent sulphur. Due to the less soluble nature of the gypsum in water, slow release of Ca. into the sites is the principle reason for the Ca increment in the soil. Apart from gypsum, the added organic amendments like green manure and natural addition of *Casuarina* litter in the soil also improved appreciable quantity of minerals. The incorporation of these organic materials influences the soil micro environment resulting reduction in pH, increase in pCO_2, decrease in redox potential and other physicochemical changes [1] and thereby increasing the availability of cationic ions in the soil. The component Ca and Mg of the organic amendments released during the chemical/ microbial decomposition made significant

contribution in increasing the exchangeable Ca and Mg. The mechanism of high adsorptive capacity of green manure and *Casuarina* litter have adsorbed the Ca and Mg that would otherwise be leached thus making them available in the soil [11]. The increased exchangeable potassium in the soil is due to the release of K ions from the primary minerals as formation of various organic and inorganic acids during the course of decomposition of added organic amendments. The Ca released from the gypsum replaced the Na ions in the soil exchangeable sites and the free Na ions in the soil solution were leached. Udayasoorian *et al*. (2009) [13] and Kauhsik *et al*. (2005) [8] were also reported similar finding of reducing sodium ions with the addition of organic amendments. The gypsum and organic amendments applied in the soil also contain remarkable quantity of sulphur. The organic amendments release CO_2 during the process of decomposition. The sulphur as well as CO_2 readily reacts with the water to form sulphuric and carbonic acids respectively and these acids solubilize the soil calcium carbonate to release calcium for exchange with sodium in the soil exchange complex. The incorporation of organic amendments resulted in the binding or chelation of the cation (Na^+) to the organo- colloids formed by the decomposition of biomass, thereby decreasing the ESP.

Cation Exchange Capacity

The CEC of the agriculture drainage water irrigated soils showed a marginal increase with the incorporation of various organic amendments. The beneficial effect of applied organic amendments in increasing the CEC of soil could be due to the continued decomposition of biomass and release of humic compounds, which are known for increasing cation exchange capacity.Similar results of increased CEC in the soil due to the application of various kinds of organic materials were earlier reported by Neelam Sharad and Verma (2001)[9] and Bullock III *et al*. (2002)[2].

CONCLUSION

The present study confirms the beneficial effects of inorganic and organic amelioration of sodic soil irrigated with poor quality agricultural drainage water. Raising plantation with salt tolerant *Casuarina equsetifolia* L.clones further improved the soil chemical properties. The decrease in soil pH, electrical conductivity and exchangeable sodium percentage and increase in the organic carbon and major nutrients were evidenced in the present investigation.

ACKNOWLEDGEMENTS

The authors are very much thankful to the management of Tamil Nadu Newsprint and Papers Limited for providing the facilities to carry out this soil investigation and Institute of Forest Genetics and Tree Breeding, Coimbatore for conducting research trial on saline tolerant *Casuarina equisetifolia* L.clones.

Table 1. Characteristics of agricultural drainage water

Parameters	Unit	Result value
pH	-	7.71
Electrical conductivity	dS m^{-1}	4.57
Total dissolved solids	mg L^{-1}	4700
Total suspended solids	mg L^{-1}	36.7
Biological oxygen demand	mg L^{-1}	6.8
Chemical oxygen demand	mg L^{-1}	118.0
Organic carbon	Per cent	0.22
NH_4- N	mg L^{-1}	23.8
Olsen-P	mg L^{-1}	0.98
NH_4OAc-K	mg L^{-1}	19.6
Calcium	mg L^{-1}	186.0
Magnesium	mg L^{-1}	50.0
Total hardness as $CaCO_3$	mg L^{-1}	670.0
Sodium	mg L^{-1}	580.0
Sodium Absorption Ratio (SAR)	mg L^{-1}	9.74
Chlorides (Cl^-)	mg L^{-1}	1739.0
HCO^-_3	mg L^{-1}	793.0

Table 2. Soil profile analysis report- Moorthipalayam

Sr.No.	Parameters	Unit	Soil depth (cm)		
			0-15	16-30	31-45
I	**Physical properties**				
	Clay (< 0.002mm)	per cent	15.90	16.50	17.10
	Silt (0.05-0.002mm)	per cent	5.70	6.10	5.90
	Sand (2-0.05mm)	per cent	78.40	77.40	77.00
	Textural class	-	sl	sl	sl
	Bulk density	Mg m^{-3}	1.57	1.56	1.53
II	**Chemical properties**				
	pH (1:2.5 water)	-	8.21	9.08	8.94
	EC (1:2.5 water)	dS m^{-1}	2.56	4.2	8.67
	CaCO3	%	-	-	-
	Organic carbon	per cent	0.28	0.22	0.18
	KMnO4 – N	kg ha^{-1}	131	117	115

	Olsen-P	kg ha^{-1}	8.95	8.8	8.75
	NH4OAc – K	kg ha^{-1}	273	268	254
III	**Ion exchange properties**				
	Exchangeable Ca	cmol (P^+) kg^{-1}	5.85	6.8	10.6
	Exchangeable Mg	cmol (P^+) kg^{-1}	1.95	5.05	6.76
	Exchangeable Na	cmol (P^+) kg^{-1}	5.73	9.13	8.74
	Exchangeable K	cmol (P^+) kg^{-1}	0.92	1.54	1.45
	CEC	cmol (P^+) kg^{-1}	15.47	23.18	27.91
	ESP	-	39.65	40.54	31.72
	Ex.Ca/mg	-	3.00	1.35	1.57

Table3. Chemical composition of amendments used in this study

Parameters	Unit	Amendments		
		Green manure	Casuarina needles	Gypsum
Total N	per cent	2.07	1.10	Trace
Total P	per cent	0.48	0.28	0.23
Total K	per cent	1.96	1.21	Trace
Calcium	per cent	0.90	0.82	19.50
Magnesium	per cent	0.71	0.52	3.05
Carbon	per cent	31.2	25.8	Trace
C:N ratio	-	15.07	23.45	-

Table 4. Effect of amendments and Casuarina plantation on the soil properties (mean value of three replications)

Reclamation stages	pH	EC (dS/m)	OC (%)	KMnO4-N (kg ha-1)	Olsen-P (kg ha-1)	NH4OAc-K (kg ha-1)	ESP (%)	CEC (cmol (P +) kg-1)
Initial	8.21	2.56	0.28	130.91	8.95	273.28	39.65	14.45
After gypsum application	8.20	2.31	0.29	131.00	8.87	274.00	25.45	14.54
After GM incorporation	8.07	2.18	0.35	143.00	9.67	296.00	21.27	14.81
1 yr after Casuarina pltn.	7.99	2.22	0.41	148.00	9.82	302.33	21.85	14.92
2 yr after Casuarina pltn.	7.93	2.15	0.42	146	9.42	300	22.18	14.97
SED	0.03	0.03	0.03	5.35	0.18	5.04	0.52	0.02
CD (p=05)	0.08	0.07	0.07	12.35	0.41	11.65	1.19	NS*

* not significant

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RESEARCH ARTICLE

Effect of *Spirulina platensis* in the Management of MSG Diabetes in Swiss Albino Mouse, *Mus musculus*.

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ABSTRACT

Antidiabetic and hypolipidaemic effect of *Spirulina platensis* was investigated in Swiss albino mouse, *Mus musculus*. Administration of *S.platensis* (20mg/animal/ day) for 60 days, in MSG induced obese diabetic mice significantly decreased Lee index, blood sugar and glycohemoglobin as compared to untreated MSG diabetic group. There was significant fall in triglycerides and cholesterol in *S.platensis* treated group. In addition, treatment with *S.platensis* to MSG diabetic mice decreased excessive hexokinase and Glucose-6-phosphatase in liver. Results of this study show that *S.platensis* has hypolipidaemic and antidiabetic effects on type II diabetes.

