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RESEARCH ARTICLE

Comparative Analysis of Laccase Immobilization on Magnetic Iron Nanoparticles using Two Activating Agents: EDAC and Cyanuric Chloride

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ABSTRACT

Surface modification improves the covalent bonding of enzymes onto the magnetic nanoparticles. The present study aims to evaluate the effect of surface activators (EDAC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and Cyanuric chloride) in immobilization of enzymes. Nanoparticles prepared by co-precipitation method ranged insize from 15-20 nm. The nanoparticles possessed crystalline property as confirmed by the XRD (X-ray powder diffraction) peaks. SEM-EDS (Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy) analysis of EDAC and cyanuric acid activated nanoparticles showed atomic sulphur percent of 0.08% and 0.02%, respectively. It is concluded that EDACwas more successful in loading more enzymes than cyanuric acid. Bradford estimation of the unbound protein after first wash for ENP-EDAC and ENP-CC was 29.1 µg/mL and 132.1 µg/mL, respectively. EDAC is a potential surface modifier for enzyme immobilization process.

Keywords: Immobilization, EDAC, Cyanuric Chloride (CC), Enzyme, Nanoparticles, laccase.

INTRODUCTION

The application of nanomaterials in environmental applications is undisputed [1]. High surface to volume ration, smaller size, high reactivity, high sorption, resistance to microbial attack, tailor made nanostructures, easily modified surface properties are few of the unique properties that enable nanomaterials to be popularly explored in

contaminant remediation [2]. The use of nanomaterials as a carrier for enzymes is one field which is being widely studied in recent times [3]. Enzymes are efficient biocatalysts and have extensive application in various industries such as food, textile, chemicals, paper, detergents, health care products and pharmaceuticals. Another major application is in waste treatment, water purification, biofuel production and bioremediation [4]. The widespread application of enzymes suffers from varied limitations of instability, problems of recovery and reusability. The catalytic properties of the enzyme are lost due to factors such as pH, temperature, ionic strength and other chemical agents [5]. Immobilization techniques provided a solution to the limitations of enzyme application. The carriers and the immobilization techniques investigated in olden times showed low enzyme loading with respect to the surface area [6]. This opened a new arena to nanoparticles as carrier for enzymes. A remarkable contribution has been reported in the last few years on enzymes immobilization in nanoparticles. It was reported that covalently binding the enzyme onto the carrier material will enhance the enzyme loading and its reusability [7]. In addition, the enzymes become stable and develop tolerance to changes in pH, temperature, ionic strength etc [8]. Covalent bonding is mainly preferred for enzyme immobilization because the bonds formed between the matrix and the enzyme will be stable as a result the release of immobilized enzyme into the solution will be avoided [9]. To covalently bind the enzyme to the nanoparticles, surface activation/ modification is very essential. So far several chemicals have been used to modify the surface of the nanoparticles. Some of them include glutaraldehyde, carbodiimides and cyanuric chloride. Carbodiimides are the chemical activators which facilitates the enzyme in binding with support material via carboxyl group. An intermediate is formed when carbodiimides reacts with carboxyl which gets stabilized when reacts with amines, forming a peptide bond, without spacer. EDAC is the most popular form of carbodiimides and it serves variable purposes for example, peptide synthesis, cross-linking and immune-conjugates preparation. An advantage associated with EDAC activation is that no such lengthy linker species are involved which ultimately reduces hydrodynamic radius of the nanoparticles [10]. Cyanuric chloride is a triazine which activates the OH group of the support material for covalent bonding of the enzyme [11, 12]. In one of the study conducted by Moreno and his co-workers, (1994), it was observed that cyanuric chloride increased the stability of the enzyme. In comparison to the native enzyme, enzymes immobilized on cyanuric chloride surface modified particles showed 37 times more stability and 80 % residual activity. Conversely, Kalkan et al. (2011) reported 94.22% enzyme immobilization on EDAC activated particles. Thus, the objective of the present study was to analyse the efficiency of EDAC and CC as surface activators in immobilization of enzyme on magnetic iron nanoparticles. The efficiency of the process was assessed in terms of (i) amount of sulphur moiety in the immobilized particles (ii) Protein leaching estimation.

Anamika Das,

MATERIALS AND METHODS

Nanoparticles synthesis

Co-precipitation method was used for the preparation of nanoparticles using iron salts in a molar ratio of 2:1. The mixture was prepared in HCl and dried in oven at 80 °C for one hour, simultaneously sodium hydroxide solution was boiled and at its boiling temperature the mixture of iron salts was added. The contents were stirred continuously till a black precipitate was formed [13]. The precipitate was then dried in an oven at 100 °C. The particles obtained were washed using distilled water till the solution reaches pH 7.

Chitosan coating

For chitosan coating reversed phase suspension technique was used. 200 mg iron nanoparticles were washed in ethanol thrice before being suspended in 50 mL mineral oil containing 0.5 mL Tween 80 in a round bottom flask. For proper dispersion the suspension was sonicated for one hour and chitosan solution (1% w/v, 15.0 mL in 5.0% v/v acetic acid) was added to it. The Fe_3O_4-chitosan dispersion formed was once again sonicated for 60 mins. Chitosan coated MNPs were mechanically stirred at 1000 rpm for 5 minutes and later glutaraldehyde solution (3.0 mL, 25% w/v in water) was added for functionalization of the particles. A permanent magnet (~3500 G) was used for the

separation was magnetically stirred for 4 h at room temperature and separated using. The chitosan coated MNPs (CNPs) obtained was vacuum dried at 200 °C after repeatedly washed with acetone for five times [7].

Laccase immobilization

Surface activation of the chitosan coated nanoparticles is required for enzyme immobilization so EDAC and cyanuric chloride was used, which are carboxyl and hydroxyl activating agents respectively for the coupling of primary amines to yield amide bonds. When the surface of the chitosan coated particles is activated, the laccase enzyme was immobilized onto the functionalized nanoparticles.

Surface activation of chitosan coated nanoparticles

EDAC

To facilitate the immobilization of laccase enzyme by covalent binding onto the chitosan coated particles, activated by EDAC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) [7]. For one hour, 50 mg of Fe_3O_4-CS nanoparticles was dispersed in 2.0 mL phosphate buffer [(PB), pH 6.0, 0.04M] in an ultrasonic bath at 10-15°C. In the dispersed mixture the EDAC solution (0.5 mL, 2.5% w/v, in same buffer) was added to it and sonicated for one hour. Magnetic stirring of the mixture was performed at 1000 rpm, at 4ºC for 6 h and stored at the same temperature overnight in the refrigerator. Finally, the EDAC activated chitosan coated MNP (CNPs-EDAC) was collected using ~3500 G magnet, washed repeatedly with 2.0 mL PB for five times and used for the immobilization of laccase enzyme.

Cyanuric Chloride (CC)

In 2.5 mL CC solution (0.5% w/v in 1,4-dioxane), 50 mg of chitosan coated magnetic iron nanoparticles were dispersed and sonicated at room temperature for 30 min. Subsequently the mixture was stirred magnetically at 1000 rpm for 6 h and the mixture was stored overnight. The chitosan coated nanoparticles activated by CC were then collected using a magnet and washed with 2 mL acetone and 2 mL Phosphate Buffer. The washing was repeated three times. The CC activated particles were separated and further used for laccase immobilization [7].

Enzyme immobilization

For 40 min sonication, 50 mg of CNPs-EDAC and CNPs-CC were washed with 2.0 mL PB and 2.0 mL laccase solution was added (0.5 mg/mL, in PB, pH 6.0). After sonication, the mixture was continuously magnetically stirred at 1000 rpm for 6 h at 4ºC and stored overnight at 4 °C. A permanent magnet was used for the separation of nanoparticles after 24 h incubation and washed repeatedly (4-5 times) with PB so the unbound enzyme gets removed. Bradford assay at 595 nm was used to assess the concentrations of the enzyme in the initial and washed solutions. The percentage of immobilization was calculated using Eq. 1 [14].

$$\% \text{ immobilization} = \left(1 - \left(\frac{\text{Final protein concentration}}{\text{Initial protein concentration}}\right)\right) \times 100 \qquad (1)$$

Characterization of Nanoparticles

The magnetite nanoparticles (MNPs), chitosan coated magnetic particles (CNPs) and surface activated (EDAC and CC) laccase immobilized nanoparticles (CENPs) were characterized for their size and morphology using TEM (Transmission Electron Microscopy) (Model TECNAI G^2 20S- TWIN). The covalent bonding of the enzymes over the chitosan coated particles was confirmed using SEM-EDS (Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy) (Carl ZEISS, MERLIN compact).

RESULTS AND DISCUSSION

Characterization of Nanoparticles and Chitosan coated nanoparticles

Figure 1 depicts the TEM images of magnetic iron Nanoparticles and Chitosan coated Nanoparticles on a scale of 100 nm.The particles were irregular in shape and ranged in size from 10 to 25 nm. No significant change in size and shape was observed between magnetic iron nanoparticles and chitosan coated magnetic iron nanoparticles. The elemental composition obtained from XRD spectra of ENP-EDAC and ENP-CC (Fig. 2) showed the presence of Fe_2O_3 in the peak series 30.1°, 35.5° and 63.6°. The sharp and intense peaks confirm the crystalline property of the nanoparticles. Likewise, the broadening of peak indicates the small size of the particles. Esquivelet al. (2007) confirmed similar observationsof the spectra. Figure 3 shows the SEM image of ENP-EDAC and ENP-CC particles. From the image it is clearly evident that in image (b) the dispersion of the nanoparticles were properly achieved while in image (a) such dispersionwas not achieved. A closer look on the images provides information regarding the asymmetry of the prepared particles in both the images. The particle size were not uniform in both the cases and were clumped together.The SEM-EDS image (Fig. 4) confirms the presence of laccase enzyme on both EDAC and CC activated CNPs through the sulphur peaks. The sulphur peaks were contributed by the laccase enzyme [7]. The ratio of sulphur in EDAC and CC surface activated particles varied and provided a quantitative idea about the amount of enzyme immobilized. EDAC activated nanoparticles showed higher enzyme immobilization than CC activated particle. The atomic percent of sulphur in EDAC activated CNP and CC activated CNP were 0.08% and 0.02% (Fig. 4), respectively.

Bradford assay

Bradford assay was performed on EDAC and CC activated chitosan coated magnetic nanoparticles to obtain the percent of enzyme immobilized and the amount lost after immobilization process. The initial protein concentration for EDAC activated CNPs and CC activated CNPs was 233.9 µg/mL and 297.3 µg/mL, respectively(Figure 5). It has been observed the amount of unbound enzyme lost in case of CC activated particles is more as compared to EDAC activated particles. After first washing, 132.17 µg/mL of protein concentration was lost in case of CC activated CNPs while in case of EDAC activated CNPs only 29.13 µg/mL of protein loss was observed. Hence as per the equation (1), the amount of enzyme immobilized after washing was 79.2% and 48.8% for ENP-EDAC and ENP-CC, respectively. From this it can be concluded that the amount of enzyme immobilized on ENP-EDAC is 1.6 times higher than the ENP-CC in our study which is in accordance with previous studies where the α-chymotrypsin enzyme retained 80% of its activity at 65 ºC and at 25 ºC the enzyme retained 90% of its activity when α-chymotrypsin was covalently immobilized to the surface of magnetic iron oxide nanoparticles, by a coupling reagent used for in-situ polymerization with 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide [16]. It is a commonly used carbodiimide for enzyme immobilization which achieves higher efficiency in enzyme immobilization [17]. Cyanuric chloride activated immobilized enzyme retained 80% of residual activity[11].

CONCLUSION

From our study, it can be concluded that the EDAC surface activator is more successful in enzyme immobilization as compared to CC activator. Strong covalent binding for the laccase enzyme was provided by the EDAC and hence can successfully be used for bio-conjugation. Although in monetary terms, cyanuric chloride is cost effective as compared to EDAC but in our study it has been observed that CC is inefficient in enzyme immobilization. The solvent used for dissolving CC might be the reason for the decreased immobilization. Henceforth, further research is required to determine the role of solvent in accelerating the immobilization in CC activated particles.

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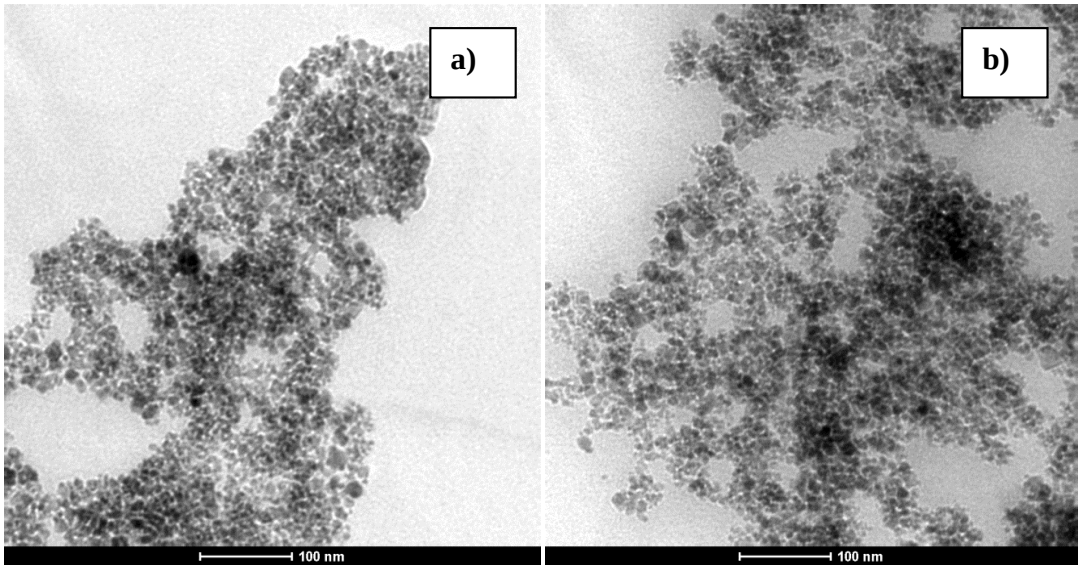


Fig. 1: TEM Images a) Magnetic Iron Nanoparticles (MNPs) b) Chitosan coated MNPs (CNPs)

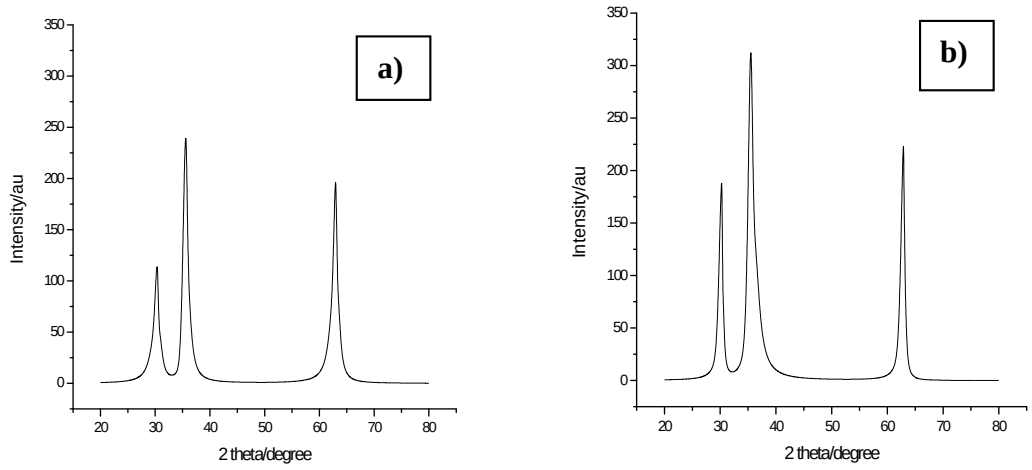


Fig. 2: a) XRD spectra of ENP-EDAC b) XRD spectra of ENP-CC

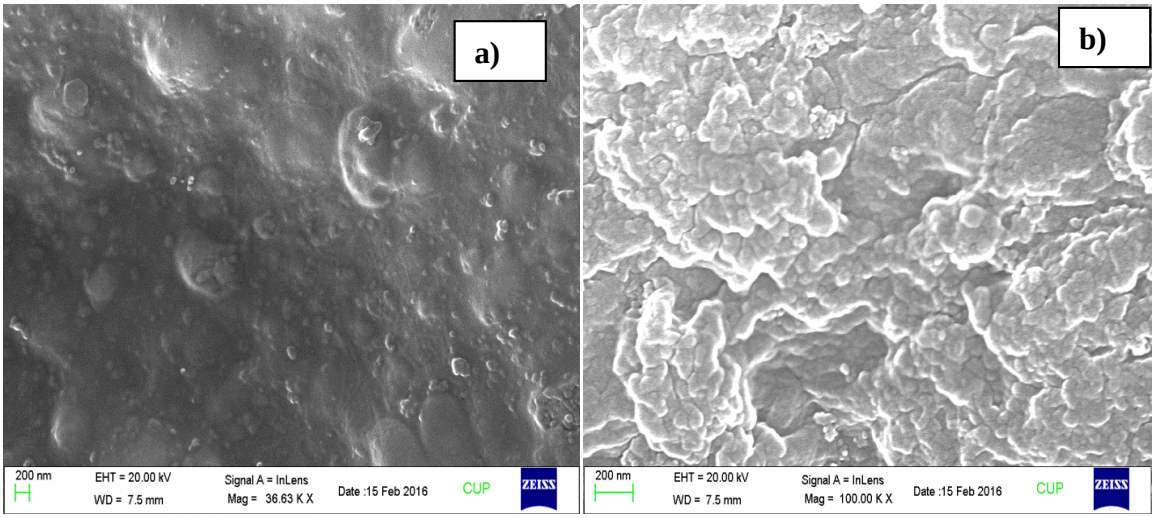
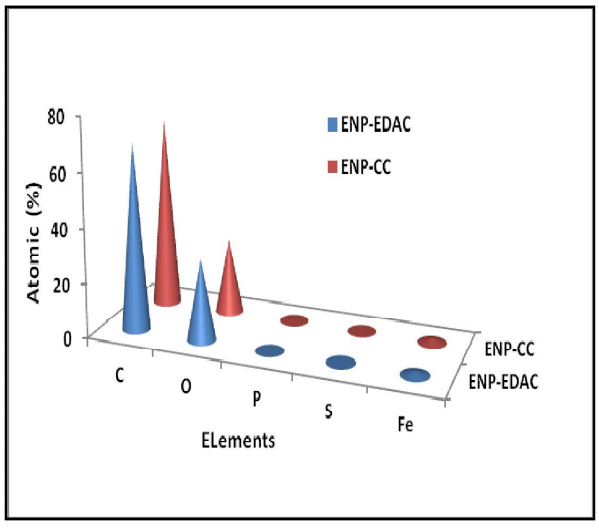


Fig. 3: SEM images a) ENP-EDAC b) ENP-CC



S. No.	Element	Atomic Weight EDAC	Atomic Weight CC
1	C	68.72	70.22
2	O	29.54	27.8
3	P	0.12	0.38
4	S	0.08	0.02
5	Fe	1.55	1.61

Fig. 4: Atomic percent of the elements (SEM-EDS)

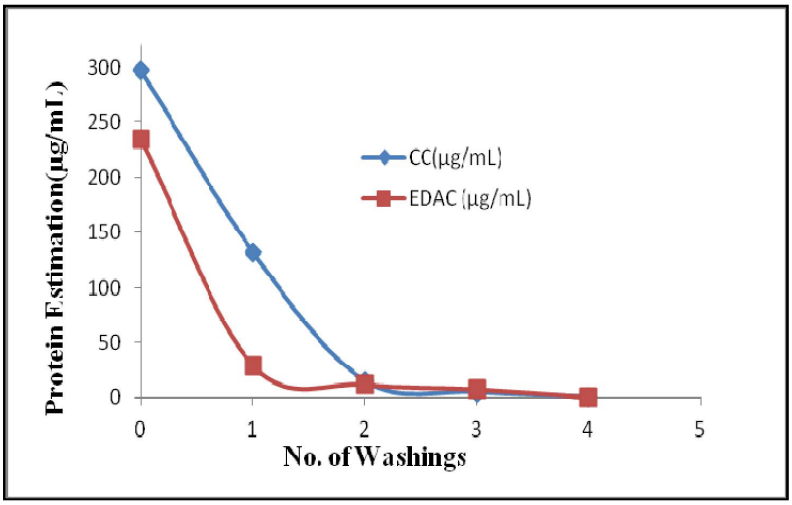


Fig. 5: Bradford assay of Enzyme Leaching

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RESEARCH ARTICLE

Effective Removal of Nitrate from Potable Water using Plant Coagulant

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ABSTRACT

Water is the elixir of life. Our earth seems to be unique among the other known celestial bodies as it has water. Water is an essential resource for life not only for Homo sapiens but also for all plants and animals. That is why our religious scriptures compare water with life and water is part and parcel of all religious rituals. The great artist Leonardo davinci once described as "water "the driver of nature. Due to increased human population, industrialization, use of fertilizers in agriculture and man-made activities cause pollution in aquatic resources. It is therefore necessary that the quality of drinking water should be checked at regular time interval because due to use of contaminated drinking water, human population suffers from a variety of water borne diseases. The report of scientists at the All India Institute of Medical Science (AIIMS) to finds an alarming prevalence of various diseases causing amoeba, bacteria, parasites, and larvae of insects in drinking water. Higher level of nitrate content in water leads to methemoglobenimea in infants. Methaemoglobinemia or blue baby disease is caused by the reaction of nitrate with haemoglobin, the oxygen carriers in blood, producing methaemoglobin, which reduce the oxygen carrying capacity of the tissues.In this present investigation, the 10 potable water samples were collected from Vilavancode Taluk, Kanyakumari Districts. The sample stations were Arumanai(well), Kulasekaran(well), Kuzhithurai(Bore well), Marthandam(Bore well), Karungal(Bore well), Puthukkadai(well), Painkulam(Bore well), Thiruvattar(well), Attoor(well) and Thengapatanam(well)) in The physical and chemical properties such as pH, EC, TS, TDS, TSS, acidity, alkalinity, nitrate, phosphate, sulphate, fluoride, chloride, calcium, hardness and magnesium were analyzed and compared with BIS standard. All the parameters are within the permissible limit except nitrate in two samples (Puthukkadai and Painkulam). The samples were treated with *Moringa oleifera* plant seed material as natural coagulants. Different dosages such as 0.5g, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g were used for nitrate reduction. After treatment, the nitrate level was reduced to within the permissible limit. The optimum dosage of