Key words: MSG diabetes, Blood glucose, Obesity, Hexokinase, *S.platensis*.

INTRODUCTION

Type 2 diabetes, which is alarmingly increasing worldwide, is caused by a combination of impaired insulin secretion from pancreatic beta cells and insulin resistance of the peripheral target tissues, especially muscle and liver. Numerous risk factors such as aging, diet, obesity, family history, physical inactivity and certain ethnicities are suggested that bring this physiological abnormality to an epidemic level[1]. Central obesity is the vital risk factor which is known to predispose individuals to insulin resistance. It is reported that approximately 80% of patients diagnosed with Type II diabetes are obese[2]. As a result vast amount of research information are getting accumulated in this area.

One of the obesity-inducing factors is reported to be the food flavoring agent, monosodium glutamate (MSG), which is used to stimulate glutamate receptors located in taste buds and enhances the taste of food[3]. MSG is now used

experimentally to induce obesity and type II diabetes[4]. It is noticed that glutamate is a neuro transmitter in the brain. It exists in the extra cellular fluid in small concentration about 8 to 12µl. When the concentration rises above this level, the neurons begin to fire abnormally. At higher concentration, the cells undergo a specialized process of delayed cell death known as excito-toxicity. A neonatal (or) young adult administration of MSG induces obesity in mice[5].

MSG has shown a number of changes related to the lack of control of the hypothalamo-pituitary axis. It has presented a raise in blood sugar, cholesterol, triglycerides, insulin (with increased resistance) and leptin levels[6]. Cyanobacteria are considered to be a rich source of novel metabolites of a great importance from a biotechnological and industrial point of view. It has become an attractive source of innovative classes of pharmacologically active compounds showing biological activities ranging from antibiotics, immunosuppressant, antiviral, anti-inflammatory to proteinase-inhibiting agents[7]. *S.platensis* is a microscopic and filamentous cyanobacterium that has a long history of use as a food for humans. *S.platensis* is a rich source of protein and vitamins, especially Vit B12, minerals, carotenoids and phycocyanins. Its safety as food has been established through toxicological study[8]. There is increasing scientific and clinical evidences for its role in controlling chronic diseases such as diabetes[9], arthritis[10], anemia[11] and cancer[12]. The *S.platensis* components which are responsible for those therapeutic properties are thought to be compounds with antioxidant abilities such as polyunsaturated fattyacids, phycocyanin and phenolics[13,14,15,16]. Of these compounds, gamma linolenic acid (C18: 3, GLA) and phycocyanin are those which have received most attention from researchers[17]. However, there are no reports on the effective phytotherapeutical agents for MSG diabetes. This study was carried out with the objective of studying the effect of *S.platensis* on the management of MSG diabetes.

MATERIALS AND METHODS

Animal care

The inbred strain of albino mice, *M.musculus* was used for this study. They were obtained from Tamil university, India. Thirty six adult mice (60 days old) of either sex weighing about 30-33g were selected for the study. The animals were housed in colony cage at an ambient temperature of 25±2°C with 12hrs light: 12hrs dark cycle. The animals were fed *ad libitum* on commercially available pelleted diet (Sai Durga Feeds, Bangalore, India). The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by the Animal Ethical Committee was followed regarding injection and other treatment of experiment (Reg.No.437/01/c/CPCSEA).

Chemicals

MSG was obtained from local market in Trichy under the name Ajinomoto. Triglyceride and cholesterol kit (manufactured by Biosystems S.A., Costa Brava 30, Barcelona, Spain) were purchased from Sri Anchana Diagnostics and Distributors, India. Glucose oxidase kit, Glyco Hemoglobin kit (manufactured by Coral Clinical Systems, Bldg. 'D', Plot No. M-46, Phase-III B, Verna Ind. Estate, Verna, Goa-403 722, India) and chemicals for enzymatic assay were purchased from Ponmani & co, Trichy.

Induction of obesity and associated diabetes

To induce obesity, MSG was administered intraperitonially in multiple (10 doses) dosage of 3.5mg/g of mice causing obesity[18]. MSG induced hyperglycemia (having fasting blood sugar level more than 190mg/dl) after 60 days of administration. These animals were used for the experiment.

Plant Material

Commercially available *S.platensis* powder from EID parry was used at the concentration of 20mg/animal/day.

Experimental procedure

Thirty six mice were divided into three groups as twelve mice in each group.
Group I: Control group has not received any treatment.
Group II: Diabetic group was injected with intraperitonial injection of MSG to induce diabetes.
Group III: Diabetic treated group administered with intraperitonial injection of MSG and were forcefully fed by Gavage method with *S.platensis* powder for 60 days.

In all the groups, Lee index, food consumption, blood sugar, total hemoglobin, glycohemoglobin, triglycerides and cholesterol were measured on 0 day and 60th day using standard procedures[19,20,21,22,23,24]. On 0 day six mice from each group were sacrificed. Liver was excised and rinsed in ice-cold saline to measure the activity of hexokinase and glucose-6-phosphatase[25]. On 60th day the mice from all the three groups were sacrificed to excise the liver for enzymatic assay. The statistical analyses were carried out using the package, SPSS for windows[26].

RESULTS

Table1 presents the data on the management of MSG diabetes with the use of *S.platensis* on the 0 and 60th days experiment. The young adult mice treated with MSG developed obesity, which was evident from the significantly increased Lee index (table1). They also developed obesity-dependant diabetes having significantly higher levels of blood sugar (P value .000 < 0.05), glycohemoglobin and lower levels of hemoglobin. There was significant increase in cholesterol and triglycerides in MSG obese mice. Administration of *S.platensis* to MSG obese mice for 60 days reduced Lee index from mean value of 367.03 to 345.95 controlling the obese condition. The Lee index of these animals was within the normal range of 320-350 on 60th day. Feeding of *S.platensis* restored the serum glucose, total hemoglobin and glycohemoglobin to almost control levels. The values of Triglycerides and cholesterol of those treated with *S.platensis* was lower than the values of MSG obese mice (P value 0.001, .000< 0.05). This showed that treatment with *S.platensis* significantly improved the lipid profile in MSG obese mice. The activities of carbohydrate enzymes such as hexokinase and glucose-6-phosphatase increased significantly in MSG obese control mice. Treatment with *S.platensis* in those mice treated with MSG decreased the hexokinase and the glucose-6-phosphatase activity (Table1).

DISCUSSION

This study was undertaken to assess the effect of *S.platensis* in the management of MSG Diabetes. The results of this study on biochemical and metabolic aspects confirmed that MSG induces obesity and obesity-dependant diabetes in young adult mice[27]. MSG is reported to cause lesion in the arcuate nucleus region of the hypothalamus, which functions in homeostasis of appetite and body weight. The abnormal response of the neurons to these signals leads to imbalance in food intake and obesity[28]. The feeding of *S.platensis* for 60 days has significantly reversed this trend. The result on Lee index indicates that MSG induced obese animals treated with *S.platensis* show a decrease in body weight gain.