Moringa oleifera for removal of nitrate from drinking water is 2 g. The removal of nitrate was attributed by the presence of coagulating protein followed by antioxidants are responsible for the reduction of nitrate contamination. The natural coagulant can be used as a substitute for chemical coagulants because chemical coagulants like alum release Aluminium cause Alzheimer disease. From the experiment, it can be concluded that nitrate contaminated water can be treated with *Moringa oleifera.*

Key words: Physicochemical parameters, Nitrate, BIS standard, *Moringa oleifera*

INTRODUCTION

Water is major and abundant natural resources of the earth and prime necessity of life. It is an essential requirement for the life supporting activities. All form of living organisms required water for their growth and development. The elevated population number and industrialization usage of chemical fertilizers in the agricultural fields and anthropogenic activities leads to water contaminations (Basavaraja simbi *et al*., 2011). Aquatic organisms need a healthy environment to live and have adequate nutrients for their growth. The productivity depends on the physicochemical characteristics of the water body. If any alteration in the physico chemical characteristics of water would become unfit for drinking and other purpose (Julie *et al*., 2010). Continuous discharges of waste water from industries, domestic sewages, dumping of solid waste pollutes both ground water and surface water and create harmful health effects and polluted water has frequently led to waterborne disease outbreaks with acute and long-term health effects ranging from diarrhea to death. Polluted water is often the main human exposure pathway to infectious pathogens and carcinogenic organic and inorganic contaminants (raja *et al*., 2002). During the last few decades, the increase in the human population as well as globalization and its effects has not only raised the quantity of waste, but has also introduced several emerging water contaminants such as pharmaceuticals, hormones, endocrine disrupting chemicals, viruses, and toxins (Xagoraraki *et al*., 2008). As analytical methods continue to improve, recent studies have revealed that emerging or re-emerging microbiological pathogens or chemicals may be present in natural or treated water bodies. Emerging contaminants, which have not historically been considered pollutants, have now raised significant concerns to public health professionals and environmental engineers and scientists. Most water pollutants originate from human activity, while a small percentage of them have their sources in natural activities such as volcanic eruptions. Primary anthropogenic sources of water pollution include poorly treated or untreated municipal sewage, individual septic systems discharge, agricultural livestock wastes, fertilizers, pesticides, industrial chemical wastes, spilled petroleum products, mine drainage, spent solvents, etc (Kuo *et al*., 2008).

Coagulation process is a traditional method for the removal of turbidity, colour, natural organic matters, form both industrial waste water and surface water. Aluminium sulphate, ferric chloride, calcium carbonate, polyaluminium chloride are the common chemical coagulants used for the waste water treatment (Muthuraman and sasikala., 2014). These chemical coagulants produce huge volume of sludge after treatment were disposed into land leads to accumulation of toxic chemicals in the environment and also aluminium sulphate and polyaluminium chloride may cause Alzheimer's diseases and other harmful effects in human. Agro-based coagulants are eco-friendly, low cost and highly biodegradable. Because of these merits, plant based coagulants such as *Moringa oleifera* seeds (drumstick) widely used for the treatment of drinking water. *Moringa oleifera* is a persisting and fast growing tree belongs to Moringaceae family.It has numerous pharmacological properties. Such as antifungal, antimicrobial, antiatherosclerotic, antifertility, relieving pain, central nervous system depressant, antiinflammatory, diuretic and regulating hyperthyroidism (Dolly Jaiswal *et al*., 2013).Objective of the present investigation was to assess the drinking water quality and to evaluate the coagulating efficiency of natural coagulant - *Moringa oleifera* seeds in water treatment.

MATERIALS AND METHODS

Sampling site

Kanyakumari disctrict is a popular international tourist spot located at southernmost tip of india. Now this district is exploited and polluted by unplanned urbanization, deforestation, large scale sand mining, brick kilns, coir retting, and discharge of waste water from industries and domestics to the water bodies directly (Rajesh et al., 2013). Kanyakumari district received rainfall from both south west and north east monsoon. The south west monsoon chiefly contributes to the rain fall (Balachandran., 2008). Drinking water samples are collected from 10 locations of southern region of Kanyakumari district (Vilavancode and Kalkulam Taluk). The sampling sites are Arumanai(well), Kulasekaran(well), Kuzhithurai(Bore well), Marthandam(Bore well), Karungal(Bore well), Puthukkadai(well), Painkulam(Bore well), Thiruvattar(well), Attoor(well) and Thengapatanam(well). The temperature of the tropical region is about 24 °C- 32 °C.

Physico-chemical characterization of drinking water

The collected drinking water samples are analyzed for major physical and chemical water quality parameter like pH, Electrical Conductivity (EC), Total solids (TS), Total Dissolved Solids (TDS), Total suspended solids (TSS), Total Alkalinity (TA), Acidity, Total Hardness (TH), Chloride (Cl-), Calcium (Ca++),Magnesium (Mg++), Fluoride (F), Nitrate (NO_3), and Sulphate (SO_4). (APHA, 2012)

Collection and preparation of plant powder

Moringa oleifera L. seed, a non-toxic plant material collected from Dindigul (Dt), Tamil Nadu, India. The collected seed was powdered using domestic blender, sieved (175 mm) and stored in air tight container. No further chemical treatment was applied for the seed powder and it was used as such for the treatment of drinking water.

Effect of *Moringa oleifera* on the removal of nitrate from drinking water

About 100 ml of drinking water sample was taken and different dosages such as 0.5g, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g were added and mixed well then it was left for a period of 2 hours. Later it was filtered through Whitmann no 41 filter paper. The filtered sample was subjected to nitrate analysis.

GC-MS analysis of *Moringa oleifera* seeds

The plant seed powder was subjected to ethanolic extract using Soxlet apparatus for GC-MS analysis.

Protein profile analysis

Protein content of the moringa seed was estimated using Bradford (1976) method. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed according to Laemmli (1970). The resolved proteins were stained with Coomassie Brilliant Blue R250.

FTIR

The plant seeds were characterized by FTIR spectroscopy to identify the functional groups. (Absorbents band was observed in the region of 600- 4000 cm^{1}.

RESULTS AND DISCUSSION

The collected ten water samples were analyzed to check the quality. The obtained values were compared with BIS (Bureau of Indian Standard) standard values. All the parameters were found within the permissible limit except nitrate in two samples.

pH

pH is an important parameter to analyze the acidic and alkaline nature of water and measures the concentration of hydrogen ions. The pH of the collected samples ranged from 6.38-7.45. The pH of all the samples was found within the permissible limit prescribed by BIS Standard pH (7 – 8.5). Neha Gupta *et al*., 2013, have been reported the similar results.

Electrical conductivity

Electrical Conductivity is a measure of water capability to transmit electric current and also it helps to assess the purity of water. Electrical Conductivity is found in the range of 0.204 to 0.408 µS/cm. Similar results have been reported by Thamarai selvi *et al*., 2014

Total solids

Total solids including dissolved solids and suspended solids indicate the presence of saline nature of water. TDS values were found to be 100 to 400 mg/L. The findings have been coincided with Sanjay 2014.

Alkalinity

Alkalinity measures the acid neutralizing capacity of water. It also imported by salts of carbonates, bi carbonates, phosphate, nitrates, borate, silicate etc., (Maharajan *et al*., 1995). The range of alkalinity for all the samples was found to be from 95 to 140 mg/L..

Total Hardness, calcium and magnesium

Total hardness of water is the addition of total alkaline earth metals such as calcium and magnesium. Both are important for the growth of flora and fauna. Dissolution of various types of rocks, industrial waste and sewage are the sources of calcium and magnesium in natural water. The maximum level of hardness leads to kidney problems and cardiac problems in human (Mary, 2012) . Hardness, calcium and magnesium of all the samples were within the permissible limit.

Nitrate

Surface water contains nitrate due to leaching of nitrate with the percolating water. Surface water can also be contaminated by sewage and other wastes rich in nitrates. The elevated level of nitrate in potable water reduces the oxygen carrying capacity of red blood cells. This leads to blue baby syndrome to infants and difficulty in breathing because of their bodies are not receiving enough oxygen. The blue baby syndrome is also known as methemoglobinemia (Sharmila *et al*., 2013). The permissible BIS standard limit for nitrate is 45mg/L. Nitrate level in most of the samples within the limit except two samples S6 and S7 (75mg/L and 60mg/L). The reason may be attributed by the discharge of sewage and domestic washing water near water source. Hence, these two samples must be subjected to water treatment before going to consumption.

Sulphate

The higher amount of sulphate -could cause a cathartic action on human beings and also cause respiratory Problems (Sujitha, *et al.,* 2012). In this study, the average sulphate concentration of the potable water sample was the range between 80 to 140mg/L. Similar results were reported by Chadetri, (2011).

Fluoride

The source of fluoride in drinking water is mainly due to weathering of rocks industries of iron, steel, petroleum refining industries and phosphate fertilizer. The maximum uptake of fluoride through drinking water leads to dental problem called fluorosis and bone diseases (Murhekar *et al.,* 2011). The BIS standard limit of fluoride is 1.5mg/L. In the present investigation, the fluoride level ranges were found to be 0.3 to 1mg/L.

Chloride

Chloride is an anion found in variable amount in groundwater. Chloride may present naturally in groundwater and may also originate from diverse sources such as weathering, leaching of sedimentary rocks and infiltration of seawater etc. The higher concentration of chloride in water leads to laxative effects (Sanjay, 2014). Chloride level for all the samples were within the standard range.

Effect of *Moringa oleifera* L on the removal of nitrate from drinking water

Two samples such as S6 and S7 have higher level of nitrate than other parameters. The samples were subjected to treatment with natural coagulant in different dosages such as 0.5g, 1g, 1.5g, 2g, 2.5g and 3g for the reduction of nitrate. The reduction percentage was depicted in table 3. The removal of nitrate was increased when increase the dosages of coagulants up to 2g. After the dosage, there was no significant reduction of nitrate was noticed. It suggests that, 2g is an optimum dose for the removal of nitrate from drinking water samples. The Moringa seed is a heterogeneous complex mixture consisting various functional groups, mainly low molecular weight organic acids (amino acids). Amino acids constitute a physiologically active group of binding agents, binding even at low concentration, (Brostlap and Schuurmans, 1988). The protein amino acids have a variety of structurally related pH dependent properties which generats negatively charged atmosphere and play a major part in the binding of metals (Costa et al., 1997). Majority of the aminoacids present in the Moringa seed have isoelectric points pH between 4.0 and 8.0 (Delvin, 2002).

GC-MS analysis of *Moringa oleifera* L. seed

The phytochemical compounds of moringa seed is presented in table 5. The GC-MS analysis revealed the presence of 9 compounds namely butoxyacetic acid has the property of haemolytic activity, tetradeconoic acid having antidiabetic activity, 9- hexodeconoic and hexodeconoic having lipogenic enzyme activities, squalene possessing biological and pharmacological activity, 1,2-bis(trimethylsilyl)benzene having antibacterial and octamethyl having anti-cancer activities. The identification of the phytochemical compounds was confirmed based on the peak area; retention time and molecular formula. Similar such results have been reported by Prasanth *et al*., (2011).

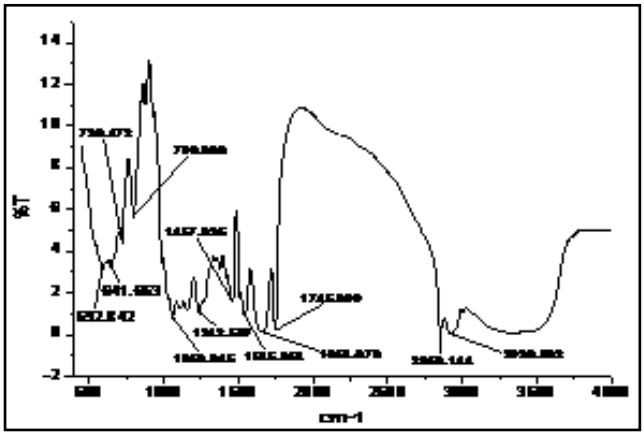
Seed protein analysis using SDS-PAGE

Electrophoresis analysis of the (SDS-PAGE) of protein extract can suggest that it is be composed of desired protein corresponding to the fraction at 50kD -10kD. The highest expressing protein was found at 30kD molecular weight (Becker et al., 2008). The SDS-electrophoresis expression of *Moringa oleifera* .L seed protein in molecular weight is presented in fig 3. Similar such results have been reported by Chandra Sekar Bhol, 2012.

FTIR analysis

The broad band centered at 3,336 cm-1 assigned to O-H stretching, this function group observed in protein and fatty acid structures present in Moringa seeds. Another N-H stretching from amide group was obtained due to the presence of high content of protein. In the region from 1400 to 1800 there are intense bands assigned to O-H stretching, C=O stretching, NO_2 asymmetric stretching. The carbonyl group is present in the fatty acid and protein structures. The presence of peak at 1457 and 1545 with N-H first overtone stretching confirms the presence of protein. Similar such results have been reported by Sasikala and Muthuraman 2015.

FTIR analysis of Moringa seeds



FTIR spectra of *Moringa oleifera* L. seed

S.No	Peak value	Stretching	Interpretation
1	3336	N-H Stretching	Primary, secondary amides
2	2927	C-H stretching	methylene
3	1747	C=O stretching	Aliphatic esters
4	1656	C=C stretching	Alkenes
5	1459	C-C stretching (in ring)	aromatic
6	1237	C-N stretching	Aliphatic amines
7	1058	C-N stretching	Aliphatic amines
8	721	CH_2 rocking band	Aliphatic hydrocarbons

CONCLUSION

The present investigation revealed the capability of *Moringa oleifera* .L seed in the reduction of nitrate from the samples. Coagulant protein present in the seed was confirmed by FTIR and SDS-PAGE analysis, which is responsible for the reduction of nitrate from drinking water.

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Fig. 1 *Moringa oleifera* L. seeds

Table1. Sampling sites

S.No.	Sampling sites	Sampling sources	Sampling code
1.	Arumanai	Well	S1
2.	Kulasekaran	Well	S2
3.	Kuzhithurai	bore well	S3
4.	Marthandam	bore well	S4
5.	Karungal	bore well	S5
6.	Puthukkadai	well	S6
7.	Painkulam	bore well	S7
8.	Thiruvattar	well	S8
9.	Attoor	well	S9
10.	Thengapatanam	well	S10

Table 2. Physical characterization of potable water sample

S.No.	Sampling sites	Physical parameters						
		pH	EC (µS/cm)	TS	TDS	TSS	Acidity	Alkalinity
1.	Arumanai	6.38	0.204	500	300	200	12.5	135
2.	Kulasekaran	7.01	0.204	400	200	200	12.5	120
3.	Kuzhithurai	7.08	0.408	600	400	200	25	100
4.	Marthandam	7.45	0.408	500	200	300	25	80
5.	Karungal	7.32	0.408	300	100	200	25	95
6.	Puthukkadai	7.03	0.204	300	100	200	25	105
7.	Painkulam	6.98	0.204	200	100	100	12.5	130
8.	Thiruvattar	6.93	0.204	300	200	100	12.5	140
9.	Attoor	6.74	0.204	200	200	100	12.5	135
10.	Thengapatanam	6.79	0.204	300	100	200	12.5	125

Table 3. Chemical characterization of potable water sample

S.No.	Sampling sites	chemical parameters						
		calcium	hardness	magnesium	chloride	Fluoride	nitrate	sulphate
1.	Arumanai	150	120	30	148	0.5	9	120
2.	Kulasekaran	170	150	20	151	0.4	31	140
3.	Kuzhithurai	120	100	20	95	0.7	27	90
4.	Marthandam	130	110	20	170	0.4	8	95
5.	Karungal	145	130	15	50	0.7	10	112
6.	Puthukkadai	95	105	20	150	0.9	60	85
7.	Painkulam	120	100	20	165	0.7	75	80
8.	Thiruvattar	95	105	10	190	0.6	29	85
9.	Attoor	170	150	20	80	1.0	30	130
10.	Thengapatanam	140	130	10	180	0.3	25	70

All the values are calculated as mg/L

Table 4. Effect of *Moringa oleifera* L on the removal of nitrate from drinking water

S. No.	Dosage (g)	% of reduction	
		S6	S7
1.	0.5	60	50
2.	1	63	59
3.	1.5	70	65
4.	2	83	76
5.	2.5	83	76
6.	3	83	75

Table.5 GC-MS analysis of Ethanolic extracted *Moringa oleifera* L. seed

S. No.	RT	Compounds name	Activity	Molecular weight
1	7.383	Butoxyacetic acid	haemolytic activity	132
2	10.233	Tetradecenoic acid	antidiabetic avtivity	264
3	11.024	9-Hexadecenoic acid	lipogenic enzyme *activities*	254
4	12.580	Hexadecanedioic acid	lipogenic enzyme *activities*	286
5	13.667	Squalene	biological and pharmacological *activities*	410
6	16.016	1,2-Bis(trimethylsilyl)benzene	antibacterial *activity*	222
7	16.275	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,	anticancer *activity*	424

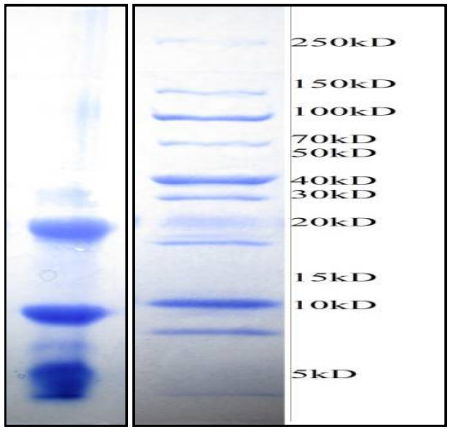

Fig. 2: SDS Electrophoresis of (A) **Moringa *oleifera* L. Seed and (B) marker**

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RESEARCH ARTICLE

Passive Smoking, DMFT Index and Antioxidant Capacity of Saliva in Children 4-6 Years

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ABSTRACT

According to the World Health Organization's report, one percent of total annual deaths worldwide are caused by passive smoking. Based on the role of saliva in oral health, the aim of this study was to investigate the association between passive smoking, as determined by dmft, total antioxidant capacity and uric acid level of saliva in children aged 4-6 year old.Seventy six passive smoker children and 76 healthy controls of children aged 4-6 year old were collected from kindergartens in Babol (north of iran). Passive smokers divided equally based on the exposure to the cigarette (PS1) less than10 cigarette daily and PS2: higher than 10 cigarettes daily). Dental examination was performed by a mirror and a probe under suitable light of the room and dmft was calculated according to the WHO criteria. The total antioxidant capacity of saliva was measured by FRAP assay and uric acid was assessed by spectrophotometry.There were no significant differences in age, maternal education, tooth-brushing status, sugary snack exposure among groups ($P > 0.05$). The mean dmft of PS1, PS2 and control groups were 5.97 ±2.07, 7.45±1.55, 5.28 ± 2.50 respectively ($P<0.001$). There was no significant difference in total antioxidant capacity and uric acid concentration among study groups.According to our findings, dental caries was more prevalent among passive smoker children than non passive smokers.

Key Words: dmft, passive smoking, saliva, Total Antioxidant capacity, Uric acid.

INTRODUCTION

According to the World Health Organization's report, one percent of total annual deaths worldwide caused by passive smoking (1). The different aspects of health maybe affected by smoking; children are more vulnerable against the negative effects of smoking because they have smaller bronchi and higher respiratory rate, and besides their immune system is not well developed [2].Cigarette Smoke has different adverse effects on health such as cancer, periodontal and dental caries (3). It is well-known than the balance between oxidants and antioxidant plays an important role in prevention of these diseases. Cigarette smoke, as an oxidative stress, can change the salivary antioxidants and may be involved in causing the diseases (4). The salivary antioxidant system has several components; one of the main components which are responsible for approximately 70% of the salivary antioxidant capacity is the uric acid (5). Although many studies were done about cigarette smoke, but there is a few studies about the indirect effects of cigarette smoke on oral health. The aim of this study was to investigate the association between passive smoking and the prevalence of dental caries, salivary total antioxidant capacity and salivary uric acid concentration in young children

MATERIALS AND METHODS

This cross-sectional study was conducted during 2012-2013 in Babol, Northern city of Iran. The study protocol was approved by the Ethical Committee of Babol University of Medical Sciences.

Patient selection

Study population was sampled by available method from the population of 4-6 years old children among kindergartens in Babol. Criteria for selecting passive smoking (PS) was having a smoker family member who smoked since the birth of the child and the amount of cigarette smoked per day. PS1 group was exposed to less than 10 cigarettes daily, and PS2 group was exposed to more than 10 cigarettes daily. The exclusion criteria including: taking antibiotics within three weeks ago, medication reducing the saliva, drugs affecting antioxidant system such as vitamins and history of fluoride therapy within the past three months, suffering from systemic diseases like diabetes, thalassemia and other blood disorders. Parents of the eligible participants were asked for giving an informed consent for participation in the study. Thirty eight individuals were sampled from each passive smoking group and 76 children were in the control group.