The plasma glucose level was high in the MSG induced obese mice. Administration of *S.platensis* to MSG obese mice reverted the level of plasma glucose to normal. The antihyperglycemic potential of this alga is well established. The phytochemicals such as beta-carotene, tocopherals and phenolic acids (phycocyanin) which posses hypoglycemic property in *S.platensis* are known to posses antidiabetic properties[29]. Glycohemoglobin concentration is proportionately increased in MSG obese mice with ambient hyperglycemia. H_2O_2 mediated increased level of iron release from glycohemoglobin may be a source of oxidative stress and cellular injuries, which may be caused by the

Fenton reaction, where glycohemoglobin level is significantly elevated. The decrease in the level of glycohemoglobin in animals treated with *S.platensis* was observed which may be due to the decreased level of plasma glucose. Another reason might be that *S.platensis*, which is a rich source of iron, contributed to the elevated levels of Hb[30].
The concentration of lipids such as Total cholesterol (TC) and Triglycerides (TGL) are significantly higher in MSG diabetic mice than in the control group. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Triglycerides and total cholesterol levels significantly decrease in the animals that received *S.platensis.* It was reported that *S.platensis* supplementation to patients with type II diabetes[31] and hyperlipidaemic nephritic syndrome[32] improved the patients lipid profile through a reduction in the total cholesterol, LDL and Triglyceride levels. This preventive effect of *S.platensis* is also observed in the induction of fatty liver and on hepatic and serum lipid levels[33,34]. In a study about the hypocholesterolemic action of *S.platensis, S.platensis* derived phycocyanin protein influence the serum cholesterol concentration, suggesting a hypocholesterolemic activity of the microalga in animals[35].

MSG has great influence with carbohydrate metabolizing enzymes. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate[36]. It is reported that hexokinase is activated by citrate and other anions. The elevation of hexokinase during MSG treatment might also be similar to the above statement, in which decrease in isocitrate dehydrogenase that causes accumulation of citrate that might have influenced the hexokinase. The administration of *S.platensis* to MSG obese mice had modulated the role of hexokinase. This may be due to the presence of flavonoids and polyphenols. The poly phenolic compound phycocyanin in *S.platensis* reportedly plays an important role in the mechanism for regulating the activities of carbohydrate-hydrolyzing enzymes.

The gluconeogenic enzyme Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the biochemical reaction of both glycogenolysis and gluconeogenesis[37]. In MSG administered mice, the increased activities of Glucose-6-phosphatase from the liver may be due to the activation (or) increased synthesis of the enzymes contributing to the increased glucose production. Also increased Glucose-6-phosphatase activity in MSG obese rats provide hydrogen, which binds with $NADP^+$ in the form of NADPH and enhances the synthesis of fats from carbohydrates (lipogenesis)[38] and finally contributes to increased blood glucose. Administration of *S.platensis* significantly decreased the activities of gluconeogenic enzymes in MSG induced obese mice by acting like insulin which functions as a suppressor of gluconeogenic enzymes and it increases the state of lipolysis which leads to decreased concentration of glucose in blood. The administration of *S.platensis* tented to bring the parameters significantly towards levels in control. It is reported that *S.platensis* has antidiabetic, hypolipidaemic property[39]. In the present study, it is observed that oral administration of *S.platensis* has reversed the diabetic and hyperlipidemic effects.

CONCLUSION

In Conclusion, *S.platensis* intervention brought in favorable effect on blood lipids, anti-oxidant capacity and anti inflammatory response in patients with type II diabetes. Our results also suggest that *S.platensis* is a promising agent as a functional food for the management of MSG diabetes. Further studies with larger sample size and longer duration are required to ascertain the mechanism of *S.platensis* action on Lipid profiles, immune variables and anti-oxidant capacity.

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Table 1: Physiological and biochemical parameters of the Control, MSG-treated and the *S.platensis*-fed obese Swiss albino mouse, *M.musculus* on the 0 and 60th day of the experiment

[Values are mean ± S.D. from six observations each]

Parameters studied	0 Day			60 Day		
	NC	MSGO	SFO	NC	MSGO	SFO
LI	312.47±10.68	339.20±10.78	340.50±12.18	339.81±8.22	367.03±7.13	345.95±6.48
FC (g/day/animal)	4.86±0.26	6.50±0.08	6.05±0.13	6.26±0.22	7.66±0.5	6.92±0.12
SG (mg/dl)	89.75±3.87	110.25±4.27	112.75±4.03	89.01±3.54	166.33±3.10*	112.92±3.87
THb (%)	11.10±0.18	9.88±0.09	10.05±0.20	10.79±0.22	7.10±0.13	9.05±0.13
GhbA1 (%)	5.05±0.13	7.95±0.13	7.35±0.13	6.15±0.13	9.9±0.13	7.95±0.13
TGL (mg/dl)	95.25±3.86	106.00±1.63	107.25±2.63	114.31±6.03	223.00±3.27	164.15±6.94
CL (mg/dl)	89.75±4.11	98.50±6.14	95.50±2.08	104.84±4.15	195.64±1.10	150.43±1.71
HK	0.177±0.004	0.185±0.004	0.189±0.003	0.189±0.006	0.371±0.003*	0.290±0.005
G6P	0.149±0.006	0.159±0.002	0.157±0.007	0.155±0.004	0.276±0.009*	0.198±0.007

NC: Normal Control, MSGO: MSG Obese, SFO: *S.platensis* fed obese. LI: Lee Index, FC: Food Consumption, SG: Serum Glucose, THb: Total Hemoglobin, GHbA1: Glyco hemoglobin, TGL: Triglycerides, CL: Cholesterol, HK: Hexokinase, G6P: Glucose6phosphatase.
HK: µmoles of glucose phosphorylated /min/mg protein
G6P: µmoles of inorganic phosphorous liberated /min/mg protein
Significant at *P< 0.05 compared with *S.platensis* fed obese.

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RESEARCH ARTICLE

Study of Optimization of *Spirulina platensis* Biomass using Response Surface Methodology.

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ABSTRACT

The cyanobacterium *Spirulina platensis* has been used by human because of its nutritional and possibly medical effects. Our study evaluated the influence of salt concentrations in the medium on the production of biomass by this cyanobacterium. Response surface methodology (RSM) based on Box-Benkhan design was used to optimize four variables affecting the production of biomass. 1) Nitrate 2) Phosphate 3) Chloride and 4) Carbonate concentration. Using multiple regression analysis a quadratic polynomial equation was established for the production of biomass. A linear relationship was found between observed and predicted values (R^2= 0.9821). The optimum conditions by RSM were found to be: Nitrate=3, Phosphate=0.75, Chloride=2.00 and Carbonate=1.50. However, experimentally the optimum values (in the same order) Nitrate=2.060, Phosphate=0.878, Chloride=1.823 and Carbonate=1.13. A maximum biomass production of was observed under these conditions.

Key words: *Spirulina platensis,* Response surface methodology, cyanobacterium, Box-Benkhan design.

INTRODUCTION

Spirulina biomass has been consumed by man for many years [2] mainly as a dietary supplement, due to the benefits following its consumption, e.g., weight loss, physical fitness and well-being [6]. Studies related to the problem of hypercholesterolemia have revealed that *Spirulina* plays a key role in weight reduction, lowering the blood cholesterol levels and improving the lipid profile of patients [4]. These positive effects could be due to the fact that the micro organism has very high protein content (about 65% w/ w) and a particular balance of vitamins and

minerals, and other components such as x-3 and x-6 polyunsaturated fatty acid, provitamins and phenolics compounds, which can have an antioxidant effect on lipid peroxidation [3]. The presence of high concentrations of c-linolenic acid in *Spirulina*, which could possibly be increased by manipulation of culture conditions, makes it one of the richest sources of this fatty acid, which is very important in the human diet [1,9].