Data collection

All the childeren participated in the study were undergone complete dental examinations which conducted by a paedodontist under natural light with the aid of a dental mirror and explorer. dmft index The total number of teeth decay (d) missing teeth (m), and filled teeth (f) were calculated according to the WHO criteria by dental mirror and an explorer in all cases;by one person no radiography was used. Information regarding demographic status and several dental habit related parameter were retrieved. Age, maternal and paternal educational level, rank of the child, tooth-brushing habits, number daily snack containing sugar exposures were recorded by a structured questionnaire for each participant. The child was forbidden from any oral stimulus such as brushing, eating and drinking for nearly 60 min prior to the sampling. Unstimulated saliva was collected from each subject then immediately transferred to the laboratory and centrifuged (1000 g, 10minutes) at 4°C to remove cell debris. The resulting supernatants were immediately stored for later analysis. Total antioxidant capacity was measured by the FRAP assay (Ferric Reduving Antioxidant Power) using TPTZ reagent (Merk™, Germany) and standards contain, ferro sulfate were assessed by spectrophotometr. The concentration of Uric acid was determined by a standard assay (Zist chem. Co. Iran) using spectrophotometry method.

Statistical analysis

All data were analysed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The differences in dental status (DMFS) between the study groups were analysed using the Kruskal-Wallis and Mann—Whitney test. Mean age and Saliva parameters including TAC and uric acid were compared between the PS1, PS2 and control group using the ANOVA and post hoc test. Chi-squared test was used to compare the education level of the parents, chil rank, tooth-brushing habits and dietary sugar exposure of the children between the PS1, PS2 and control groups. P value less than 0.05 was considered significant.

RESULTS

The mean age of the control group, P.S1 and P.S2 groups was 5.75 ± 0.43, 5.74± 0.44 and 5.71 ± 0.46 years old respectively. The demographic status of the study population was indicated in table1.There were no significant difference in baseline characteristics of the study population regarding the age, child rank, mother's education level, exposure to sugar an, but there was significant difference in father's education among groups (P = 0.019). In clinical findings, just the DMFT rate had significant difference between groups which according to Tukey test, this significant difference was between the PS 2 group and control group(P =0.001). The comparison between the mean TAC and uric acid among study groups were not significant (table 2).

DISCUSSION

It has been found that cigarette smoke affects the oral health including dental caries (6) but there is little information about the passive smoking effects; hence this study was performed to evaluate the effect of the passive smoking on oral and dental health. This study is one of the most recent studies which was simultaneously evaluated the association between passive smoking dental caries pattern and salivary antioxidants. Based on the results of this study about dmft a significant difference was found between control group and P.S2 but there was no difference between control group and P.S1. In addition, there was a significant difference between P.S1 and P.S2. Therefore, in this study a higher rate of dental caries, was observed in children who were exposed to more than 10 cigarettes per day. Likewise, in the control group, the father's education was higher than P.S groupswhichis likely to increase the preventative role of the social awareness about the effects of cigarette smoke in children.dental caries no increase in P.S1 group compared with, it can be supposed that this group was not sufficiently exposed to cigarette smoke. But different reasons were reported for induction of decay in P.S children including the effect of immunsuppressive, the reduction of immunoglobulins of saliva and buffering capacity of saliva caused by cigarette smoke (7-8). In addition, it has been observed that nicotine could accelerate the salivary growth of streptococcus mutans based on in-vitro studies (9). The findings of peresent study is similar to the previous studies conducted by Hanioka et al. and Ausar et al. in which the P.S was associated with the increased prevalence of dental caries (10-11). Also, P.S can decrease the level of Vitamin C which is associated with the growth of cariogenic bacteria (12). The total antioxidant capacity in P.S1 group was decreased compared with control group and was increased in P.S2; but not significant statistically. The results of Charabopoules'and etal on the effect of direct smoke on TAC plasma and saliva showed the increase of TAC in plasma but no in the saliva which is similar to our study (13). It is reported that the antioxidant changes and oxidant stress caused by cigarette can have an important role on health. But the result of Greabu et al.'s study on the effects of direct smoke on the total antioxidant capacity of saliva showed that the directly exposure to cigarette smoke can significantly decrease the salivary antioxidants (5)which seems that free radicals and changes in oxidants and antioxidants balance can have an essential role in the pathogenesis of smoking-related diseases. Free radicals have the ability to directly and indirectly stimulate the oxidative stress, thus they can disrupt the oxidative balance (14). A lot of oxidants and free radicals are released after smoking. This can cause oxidative damages resulting in the atrophy of lung, heart disease and cancer (15).Chelchowska et al reported that the TAC level in blood samples of

smoker mothers'baby was significantly reduced (16). Also, Aycicek et al showed that the plasma level of total antioxidant capacity in P.S people was significantly lower than the control group (17).

In this study, we also assessed the level of uric acid in saliva. Uric acid one of the most important component of the salivary antioxidants. There was no significant difference between the mean level of salivary uric acid in different groups Results from similar studies revealed controversial results. Abdolsamadi et al. revealed that there was no difference between smokers and non smokers in salivary uric acid (19) which was in disagreement with the findings of Greabu M, et al. (20) and Zappacosta et al (21) who demonstrated the significant decrease in level of uric acid among adult smokers in comparison to non-smokers.While our study was conducted among children passive smokers. The present study is one of the first studies examining the effects of passive smoking on salivary antioxidants among children; however, in this study we encountered several limitations. Future studies with larger sample size and follow up design will produce more accurate results. Also, using more sensitive test for measuring antioxidant activity is suggested.

CONCLUSION

According to our findings smoking more than 10 cigarettes daily by parents can increase the susceptibility to dental caries in children. But, did not associated with significant significant decrease in salivary antioxidant concentrations

Conflict of interest: Authors declared no conflict of interest**.**

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Table 1: Demographic characteristics of study population

Groups Variables	Controls N=76	PS 1 N=38	PS 2 N=38	P value
Age(Mean)	5.74±0.44	5.71±0.46	5.74±0.44	1
Child rank				
First	38(%50)	19(%50)	21(%55)	0.86
Second	28(%37)	15(%39)	15(%40)	
Third	9(%12)	3(%8)	2(%5)	
Fourth	1(%1)	1(%3)	0(%0)	
Maternal education				
guidance	10(%13)	7(%18)	10(%27)	0.059
High school	39(%52)	19(%50)	24(%63)	
Higher diploma	27(%35)	12(%32)	4(%10)	
Paternal education				
guidance	7(%9)	3(%9)	5(%13)	0.019*
High school	33(%43)	23(%60)	27(%71)	
Higher diploma	36(%48)	12(%31)	6(%16)	
Number of daily brushing				
Less than once	40(%52)	19(%50)	24(%63)	0.45
Once or more	36(%48)	19(%50)	14(%37)	
Number of snacks containing sugar				
Zero to 2	18(%23)	8(%21)	7(%19)	0.80
3 or more	58(%57)	30(%79)	31(%81)	

*Significant level was at less than 0.05

Table2: Comparison of mean dmft, total antioxidant capacity and uric acid concentration in the study groups

Groups Variables	Control	PS 1	PS 2	P value
dmft	5.28±2.50	5.97±2.07	7.54±1.55	0.001*
TAC	700±157	681±175	725±85	0.804+
Uric acid	331±82	331±76	359±59	0.138+

* Kruskal-Willis test + ANOVA test

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RESEARCH ARTICLE

Influence of Zinc Deficiency on Yield and Yield Attributing Traits in F_2 Population of Rice under Water Logged Conditions

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ABSTRACT

Zinc deficiency is the most common micronutrient deficiency affecting rice yields in India. Breeding for zinc deficiency tolerance demands understanding of the tolerance mechanism. This requires studies involving specific population developed using zinc deficiency tolerant and susceptible parents pertaining to the regions, which are not sufficient at present. In our present study, we developed F_2 population by crossing zinc deficiency tolerant landrace of the Krishnagiri region Kotta Nel and zinc deficiency susceptible variety ADT-39. The progenies were raised under zinc deficient field with continuous water logging to evaluate their performance under zinc deficiency using Zn Def Scoring and to understand the effect of zinc deficiency on yield and yield attributing traits. The F_2 progenies exhibited varying performance under zinc deficiency and the scores were normally distributed indicating the chance of selecting extreme tolerant and susceptible lines. Zinc deficiency induced significant effect on all the traits contributing to yield. The plants with high zinc deficiency scores displayed high reduction in yield and yield attributing traits indicating direct association between Zn deficiency scores and the yield which emphasizes the impact of zinc deficiency on yield and also suggests the utilization of Zn Def score and yield as important measures for selection of zinc deficiency tolerance while screening huge populations.

Keywords: - Zinc, Rice, Screening, Segregating population, scoring, yield

INTRODUCTION

Zinc deficiency is one of the most widespread micronutrient disorder of rice, affecting more than 50% of the crop production worldwide (Quijano – Guerta *et al.,* 2002) which results in poor yield. It was first identified as a growth-limiting factor on calcareous soils of northern India known as Khaira disease (Nene, 1966) and it became greater concern since green revolution (Welch and Graham 2002) during which the focus was on yield. Globally, about half of the agricultural soils are zinc deficient (FAO). In India, about 50% of the soils are deficient and is expected to increase to 63% in 2025 if the current trend continues (Das and Green, 2013).

Zinc deficiency can be the result of low total soil-Zn content of the parent material, but is more frequently caused by Zn immobilization due to various changes in soil physical and chemical properties *i.e.* pH, redox potential, organic matter, pedogenic oxide and soil sulfur contents as they have strong influence on these adsorption-desorption reactions by regulating Zn solubility and fractionation (Alloway, 2009). In rice, Zinc deficiency occurs predominantly in lowland conditions as submergence of a well-drained paddy soil depletes oxygen, decreases redox potential and increases pH in acidic soils (Renkou *et al.,* 2003). Other factors associated with prolonged submergence such as high contents of bicarbonate and organic matter and high Mg:Ca ratio also severely affects availability of Zn to rice (Neue and Lantin, 1994). Since Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients, its deficiency in plants causes many symptoms, including chlorosis, bronzing, rosetting and goblet leaves (Broadley *et al.,* 2007). In rice the symptoms usually appears 2-3 weeks after seedling transplanting *viz.,* leaf bronzing, stunting and mortality in severe conditions. Flowering is normally delayed or even hindered in severe cases. Plants may recover in 4-6 weeks but delays in maturity and yield reduction are common (Yoshida and Tanaka, 1969). It is claimed that Zinc deficiency can be corrected by adding Zn compounds to the soils or plant but the high cost associated with applying fertilizers in sufficient quantities to overcome Zn deficiency, places considerable burden on resource poor farmers (Singh *et al,* 2003). This necessitates development of permanent and plant based improvement in Zn uptake and utilization through breeding programs (Rehman *et al.,* 2012). Understanding the mechanisms of zinc acquisition from soils with low available zinc and increased internal utilization efficiency are essential to exploit the genotypic variations (Frossard *et al.,* 2000). Identification of the suitable donors from the vast variation for Zn deficiency tolerance in germplasm is the prerequisite to develop the large number of segregating population that can be employed for understanding the mechanisms underlying tolerance. In our previous study, we identified Kotta Nel and Savulu Samba, the local landraces of the Krishnagiri district as the potential donors for zinc deficiency tolerance under continuous water logging and salinity respectively and confirmed their tolerance using simplified field plot technique which could be utilized for screening large number of germplasm lines (Salini *et al.,* 2015). In this study we have attempted to analyse the performance of F_2 progenies of the cross between Zn deficiency tolerant donor parent Kotta Nel and the susceptible variety ADT-39, raising them under zinc deficient field conditions.