Spirulina species are cyanobacteria (family Oscillatoriaceae) with helical, Multicellular filaments, which may be 50–300 lm long and 10 lm in diameter [7]. These species are found in a wide variety of diverse environments, such as saline lakes, soil, marshes, brackish water, seawater, thermal springs and fresh water [5,6]. Due to the fact that this organism grows in highly selective conditions, mainly in high saline concentrations, it can be cultivated in open-air cultures and still remain relatively free from contamination by algae and protozoa [1]. Self-shading restricts light availability, severely limiting biomass production, and low cell densities prevent efficient harvesting of the cells. Together, these factors have restricted large-scale cultivation of microalgae to a small subset of genera that includes Spirulina and Dunaliella [11].

The factors affecting the production of Spirulina platensis have been the subject of extensive study and review. Several factors can influence the growth and composition of Spirulina platensis, such as pH, salinity, light intensity, temperature and the presence of bicarbonate ions [8]. The species that present commercial interest are *S. platensis* and *S.maxima*, which profusely populate certain alkaline lakes in Africa and Mexico. The main characteristics necessary to promote growth in natural waters are an alkaline pH and very high salt concentrations, particularly of sodium carbonates. Moreover, the use of natural sources of nutrients for the growth of microalgae decreases production costs.

Response surface methodology (RSM) was used to study the effect of the addition of nutrients on the biomass concentration of Spirulina platensis through a batch culture. Nitrate, phosphate, carbonate and chloride were selected as experimental factors. A Box-Benkhan and full-factorial design consisting of four factors and 29 runs were used. The experimental data were analyzed by the response surface regression procedure.

MATERIALS AND METHODS

Cyanobacterium strains

An original strain of *Spirulina platensis* was kindly provided by Spirulina Foundation®, Tumkur,India.

Medium

A modified Zarrouk's medium containing. $NaNO_3$=2.5g/l; $K2HPO_4$=0.5 g/l; $NaHCO_3$=10 g/l; NaCl=1 g/l; $MgSO_4$.$7H_2O$=0.2 g/l; $CaCl_2.2H_2O$=0.02 g/l; $FeSO_4.7H_2O$=0.01 g/l was used to grow the spirulina culture [10].

Culture conditions and Growth

The alga was cultured in Zarrouk liquid medium at pH 10 at room temperature (26-28^{o}C). 500 ml conical flasks containing 300 ml media were placed in an enclosed wooden illumination chamber which is fitted with 18 cool, white fluorescent tubelights (Philips, trulite). The light intensity in this chamber could be controlled by switching on required number of tubelights and measured using lux meter (Model-LX 101, Taiwan). Algal developments were postulated by changes in the concentrations of Nitrate, Phosphate, Chloride and Carbonate. Each batch culture was inoculated with an initial *spirulina* culture of 15%. Cultures were grown at a light intensity of 4 k lux and aerated continuously by an aquarium pump. The cultures were grown for a period of 10 days under a 12h light/12h dark

photoperiod. Cell growth rate was measured gravimetrically. After the stationary phase (10 days), the cells were harvested by centrifugation at 10,000 rpm for 10 min.

Design of Experiment

RSM was used to study the influence of four variables on the production of biomass of *spirulina platensis*: Concentration of 1) Nitrate, 2) Phosphate, 3) Chloride and 4) Carbonate. A four level four factor Box-Benkhan Design was used. A total of 29 experiments were conducted. The coded and uncoded experimental factors and their respective test ranges are given in table 1. These ranges were established based on the results obtained by preliminary experiments. The response surfaces and contour plots were generated using design expert software (Version 8, Stat Ease, and USA). The accuracy of the fitted model was tested by the coefficient of determination (R^2). The quadratic model was partially differentiated with respected to each variable, and the resulting linear equations were set to zero. The solution to these equations represented the optimum values of parameters.

RESULTS AND DISCUSSION

Optimization of Parameters by RSM

The experimental plan for four independent variables at four levels is shown in Table 2. It depicts the uncoded experimental parameters and their levels along with the observed responses.

All experiments were conducted and the results were analyzed by multiple regressions using design expert 8 software. The final estimative model for the percentage of production of biomass in terms of uncoded factors was as follows:

R1 = +0.96047+0.045539 *Nitrate-0.29574*Phosphate-0.3745*Chloride+0.083015*

Carbonate-0.10000*Nitrate*Phosphate+0.080000*Nitrate*Chloride+0.11000*Nitrate*Carbonate+0.53605*

Phosphate*Chloride+0.27654*Phosphate*Carbonat-0.021728*Chloride*Carbonate-1.34797E-004*$Nitrate^2$-

0.34611*Phosphate +0.023474*$Chloride^2$-0.081403* $Carbonate^2$ ------- (1)

Where R1 is the predicted response variable and Nitrate, Phosphate, Chloride and Carbonate are the actual values of independent variables. Table .3 describes the ANOVA results for the second order response surface model. The value of Fischer variance ratio was very high (F=89.92) with low probability ($p< 0.0001$) which demonstrates the high significance of the model. The significance of each term was determined by their respective p-value. The smaller the p-value, the more significant is the corresponding coefficient. It can be observed from Table. 3, all linear terms are statistically significant. In this case A, B, C, D, AC, AD, BC, BD are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 44.45 implies the Lack of Fit is significant. Standard deviation was found to be 0.031. A low value of coefficient of variation (CV=2.32%) indicated that model would accurately predict the relationships between the parameters

Figure 1 reveals that the predicted values of production of spirulina biomass by model (Equation 1) are in close tolerance with actual values (R^2=0.9890). In addition, the adjusted R^2 (0.9780) and predicted R^2 (0.9392) are also high. The normal probability residual plot (figure 2) is a straight line, confirming the normality of the data. Adequate

precision measures the signal-to-noise ratio. In the present work, it was found to be 34.365 which indicated an adequate signal.

Since the model was found to be quite satisfactory, it was employed to optimize the parameters. The optimum conditions for the production of Biomass (by RSM) were found to be; Nitrate=3, Phosphate=0.75, Chloride=2 and Carbonate=1.50. Experiments were conducted by slightly varying the parameters from predicted values of RSM. It was found that, experimentally, the optimum conditions were; Nitrate=2.060, Phosphate=0.878, Chloride=1.823 and Carbonate=1.13.

Analysis of Response surface Plots

Figures represent the response surface curves generated by the design expert software. Response surface plots are useful for understanding the interaction of two test variables and determining their optimum levels by holding other test variables constant.

Effect of Phosphate and Nitrate and their interactive effect on production of Biomass

The effect of Concentration of Nitrate and Phosphate in the production of Biomass is shown in figure 3 and 4. It can be observed that the total biomass production increases with the increase in concentration of Nitrate and Phosphate concentration upto 1.606.

Effect of Chloride and Nitrate and their interactive effect on production of Biomass

The effect of Concentration of Nitrate and Chloride in the production of Biomass is shown in figure 5 and 6. It can be observed that the total biomass production increases with the increase in concentration of Nitrate and chloride concentration upto 1.75. Shows the highest production of *spirulina platensis* biomass.

High biomass production by *Spirulina platensis* could be due to change in availability of nutrient ional value such as rich in protein content, polyunsaturated fatty acids, pigments, vitamins and phenolics. Besides, notable high algal biomass, this cyanobacterium could be used for the removal of unwanted materials from artificial waste water, which confirmed from biotechnological studies.

Effect of Carbonate and Nitrate and their interactive effect on production of Biomass

The effect of Concentration of Nitrate and Chloride in the production of Biomass is shown in figure 7 and 8. It can be observed that the total biomass production increases with the increase in concentration of Nitrate and chloride concentration upto 1.7

CONCLUSION

The optimum conditions for maximum production of biomass were found to be: Nitrate=2.060, Phosphate=0.878, Chloride=1.823 and Carbonate=1.13. Experiments conducted under these conditions showed a 1.76 g/l Production of Biomass. Analysis of response surface plot and contour plots indicated that Nitrate and Chloride exerted significant effect on the process as compared to remaining parameters.

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Table 1: Experimental Range and levels of variables

Factor	Concentrations	Minimum	Maximum
A	Nitrate	1	3
B	Phosphate	0.5	1
C	Chloride	1	2
D	Carbonate	1	2

Table 2: BBD Showing Real values along with Experimental Biomass Production

SI.No	Nitrate(g/l)	Phosphate(g/l)	Chloride(g/l)	Carbonate(g/l)	Biomass (g/l)	Response R1
1	2	0.75	2.00	1.00	1.35	1.33
2	2	1	1.50	1.00	1.18	1.20
3	2	1	2.00	1.50	1.56	1.56
4	2	0.75	1.00	1.00	1.06	1.10
5	1	0.75	1.50	1.00	1.03	1.01
6	2	0.75	1.50	1.50	1.35	1.35
7	1	0.50	1.50	1.50	1.02	0.99
8	2	0.75	1.50	1.50	1.35	1.35
9	3	0.75	1.50	2.00	1.75	1.74
10	2	0.75	1.00	2.00	1.35	1.34
11	2	0.50	2.00	1.50	1.3	1.33
12	2	0.75	2.00	2.00	1.6	1.56
13	1	0.75	1.50	2.00	1.1	1.13
14	1	1.00	1.50	1.50	1.17	1.15
15	3	0.75	1.00	1.50	1.45	1.46
16	2	0.75	1.50	1.50	1.36	1.35
17	2	1.00	1.50	2.00	1.5	1.51
18	3	1.00	1.50	1.50	1.6	1.61
19	2	1.00	1.00	1.50	1.25	1.20
20	1	0.75	2.00	1.50	1.17	1.17
21	2	0.75	1.50	1.50	1.36	1.35
22	3	0.75	1.50	1.00	1.46	1.41
23	2	0.50	1.50	1.00	1.16	1.17
24	3	0.75	2.00	1.50	1.76	1.76
25	2	0.75	1.50	1.50	1.35	1.35
26	3	0.50	1.50	1.50	1.55	1.55
27	2	0.50	1.00	1.50	1.15	1.13
28	2	0.50	1.50	2.00	1.36	1.34
29	1	0.75	1.00	1.50	1.02	1.03

Table: 3 Analysis of variance (ANOVA) for Response surface Quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prod>F
Model	1.21	14	0.086	89.92	<0.0001 *significant*
A-Nitrate	0.78	1	0.78	811.98	<0.0001
B-Phosphate	0.030	1	0.030	30.93	<0.0001
C-Chloride	0.15	1	0.15	150.94	<0.0001
D-Chloride	0.17	1	0.17	175.35	<0.0001
AB	2.500E-003	1	2.500E-003	2.60	0.1291
AC	6.400E-003	1	6.400E-003	6.66	0.0218
AD	0.012	1	0.012	12.59	0.0032
BC	0.016	1	0.016	16.24	0.0012
BD	5.224E-003	1	5.224E-003	5.44	0.0352
CD	1.290E-004	1	1.290E-004	0.13	0.7196
A^2	1.159E-007	1	1.159E-007	1.206E-004	0.9914
B^2	3.033E-003	1	3.033E-003	3.16	0.0973
C^2	2.233E-004	1	2.233E-004	0.23	0.6373
D^2	2.635E-003	1	2.635E-003	2.74	0.1200
Residual	0.013	14	9.610E-004		
Lack of Fit	0.013	10	1.333E-003	44.45	0.0012 *significant*
Pure Error	1.200E-004	4	3.000E-005		
Cor Total	1.22	28			

Note: Mean= 1.33; CV=2.32%; Std.Dev=0.031; R^2=0.9890; Adj.R^2=0.9780; Predicted R^2=0.9392; Adequate Precision=34.365.