MATERIALS AND METHODS

The study was conducted at the Regional Research Station, Paiyur, during *kharif*, 2014. A total of 216 F_2 progenies derived from cross ADT-39 x Kotta Nel were used in this study for evaluation of zinc deficiency tolerance. ADT-39 was found to be highly susceptible to zinc deficiency and the landrace Kotta Nel was tolerant (Salini *et al.,* 2015). The seeds was sown on raised bed nursery without addition of zinc in the soil and transplanted at 15 DAS to the zinc deficient plot (Table 1) with one seedling per hill, adapting a spacing of 22.5 cm x 22.5 cm. The fertilizers of N, P, K were applied based on STCR recommendations at appropriate growth stages. The genotypes were scored for zinc deficiency symptoms on 10^{th}, 25^{th}, 45^{th} DAP based on standard evaluation system for rice (IRRI, 2007). The yield and yield attributing traits were recorded at different stages of crop development. Genotypic and phenotypic correlations

were partitioned into path coefficient analysis using the technique outlined by Dewey and Lu (1959). The TNAU STAT, statistical package was employed to perform correlation analysis and path analysis.

RESULTS AND DISCUSSION

The F_2 population of ADT-39 x Kotta nel recorded significant variations for all the traits observed in the study. The Zn def scores ranged from 0 to 9. The scores at 10 DAP, 25 DAP and 45 DAP displayed normal distribution when fit into curve, depicting the presence of significant variation for zinc deficiency tolerance among the F_2 progenies (Fig. 1). At 45 DAP, 10 per cent of the F_2 progenies scored 0, 21 per cent scored 1, 25 per cent scored 3, 20 per cent scored 5, 11 per cent scored 7 and 10 per cent scored 9. The plants which showed severe symptoms of zinc deficiency such as high leaf bronzing, drastic reduction in height and very poor tillering died subsequently. Mortality rate of 12 per cent was recorded. The plants which died had a score of predominantly 9 and 7. This demonstrates that Zn def scores could be used for identifying plants highly susceptible to zinc deficiency. Similar findings has reported that the effect of low Zn availability in rice have usually been regarded with the occurrence and extent of leaf bronzing as indications of zinc deficiency (Cayton *et al.,* 1985; Qadar, 2002). Quijano- Guerto *et al.,* 2002 compared mean yields under non-Zn-deficient conditions of Zn-deficiency tolerant and susceptible lines in field trials and classified the lines as susceptible or tolerant based on their mean scores over years stating that tolerance can be identified satisfactorily by scoring for visual symptoms in early growth stages in well-managed Zn-deficient soils and this simple and effective screening procedure lends itself to large-scale screening, for example mutant or other populations to map genes responsible for tolerance.

Single plant yield recorded highly significant positive correlation with days to 50% flowering (0.937**), days to maturity (0.912**), plant height (0.813**), number of productive tillers (0.827**), panicle length (0.791**), number of filled grains (0.699**), spikelet fertility percentage (0.814**) and plant biomass (0.816**). Suma *et al.,* 2014 reported that plant height, panicle length, number of tillers and number of productive tillers exhibited significant positive correlation with grain yield per plant under zinc deficiency. In the study to understand the effect of Zn deficiency, of 67 observations a significant difference in mean maturity of Zn tolerant and susceptible lines (t=2.1927**) and also the tolerant group had a shorter maturity (105 days) than the susceptible (122 days) by Quijano-Guerto *et al.,* 2002, recording the effects of zinc deficiency on days to maturity. They hypothesized that the early maturing cultivars are able to capture Zn when Zn is most available in the soil early in the season as the concentrations of water-soluble Zn in soil are largest at the start of soil flooding and decrease over time (Ponnamperuma, 1985). Bekele *et al.,* 2013 reported highly significant and positive correlation for grain yield per plant with number of productive tillers (r = 0.5) under Zn deficiency. Single plant yield recorded highly significant negative correlations with Zn def scores at 10 DAP, 25 DAP and 45 DAP. The relevance of relationship between Zn score and grain yield is in accordance with the finding in barley by Genc *et al.,* 2002 reporting that visual symptoms of Zn deficiency varied considerably between barley genotypes and are significantly correlated with Zn efficiency and grain yield and the visual score was used to find the gene for increased tolerance in Zinc efficient genotype. Hence, visual Zn deficiency scores are useful for genetic analysis of tolerance to Zn deficiency. The Zn def score showed highly significant negative correlation with all the yield attributing traits *viz.,* days to 50% flowering, days to maturity, plant height, number of productive tillers, panicle length, number of filled grains, spikelet fertility percentage and plant biomass highlighting that the selection for these traits under zinc deficiency could result in identifying genotypes with tolerance to zinc deficiency. Impa *et al.,* 2013 stated that Zn efficiency, which is used synonymously with Zn deficiency tolerance, reflects the ability of a plant to grow and yield well under Zn-deficient conditions. Also Singh *et al.,* 2005 reported that for a genotype to be zinc-efficient, it should not only be able to absorb more zinc from deficient soils, but should also produce more dry matter and grain yield. Improved Zn efficient types are likely to give a higher grain yield and grain Zn on Zn-deficient soil.Path analysis partitions the total correlation coefficient into direct and indirect effects and measures the relative importance of the causal factor individually (Dewey and Lu, 1959. The results of path coefficient analysis of different characters with single plant yield of rice in F_2 progenies of ADT-39 x Kotta Nel are presented in Table 3. The

lower residual effect (0.254) indicated that the characters chosen for path analysis were adequate and appropriate. Days to 50% flowering, number of productive tillers, number of filled grains and biological yield were found to have direct positive effect on single plant yield suggesting the scope for enhancement of yield by selection of these traits. These results are in agreements with earlier reports of Krishna *et al.* (2008), Panwar and Ali (2007) and Reddy *et al.* (2008).

CONCLUSION

Our study confirms that Zinc deficiency scoring is an important measure for identification of zinc deficiency tolerance and could be employed for screening large number of genotypes for zinc deficiency tolerance. It is also evident from the study that zinc deficiency severely affected all the yield attributing traits, emphasizing the importance of Zn for the proper growth, functioning and yield at all the stages of crop growth. Hence, yield under zinc deficiency could be used as the selection criteria for developing zinc deficiency tolerant rice varieties. Screening under zinc deficiency could be employed in rice breeding and included as the essential breeding objective for bridging the yield gap between the actual and the potential yield.

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Table 1. Initial Soil Characteristics

Parameter	Value	Unit	Comments
Organic carbon	0.52	(%)	Medium
pH	8.20		Slightly alkaline
EC	0.34	dS m^{-1}	Non saline
Available N	171	Kg ha^{-1}	Low
Available P (Olsen's)	7.0	Kg ha^{-1}	Low
Available P (Bray)	-		
Available K	223	Kg ha^{-1}	Medium
Available Zn	0.64	Mg kg^{-1}	Deficient

Soil texture: Sandy loam Soil type: Black Lime status: Calcareous

Table 2. Correlation Coefficients among Different Characters in 216 Individuals in F_2 Progenies of Adt-39 X Kotta Nel

Characters	10th day	25th day	45th day	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of productive tillers	Panicle length (cm)	Number of filled grains	Spikelet fertility	Plant Biomass (g)	Single plant yield (g)
10th day	1.000	0.760**	0.727**	-0.547**	-0.545**	-0.503**	-0.479**	-0.479**	-0.438**	-0.509**	-0.440**	-0.592**
25th day		1.000	0.821**	-0.655**	-0.658**	-0.614**	-0.575**	-0.610**	-0.553**	-0.633**	-0.583**	-0.672**
45th day			1.000	-0.725**	-0.713**	-0.644**	-0.639**	-0.626**	-0.568**	-0.648**	-0.640**	-0.762**
Days to 50% flowering				1.000	0.995**	0.938**	0.877**	0.919**	0.792**	0.937**	0.836**	0.937**
Days to maturity					1.000	0.959**	0.879**	0.943**	0.819**	0.962**	0.832**	0.912**
Plant height (cm)						1.000	0.836**	0.969**	0.869**	0.979**	0.788**	0.813**
Number of productive tillers							1.000	0.822**	0.740**	0.853**	0.850**	0.827**
Panicle length (cm)								1.000	0.848**	0.970**	0.777**	0.791**
Number of filled grains									1.000	0.872**	0.697**	0.699**
Spikelet fertility										1.000	0.789**	0.814**
Plant Biomass (g)											1.000	0.816**
Single Plant Yield (g)												1.000

** Significant at 1% level * Significant at 5% level

Table 3. Path Coefficient Analysis of Different Characters with Single Plant Yield Of Rice in F_2 Progenies of Adt 39 X Kotta Nel

Characters	10th day	25th day	45th day	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of productive tillers	Panicle length (cm)	Number of filled grains	Spikelet fertility	Biological yield (g)	Single plant yield (g)
10th day	**-0.076**	-0.012	-0.040	-1.151	0.577	0.144	-0.003	0.041	-0.042	0.016	-0.046	-0.592**
25th day	-0.058	**-0.015**	-0.045	-1.380	0.697	0.176	-0.003	0.052	-0.053	0.020	-0.061	-0.672**
45th day	-0.055	-0.013	**-0.055**	-1.527	0.755	0.184	-0.004	0.054	-0.055	0.020	-0.067	-0.762**
Days to 50% flowering	0.042	0.010	0.040	**2.106**	-1.054	-0.268	0.005	-0.079	0.077	-0.029	0.088	0.937**
Days to maturity	0.041	0.010	0.039	2.095	**-1.059**	-0.275	0.005	-0.081	0.079	-0.030	0.087	0.912**
Plant height (cm)	0.038	0.009	0.036	1.975	-1.016	**-0.286**	0.005	-0.083	0.084	-0.031	0.083	0.813**
Number of productive tillers	0.036	0.009	0.035	1.848	-0.931	-0.239	**0.006**	-0.071	0.072	-0.027	0.089	0.827**
Panicle length (cm)	0.036	0.009	0.035	1.935	-0.999	-0.277	0.005	**-0.086**	0.082	-0.030	0.081	0.791**
Number of filled grains	0.033	0.008	0.031	1.668	-0.868	-0.249	0.004	-0.073	**0.097**	-0.027	0.073	0.699**
Spikelet fertility	0.039	0.010	0.036	1.973	-1.019	-0.280	0.005	-0.083	0.084	**-0.031**	0.083	0.814**
Biological yield (g)	0.033	0.009	0.035	1.760	-0.881	-0.226	0.005	-0.067	0.067	-0.025	**0.105**	0.816**

Residual effect = 0.254 *, ** Significant at 5 and 1 percent level respectively Diagonal values (bold) are direct effects

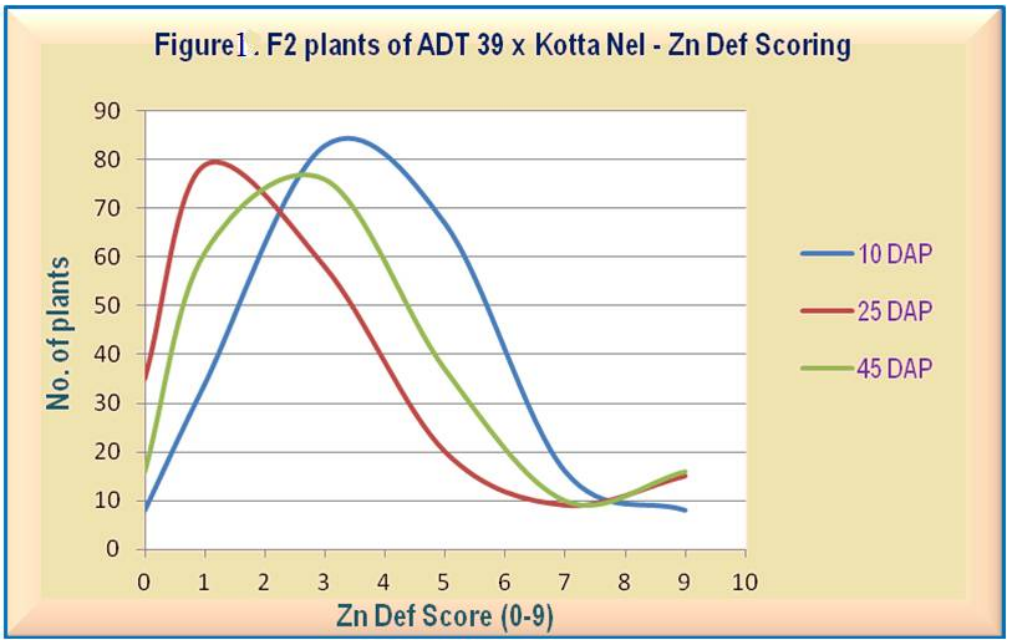

Figure 1. Normal Distribution of F_2 Progenies of Adt-39 X Kotta Nel For Zn Def Scoring

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RESEARCH ARTICLE

Character Association for Fruit Yield and Yield Traits in *Decalepis hamiltonii* Wight.&Arn.

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ABSTRACT

An Experiment was undertaken to study the correlation and path analysis in six accessions of *Decalepishamiltonii*. Correlation studyrevealed that petiole length, pedicel length and fruit diameter had significant positive correlation with yield per plant.According to path analysis, leaf width, petiole length and fruit lengthhad high positive direct effects on yield per plant. Plant height, leaf length, pedicel length, fruit diameter, thickness of pericarp and thickness of mesocarphad negative direct effects on yield. Thus based oncorrelation and path analysis, the traits viz., leaf width, petiole length, fruit length, plant height, leaf length, pedicel length, fruit diameter, thickness of pericarp and thickness of mesocarp may be considered as selection indices for high yield.

Key Words: *Decalepishamiltonii*, Correlation, Path analysis, Fruit yield.

INTRODUCTION

Decalepis hamiltonii Wight &Arn.also known as swallow root,is a woody climber belonging to family Asclepiadaceae. Its grown mostly in moist as well as dry deciduous forest of peninsular India (Gamble and Fischer, 1957) In local languages, this plant is known as of MareduKommulu, NannariKommulu, MadinaKommulu, BarreSugandhi and MareduGaddalu. It is found growing between altitude of 300 and 1200m (Reddy and Murthy, 2013). It has got medicinal importance and hence its used in various drug preparation. Moreover, it is also used as substrate for vanillin and hence icecreams andother food items are prepared out of it.

As this is a threatened plant species globally and comes under the Red list of medicinal plant as prepared by IUCN based on the threat they are facing for their existence, veryless attention has been paid for its improvement.Crop improvement through selection depends on theinterrelationship of number of componentcharacters. In the present study an attempt wasmade to evaluate the direct and indirect associationamong the various variables of six *D. hamiltonii* accessions through correlation and path analysis.

MATERIALS AND METHODS

Theexperiment was conducted at the Field Gene Bank of Division of Plant Genetic Resources, Indian Institute of HorticulturalResearch (IIHR), Bengaluru. The six accessions of *D. hamiltoniiviz.,*RET,KAR-180, RET,KAR-172, RET,KAR-178, RET,KAR-175, RET,KAR-185 and RET,KAR-54 were collected from wild by exploration and maintained here organically. Recommended cultural practices were adopted for proper growth and stand of the plants.The observations were recorded on 10 traits from five randomly selected plants from each of the accessions and itsreplications. The characters viz., plant height, leaf length, leaf width, petiole length, pedicel length, fruit length, fruit diameter, thickness of pericarp, thickness of mesocarp and fruit yield were studied.The recorded data were analysed as suggested byAl-jibouri*et al.* (1958) for correlation coefficientanalysis and by Deway and Lu (1959) for pathcoefficient analysis.

RESULTS AND DISCUSSION

The correlation study reveals the degree of interrelationshipof plant characters for improvement ofyield as well as important quality parameters in any breeding programme (Table 1). Fruit yield per plant had positive correlation with petiole length (0.17), pedicel length (0.28) and fruit diameter (0.14).Linear relationship between these mentioned characters and fruit yield per plant suggest that selection method ofcrop improvement should mainly be focussed over these characteristics.

The path analysis shows that the association ofthe independent character with dependent variableis due to their direct effect on it. If the correlation between dependentvariable and independent character is due to directeffects of the character, it reflects a truerelationship between them and hence selections can bemade for such character to improvedependent variable. But, if the association ismainly through indirect effect of the character *i.e.,*through another component character, the breederhas to select for the later through which the directeffect is exerted.In the present experiment, path analysis was done for fruit yield per plant (Table 2).Leaf width (1.40383) had highest positive effect on fruit yield followed by petiole length (0.42992) and fruit length (0.24801).Plant height (-1.60440) had highest directnegative effect on fruit yield per plant followed by leaf length (-1.46785) and pedicel length (-0.71778).Hence, based on correlation and path analysis, the characters viz., leaf width, petiole length, fruit length, plant height, leaf length, pedicel length, fruit diameter, thickness of pericarp and thickness of mesocarp may be considered as selection indices for high fruit yield.

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Table 1: Simple correlation co-efficient among important quantitative character in *Decalepis hemiltonii*.

1	-0.05	0.15**	-0.43**	-0.66**	0.45**	-0.55**	-0.18**	-0.38**	-0.65**
	1	0.97**	0.89**	0.54**	0.44**	0.13*	-0.65**	-0.1	0
		1	0.8**	0.31**	0.42**	-0.11	-0.58**	-0.1	-0.12*
			1	0.71**	0.1	0.29**	-0.4**	0.19**	0.17**
				1	0.21**	0.88**	-0.44**	0.04	0.28**
					1	0.18**	-0.94**	-0.88**	0.01
						1	-0.28**	0	0.14*
							1	0.79**	-0.21**
								1	-0.32**
									1

Critical r value at 5%= 0.47*Significant at p= 0.05, Critical r value at 1%= 0.59 **Significant at p= 0.01

1. Plant height 2. Leaf length 3. Leaf width 4. Petiole length 5. Pedicel length 6. Fruit length
7. Fruit diameter 8. Thickness of pericarp 9. Thickness of mesocarp 10. Fruit yield

Table 2: Path analysis for yield

	1	2	3	4	5	6	7	8	9
1	**-1.60440**	0.10699	0.14676	-0.22314	0.73134	0.12872	-0.04727	0.05871	0.00686
2	0.11694	**-1.46785**	1.41158	0.40062	-0.56212	0.11484	0.00123	-0.15379	0.12753
3	-0.16773	-1.47595	**1.40383**	0.35863	-0.32765	0.11297	-0.00854	-0.12282	0.08469
4	0.83272	-1.36783	1.17106	**0.42992**	-0.64042	0.02408	0.02960	-0.07892	-0.22179
5	1.63472	-1.14954	0.64082	0.38359	**-0.71778**	0.07479	0.11049	-0.05379	-0.47491
6	-0.83269	-0.67970	0.63948	0.04174	-0.21645	**0.24801**	0.01735	-0.20943	0.96392
7	0.65079	-0.01550	-0.10289	0.10919	-0.68046	0.03693	**-0.11655**	0.02960	0.06748
8	-0.69614	1.66848	-1.27440	-0.25079	0.28536	-0.38390	0.02550	**-0.13530**	0.31858
9	2.44689	2.06521	-1.58965	1.02536	-1.26548	-1.03564	-1.74772	-1.63254	**-0.00450**

Residual Effect = 0.2713

1. Plant height 2. Leaf length 3.Leaf width 4. Petiole length
5. Pedicel length 6. Fruit length 7.Fruit diameter 8. Thickness of pericarp
9. Thickness of mesocarp

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RESEARCH ARTICLE

Antimicrobial Activity of *Actinomycetes* from Soil Samples of Some Micro-Ecosystems of Satara District, Maharastra, India against Selected Human Pathogens

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ABSTRACT

In the present study, antagonistic actinomycetes from different soil samples were isolated, screened for their inhibitory activity, identified and their anti-microbial profile against different human test pathogens was determined. Three different micro-ecosystems namely, Forest area, Krishna river bank and Sugar cane farm from Satara district, Maharastra, India were chosen for the isolation of Actinomycetes from soil samples. A total of 83 actinomycetes were isolated from 45 soil samples and only five isolates (6.02%) with inhibitory activity against the sensitive strain of *Escherichia coli* (MTCC 739, IMTECH, Chandigarh) were isolated. Soil samples from the forest area, yielded greater number of antagonistic actinomycetes (10.00%) followed by the samples from Krishna river bank (7.14%) while, samples from sugarcane farm did not yield any antagonistic actinomycetes. All the five antagonistic isolates showed (100%) inhibitory activity against the test pathogen, *Shigella sonnei* and four antagonistic actinomycete isolates (80%) were inhibitory to the test pathogen, *Candida albicans*. The antagonistic actinomycete isolate FO-3 exhibited antagonism towards all the test pathogens at different levels and was subjected to chemo-taxonomical studies. Thin layer chromatography studies revealed that the isolate FO-3 belongs to cell wall chemotype-I and G:C content of genomic DNA was determined to be 70.03%.The isolate FO-3 was identified to be belonging to the genus *Streptomyces*. The outcome of the present study clearly suggests that, ecosystems rich in organic matter with a high percentage of carbon are the great sources of antagonistic actinomycetes producing novel anti-microbial compounds.