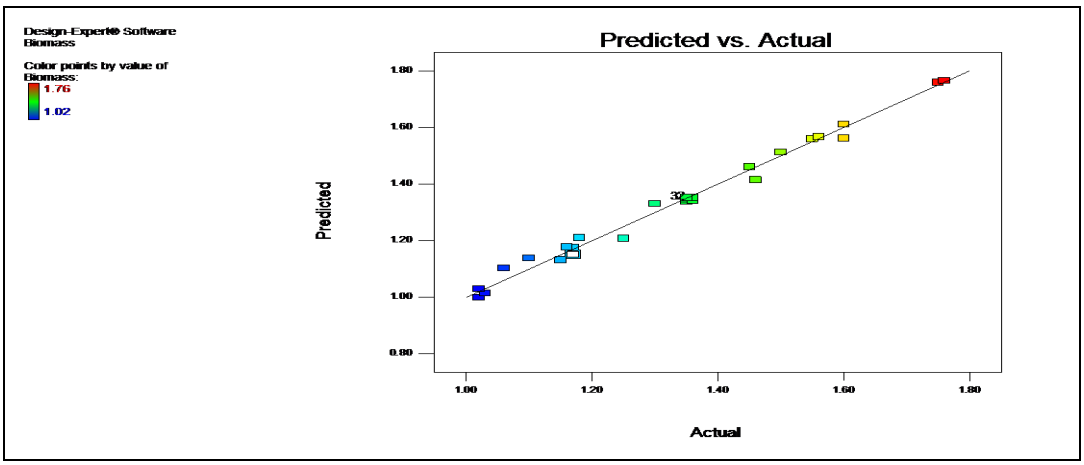

Figure 1. Experimental versus of Predicted Biomass Production

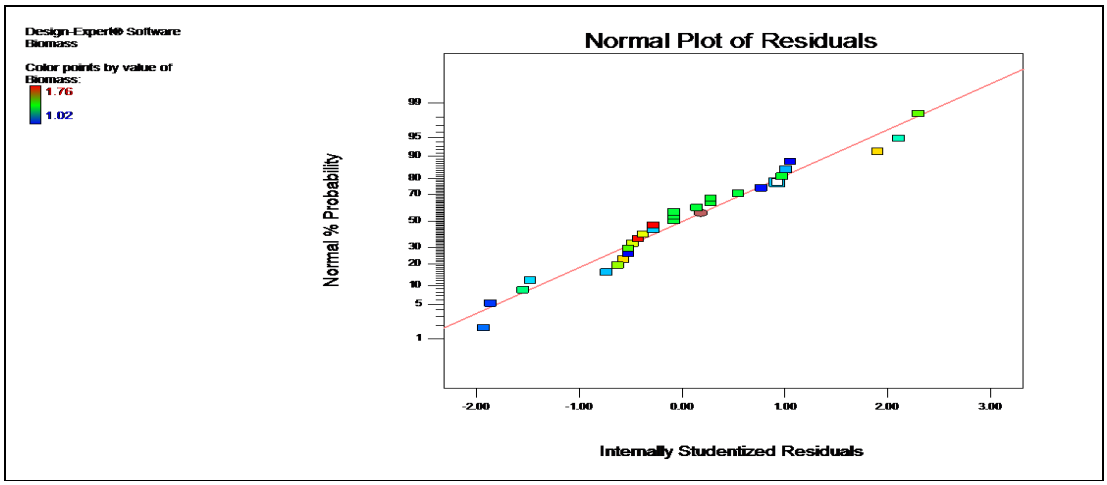

Figure 2: Normal Probability Plot of Residuals

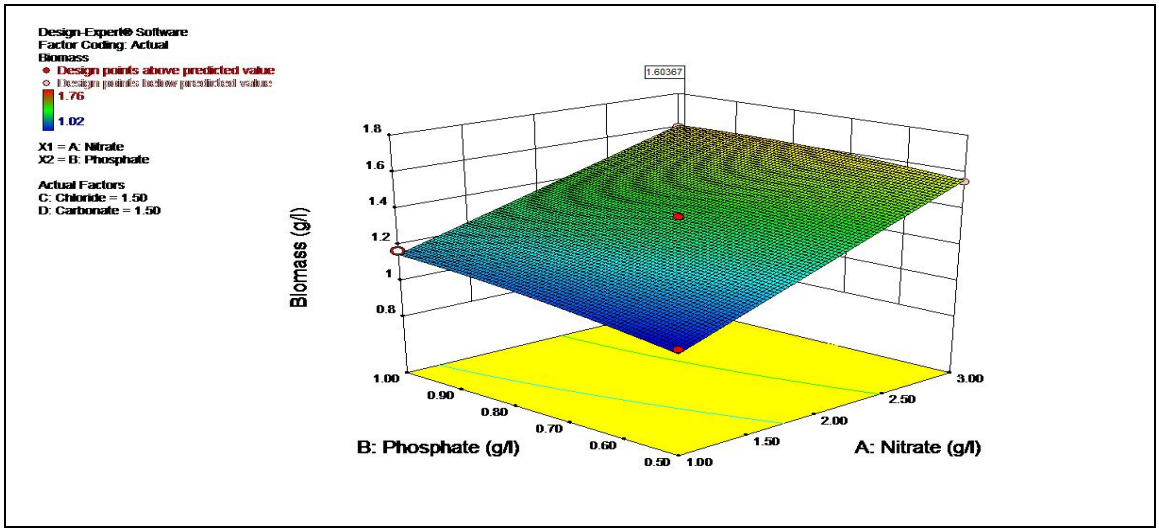

Figure 3: Response surface plot showing the effect of Phosphate and Nitrate and their interactive effect on production of Biomass

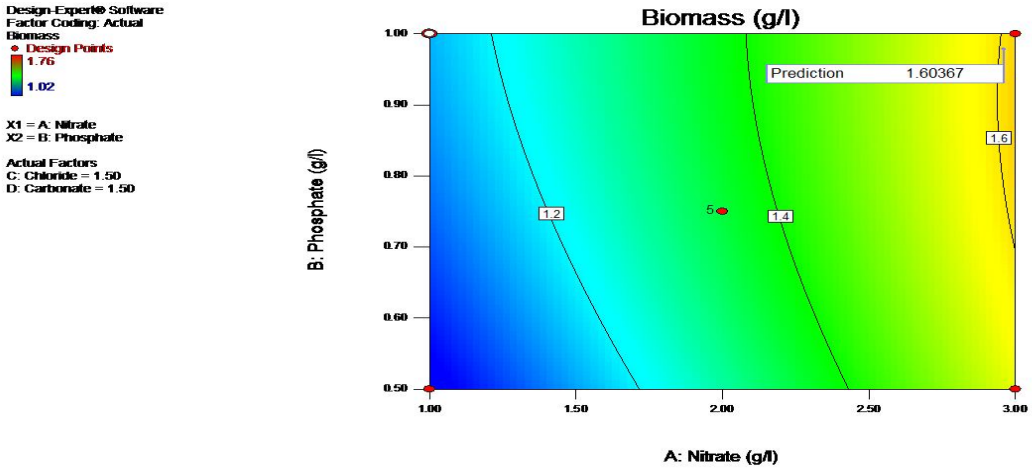

Figure 4: Contour plot showing the effect of Phosphate and Nitrate and their interactive effect on production of Biomass

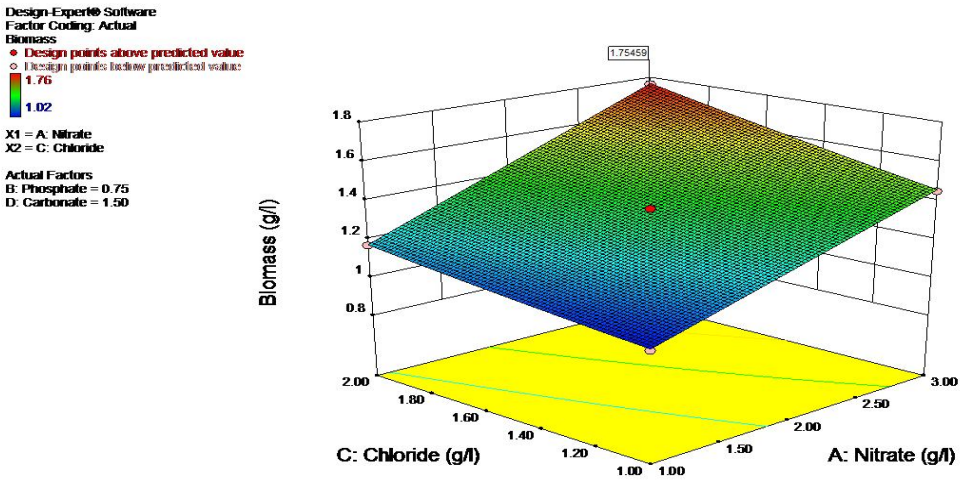

Figure 5: Response surface plot showing the effect of Chloride and Nitrate and their interactive effect on production of Biomass

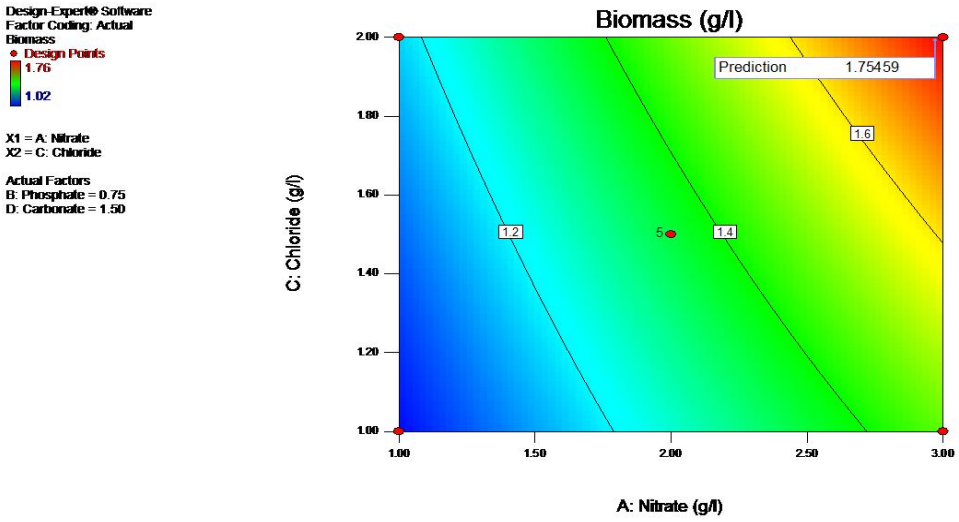

Figure 6: Contour plot showing the effect of Chloride and Nitrate and their interactive effect on production of Biomass

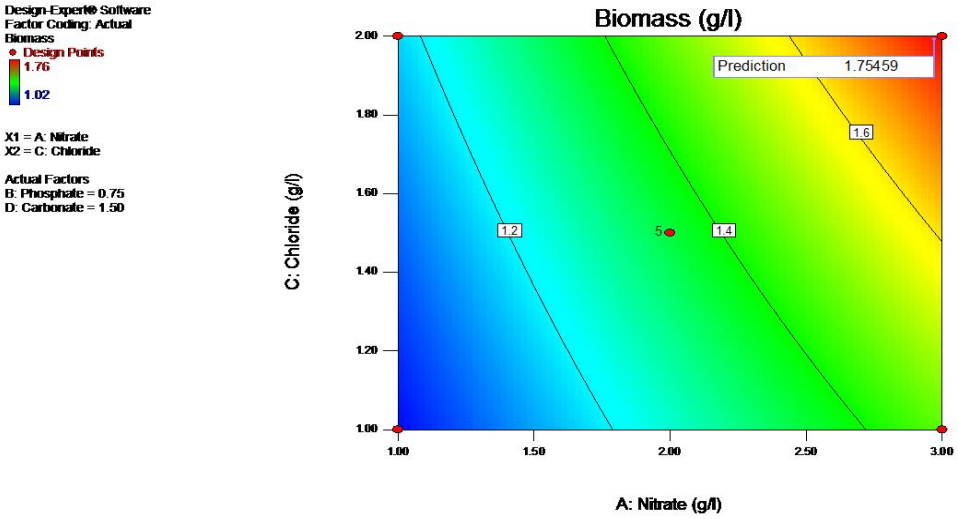

Figure 7: Response surface plot showing the effect of Carbonate and Nitrate and their interactive effect on production of Biomass.

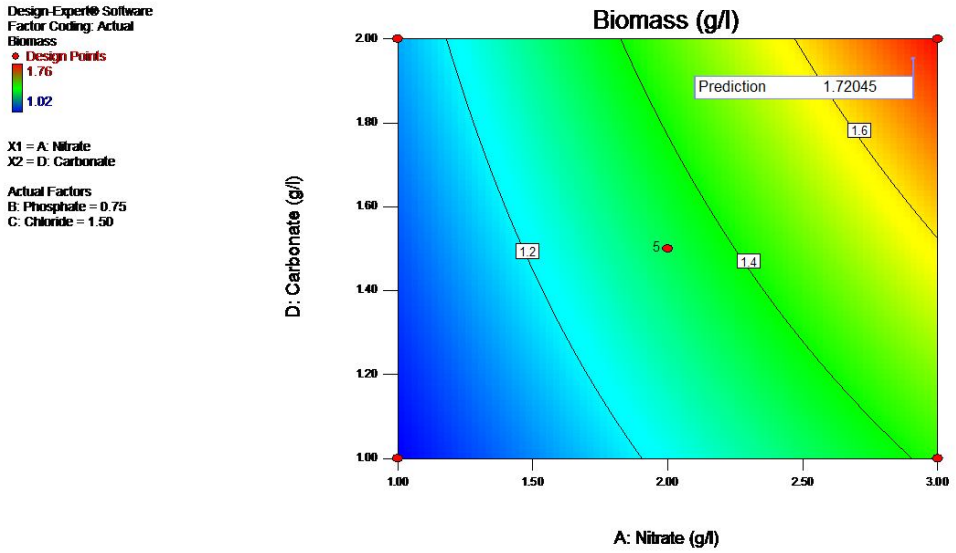

Figure 8: Contour plot showing the effect of Carbonate and Nitrate and their interactive effect on production of Biomass.

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RESEARCH ARTICLE

Study on the Quality of Vermicomposting by *Lampito mauritii* from Pondicherry and *Eisenia foetida* from Cochin and Ooty,India.

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ABSTRACT

Earthworms recycle millions of tons of organic wastes; almost any organic wastes can be converted into compost within a minimum time span. The present study aims at determining the quality of composting using *Lampito mauritii* from Pondicherry and *Eisenia foetida* from Cochin and Ooty. The worms from all three locations had excellent potentials on a comparative basis for vermicomposting.

Key words: Vermicomposting, *Lampito mauritii, Eisenia foetida*

INTRODUCTION

Earthworms represent a key component in the biological strategies of nutrient cycling in soil and the structure of their communities gives a clear indication of the type soil system they inhabit. Earthworms recycle millions of tons of organic wastes, such as food waste, agricultural waste, city garbage; kitchen waste etc.The present study aims at determining the quality of vermicomposting by *Lampito mauritii* from Pondicherry and *Eisenia foetida* from Cochin and Ooty, India.

MATERIALS AND METHODS

Collection of earthworms

The earthworms *Lampito mauritii were* collected from the East Coast Region Pondicherry and *Eisenia foetida* was collected from West Coastal region that is Cochin and a hilly region Ooty. The worms were collected by hand sorting using spade. Around 50 earthworms were collected from each location in specific from agricultural areas.

Substrate

Cowdung is one of best food for earthworms and the same was used as substrate for the worms, mixed with agricultural soil in the ratio 1:1.

Predigestion of substrate

Raw wastes or fresh wastes are not suitable food for worms. Hence predigestion of the raw substrates is essential for the process of composting. Four tanks with 3 for the three locations mentioned in collection of earthworms section and one tank with substrate and no worms released into it were used as control. 1kg of cow dung mixed with 1 Kg ofAgricultural Soil in the ratio 1:1 was taken in a cement tank of 60cm diameter and 70cm depth. The mixture was mixed regularly sprinkled with water to maintain moisture. The sample was digested in 30days. The predigested 30days old decomposed substrate serves best food for earthworms.

Preparation of Vermibed

Cement tanks (60×70cm) were chosen for the study. The bed was layered with stones for 3cm to let water flow down; overlaid with 200gm of husk to prevent escape of worms followed by 3cm of sand to serve similar purpose and finally covered with the predigested substrate and 1kg of soil mixture. 50 of the worms collected from each locality were released into each tank separately, labeled and the time noted as day 0. About 250 to 500ml of water was sprinkled on the surface daily, to maintain moisture which is essential for worm growth.

Vermicompost recovery

At 45th day after introduction of worms the compost was ready for use. This was evident by its physical appearance as judged by the development of the dark brown colored loose granular mass with uniformly disintegrated structure, watering was stopped, after one or two days the compost was removed from the cement tank together with the worms, heaped on a plastic sheet and kept in the shade. The compost was removed from the top leaving the earthworms in the form of a bundle at the bottom. The compost was then sieved, dried in shade and packaged.

Chemical characteristics of the Vermicompost

The fresh castings ejected by the earthworms were collected from each of the vermibed containers and separately packed in air tight plastic bags and tested in the laboratory for Physical parameters – pH, macronutrients- N,P,K, and C.

RESULTS

The present study is an attempt to evaluate the potency of two worms from three localities for their composting abilities.The results obtained are depicted in table 1. Composting was completed by day 45 for *Lampito mauritii* - Pondicherry tank and by day 40 for *Eisenia foetida* Cochin and Ooty. Control tank was also taken for the analysis on day 45. Worms from all three localities composed the substrates at an alkaline pH of 8.5 while that of control was recorded to be 6 a slightly acidic range.

Nitrogen levels was maximum in Cochin318.5mg/g compost and minimum in Ooty compost 298.9mg/g Pondicherry compost had a value of 309.5mg/g, where as the control had a value of 407.9 mg/g respectively and the levels of Magnesium was maximum 988.6mg/g in Pondicherry compost followed by Cochin with 518.6 mg/g and minimum in ooty compost 243.5mg/g while that of control was found to be 306.8 mg/g. Pottassim levels were minimum in Ooty compost and maximum in Pondicherry followed by Cochin compost with values of 146.5mg/g, 212.6mg/g amd 211.6 mg/g respectively and 268.7 mg/g in control. Phosphorus levels were maximum in Cochin 0.331mg/g and minimum in Ooty 0.209 mg/g and moderate in Pondicherry 0.239 mg/g while that in control was and 0.188mg/g.

Organic Carbon levels were found to be maximum in compost by Ooty (0.0345g/kg) worms and minimum by compost from Cochin (0.0165g/kg). The Pondicherry worms showed a mid range between the two (0.027g/kg), in control the amount of Organic Carbon recorded was .028g/kg.Organic matter levels were found to be maximum in compost by Ooty (0.079g/kg) worms and minimum by compost from Cochin (0.038g/kg). The Pondicherry worms showed a mid range between the two (0.0624g/kg), in control the amount of Organic Carbon recorded was .038g/kg.

DISCUSSION

Exotic worms take 40 days for composting while native varieties take 50 days for the process. Increasing substitutions of substrates in composting causes an increase in pH and macronutrients [1]. From earlier studies it is evident that vermicomposting provides all the nutrients in readily available form. Sreenivas et. al.,(2000) [17]studied that available N, P K and Mg uptake increased on application of vermicompost. Vermicomposting reduces the C:N ratio and retains more N than the traditional methods of preparing compost [3,4].

The soil pH is important because it affects the availability of nutrients in the soil. Many plant nutrients are not readily available to plants in highly alkaline or acidic soils. These essential nutrients are most available to most plants at a pH between 6 and 7.5. pH for compost from filter mud using polycultures showed a range from 6.06-6.7 and that of monoculture from 6.16-6.06 initially[10]. Goraknath and Keshavsingh,(2009)[6], also reported that composting of different combinations of animal, agro and kitchen wastes caused a change in pH from alkaline to an acidic or neutral range 7.6 - 6.5. The shift in pH from alkaline to acidic range is attributed to the bioconversion of organic materials into organic acids[14]. In the present experiment the pH was found to be slightly alkaline for all studied ranging between 7.17 – 7.34. A similar result with alkaline pH was obtained by Karthikeyan, et.al, 2007[8], wherein pH of 8.33; 8.49 and 8.3 when combinations of market waste and cowdung waste in 1: 0.5, 1:1and1:1.5 ratios respectively were used. Mainoo,NO, *et.al*,2009[12], showed that fresh pineapple waste had a pH of 4.4 but after 24 weeks the pH increased to about 7.2- 9.2, *ie*, an alkaline range, pineapple composting in the present study had a pH of 7.34 thus supporting our results. Chemical analysis of vermicomposting from dry leaves of fruit trees showed a pH ranging between 6.89 & 8.60[16].

Increased content of Nitrogen may be attributed to the release of nitrogenous products of earthworm metabolism through the casts (excreta), urine as well as mucoproteins [2,20]. Mainoo,NO, *et.al*,2009[12], showed that fresh pineapple waste contained as much as 0.4% total Nitrogen, The decomposition efficiency of *Perionyx sansibaricus* on a variety of wastes such as agriculture waste, farmyard manure and urban solid waste was studied by Suthar,S,

2006[18] . Composting resulted in significant increase in total N (80.8-142.3%), P (33.31-114.6%) and K(26.32-125.2%), whereas decrease in organic carbon(14.0-37.0%) as well as C:N ratio(52.4:69.8%). The total Nitrogen content was found to be 1.08% - 1.55% minimal for pineapple and maximal for corn bract. *Leucaena leuccophela* Lamk, leaves were composted in two months and contained N, 0.9-1.9%; P, 0.5 – 1.2% and K, 0.9 – 2.5% while that of *Bombax ceiba* yielded 1.2%; 0.6% and 1.8% of N, P and K respectively[16]. High Nitrogen content were obtained in combinations of market waste and cowdung waste (1:0.5, 1:1 and 1:1.5), were about 1.78%; 1.88% and 1.82% respectively[8]. The enhanced microflora present in the gut of earthworms, during composting process plays an important role in the increase in Potassium and other nutrient [9]. Hemalatha and Meena,(2006)[7], decomposed Municipal solid waste, vegetable waste, raw dairy distillery effluents and showed that combination with dairy effluent gave high levels of macronutrients, especially, Potassium. Suthar (2009)[19]decomposed yard manure and urban solid waste, and reported an increase in total potassium from 26.3 to 125.2% on using *Perionyx sansibaricus* .Vermicomposting and garden compost resulted in 0.15-0.73% and 0.48% levels of Potassium, respectively[13].Mainoo,NO, *et.al*,2009, [12]showed that fresh pineapple waste contained as much as 0.9% total potassium. In the present experiment the total potassium was found to be highest for pineapple about 1.75% an least in corn bract 0.82%. A low Potassium content was obtained in combinations of market waste and cowdung waste (1:0.5, 1:1 and 1:1.5), which was about 1.56%; 1.62% and 1.52% respectively against an initial level of 1.85%[11,15.]. Hence as a concluding remark vermitechnology can be viewed as an alternate resource technology and as a means to save the environment. Let us take one step towards organic farming and save mother earth from pollution hazards.

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Tab.1: Vermicompost analysis

S. No	**Name of the Test**	***Eisenia foetida* (Cochin)**	***Lampito mauritii* (Pondicherry)**	***Eisenia foetida* (Ooty)**	**Control**
1	pH	8.5	8.5	8.5	6.0
2	Nitrogen mg/ g	318.5	309.5	298.9	407.9
3	Phosphorous mg/g	0.331	0.239	0.209	0.188
4	Potassium mg/g	211.6	212.6	146.5	268.7
5	Organic Carbon g/kg	0.0165	0.027	0.0345	0.028
6	Organic Matter g/kg	0.038	0.0624	0.079	0.038

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Manuscripts should be concisely written and conform to the following general requirements: Manuscripts should be typewritten in double-space in A4 sized sheets, only on one side, with a 2 cm margin on both sides. Research Papers should have more than 15 pages, Review Articles in the range of 15-30 pages and Short Communications up to 15 pages, inclusive of illustrations. Pages should be numbered consecutively, starting with the title page and the matter arranged in the following order: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Acknowledgements, References, Illustrations (Tables and figures including chemistry schemes along with titles and legends) and figure and Table titles and legends. Abstract should start on a separate page and each table or figure should be on separate sheets. The titles "Abstract" and "Introduction" need not be mentioned. All other section titles should be in capital letters while subtitles in each section shall be in bold face lower case followed by a colon.

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Abstract - Should start on a new page after the title page and should be typed in single-space to distinguish it from the Introduction. Abstracts should briefly reflect all aspects of the study, as most databases list mainly abstracts. Short Communications as well as Review Articles should have an Abstract.

Key-words - Provide four to ten appropriate key words after abstract.

Introduction - Shall start immediately after the Abstract, as the next paragraph, but should be typed in double-space. The Introduction should lead the reader to the importance of the study; tie-up published literature with the aims of the study and clearly states the rationale behind the investigation.

Materials and Methods - Shall start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned. The main methods used shall be briefly described, citing references. Trivial details may be avoided. New methods or substantially modified methods may be described in sufficient detail. The statistical method and the level of significance chosen shall be clearly stated.

Results - All findings presented in tabular or graphical form shall be described in this section. The data should be statistically analyzed and the level of significance stated. Data that is not statistically significant need only to be mentioned in the text - no illustration is necessary. All Tables and figures must have a title or caption and a legend to make them self-explanatory. Results section shall start after materials and methods section on the same page.

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Discussion - This section should follow results, deal with the interpretation of results, convey how they help increase current understanding of the problem and should be logical. Unsupported hypothesis should be avoided. The Discussion should state the possibilities the results uncover, that need to be further explored. There is no need to include another title such as "Conclusions" at the end of Discussion. Results and discussion of results can also be combined under one section, Results and Discussion.

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Articles in Journals

1. Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.
   List the first six contributors followed by *et al*.
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1, Suppl 2):89-97.

Books and other Monographs

4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.
6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

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