Key words: soil, antagonistic, actinomycetes, human pathogens, TLC, hyperchromicity, *Streptomyces*

INTRODUCTION

Ever since the discovery of penicillin by Alexander Fleming in 1928, diverse classes of antimicrobial compounds from various groups of living organisms both terrestrial and aquatic, are being extensively used for treating various diseases. Unfortunately, reports on the emergence of human pathogens with multiple drug-resistance are ever increasing (Spellberg *et al.,* 2004). Human pathogens which are drug resistant, pose a huge threat human medicine (Luzhetskyy *et al.,* 2007) and this has led to the exploration for novel bio-active compounds with anti-microbial properties, by the scientific community. Actinomycetes are the gram-positive, filamentous bacteria and they have been described as the greatest source of antibiotics. Diverse classes of antimicrobial compounds like, Aminoglycosides, Anthracyclines, Chloramphenicol, β-lactams, Macrolides and Tetracyclines have been isolated from this single group of bacteria and they are the sources of more than 4,000 of the naturally occurring antibiotics (Okami and Hotta, 1988). The origin of almost 80% of the world's antibiotics can be traced to actinomycetes (Pandey *et al*., 2004). Terrestrial soil samples have been a rich source of actinomycetes (Okazaki and Natio, 1986). Hence, the present study was undertaken with the objectives of isolation of actinomycetes from different soil samples, detection of inhibitory strains, determination of their antagonistic profile against selected human test pathogens and their identification using standard chemotaxonomic schemes.

MATERIALS AND METHODS

Collection of samples

Sterile polypropylene bags were used for the collection of 5-10 grams of the soil samples. Soil samples were collected aseptically from three different sampling stations namely, Forest area, Krishna river bank and Sugarcane farm from Satara district, Maharastra, India. 45 soil samples in total, with 15 samples from each of the three sampling stations, were collected aseptically and stored at refrigeration temperature till further use.

Isolation of actinomycetes

The collected soil samples were diluted using ten fold serial dilution method with sterile saline. The dilutions were thoroughly mixed with a vortex mixer for a minute and inoculation was done using spread plating onto a selective medium, Starch-Casein Agar (SCA)(Hi-Media Pvt. Ltd., Mumbai) (Table 1). SCA medium was used with two anti-fungal agents, Cycloheximide and Nystatin @ 50 μg/ml (Hi-Media Pvt. Ltd. Mumbai). Plates were incubated at room temperature for 6-7 days. The actinomycete isolates were selected based on their colony morphology with a typical chalky to leathery appearance (IMTECH, 1998) followed by gram staining, acid fast staining and subjected to light microscopy for filamentous nature, width of hyphae, nature of aerial and substrate mycelium (Cappucino and Sherman, 2004). The Gram-positive, non-acid fast isolates with aseptate hyphae were picked up and purified onto Starch Casein Agar (SCA) plates. The purified isolates were sub-cultured on SCA slants, incubated at room temperature for 6-7 days and stored at refrigeration temperature till further use.

Detection of antagonistic actinomycetes

A modified spot inoculation method of James *et al*.(1996), was employed for the primary screening of purified actinomycete isolates for their inhibitory activity, against a sensitive strain of *Escherichia coli* (MTCC 739) (IMTECH, Chandigarh). Spot inoculation of actinomycete isolates was done at the center of the Antibiotic Assay Medium (AAM) (Hi-Media Pvt. Ltd. Mumbai) (Table 2). After incubation for 6-7 days at room temperature, the plates were flooded with an overnight broth culture of the sensitive strain of *E. coli* and incubated at 37^0C for 24-48 hours. The antagonistic nature of actinomycete strains was detected by the presence of clear zones of growth inhibition of the sensitive *E. coli* strain, around their colony. Based on the extent of the zone of inhibition, the degree of antagonism of

actinomycete isolates was evaluated on a 5-point scale. Antagonistic actinomycete isolates with higher antagonistic activity were used for the secondary screening against selected human test pathogens.

Determination of Inhibitory activity of antagonistic actinomycetes

The actinomycete isolates found out to be antagonistic, were subjected to secondary screening to determine their inhibitory profile against selected Gram-negative, Gram-positive bacterial and fungal human pathogens following the modified cross-streak assay of Lemos *et al*. (1985). Nine human test pathogens, *Salmonella typhi* (MTCC 734), *Vibrio parahaemolyticus* (MTCC 451), *Salmonella paratyphi* A (MTCC 735), *Shigella flexneri* (MTCC 1457), *Shigella sonnei* (MTCC 2957), *Salmonella typhimurium* (MTCC 98), *Klebsiella pneumoniae* (MTCC 109), *Staphylococcus aureus* (MTCC 96) and *Candida albicans* (MTCC 227), procured from IMTECH ,Chandigarh were used in the assay. Inoculum from each of these inhibitory actinomycete isolates was used for making a diagonal streak onto Antibiotic Assay Medium (AAM) agar plate and incubated at room temperature for a period of 6-7 days. For streaking, the inoculation loop was straightened out and bent into L-shape so as to get a streak of 8-10 mm width. After the incubation, young culture of each of the selected human test pathogens was streaked perpendicular to the central streak of the actinomycetes culture, leaving a gap of 2 mm from the central streak. After incubation at 37^0C for 24h, the inhibitory activity of actinomycete isolates was indicated by the clear zones of growth inhibition of test pathogens near the central streak and clear zones of various test pathogens was measured in millimeters (mm). The AAM agar plates with only the test pathogens served as control.

Identification of antagonistic actinomycetes

Standard chemotaxonomic schemes of IMTECH (1998) and Goodfellow (1989) were used for the identification of actinomycete isolates with greater inhibitory activity.

Light microscopy

Actinomycete isolates with prominent antagonistic activity were sub-cultured using cover slip culture technique onto SCA medium. A novel, indigenously designed cover slip holder was used for scanning the field for the nature of aerial and substrate mycelium using a phase-contrast Nikon-make microscope (Cappucino and Sherman, 2004).

Biochemical tests and determination of cell wall chemotypes

The actinomycete isolates were subjected to casein, xanthine, urea, xylose and lactose utilization tests (Schaal, 1985). Thin Layer Chromatographic (TLC) analysis of the extracted cell wall amino acids was carried out using cellulose coated thin layer chromatography sheet, LL-Diamino Pimelic Acid (DPA), meso-DAP, DD-DAP isomer standards(Qualigens, India) and methanol: water: 6 N HCl : Pyridine (80: 26: 4: 10 v/v) as mobile phase. Visualization was carried out by spraying the plates with 0.2% (w/v) ninhydrin in acetone. The plates were heated at 105^0C for 5 minutes. Rf values of amino acids in the samples were calculated, compared with standards and identified. TLC was also carried out for the detection of characteristic sugars in the cell wall of the actinomycete isolates. Samples were run on silica gel coated TLC sheet with Glucose, Mannose, Rhamnose, Galactose, Ribose, Arabinose, Xylose as sugar standards (Qualigens, India), and acetonitrile:water (92.5:7.5 v/v) as mobile phase. The spots were visualized by spraying aniline phthalate reagent (prepared using aniline 2 ml, phthalic acid 3.3g and water saturated butanol 100 ml) and heating the plates at 100^0C for 5 minutes for visualisation. Rf values of samples were calculated, compared with standards and the sugars in the samples were identified.

Determination of the G+C content of DNA of antagonistic actinomycete isolates

The protocol of Wilson (2003) was used for the extraction of chromosomal DNA from antagonistic actinomycete isolates. For determining the temperature of melting (Tm) through hypochromicity curve, the chromosomal DNA of the isolate was heated at different increasing temperatures and the absorbance was recorded at 260 nm. The temperature was raised in increments of 2^0C from 40^0C to 96^0C and the absorbance was measured using Agilent 8453 UV-Visible Spectrophotometer with thermostat controlled cell housing and a water bath. The absorbance was recorded until its value got stabilized. The hypochromicity curve was obtained by plotting hypochromicity value (A_t /A_{25}) against temperature. Midpoint of hypochromicity curve was taken as the Tm and calculation of G+C content was done according to the formula G+C=(Tm-53.9)2.44 (Mandel & Murmur, 1968).

RESULTS

Isolation of actinomycetes

A total of 83 actinomycetes were isolated from 45 soil samples. High number of actinomycete isolates were obtained from forest area followed by Krishna river bank and Sugarcane farm (Table 3).

Detection of antagonistic actinomycetes

Out of 83 actinomycete isolates from the three sampling stations, only five isolates inhibited the growth of sensitive *E. coli* strain, constituting to 6.02% of the total number of isolates in the primary screening for the inhibitory actinomycetes (Table 4). Of the three sampling stations, the soil samples from the Forest area yielded the highest number of antagonistic actinomycetes (10.00%) followed by Krishna river bank (7.14%)(Table 4). Samples from the Sugarcane farm did not yield any of the inhibitory actinomycetes. The isolate FO-3 recorded the highest inhibitory score of 5 on a 5-point scale against the sensitive *E. coli* strain followed by FO-4 with a score of 3 and KR-2 & KR-25 each with a score of 2 (Fig 1).

Inhibitory activity of the actinomycete isolates against various test pathogens

All the five inhibitory actinomycete isolates were subjected to secondary screening to determine their inhibitory profile against selected human test pathogens. The isolate FO-3 from the Forest area exhibited prominent inhibitory activity with a zone of inhibition of ≥ 20 mm against most of the test pathogens and inhibited all the test pathogens. The isolates FO-4 and KR-25, also recorded a higher zone of inhibition of ≥ 20 mm, but against three and two of the test pathogens respectively. The isolates FO-9 and KR-2 inhibited two of the test pathogens each. The test pathogens Tp-2, *Vibrio parahaemolyticus* and Tp-6, *Shigella flexneri* did not show any growth on AAM medium and hence were not considered in the assay results(Table 5) (Fig. 2).

Identification of actinomycete isolates with antagonistic activity

All the five isolates with inhibitory activity against the sensitive strain of *E. coli* (MTCC 739) were gram positive and non-acid fast. The isolate, FO-3 which showed prominent inhibitory activity against all the test pathogens, was subjected to chemotaxonomic scheme of identification. LL-DAP and glycine were present in the cell wall hydrolysate of this isolate and hence it was classified under Cell wall chemotype-I. The whole cell sugar analysis of this isolate showed absence of characteristic sugars (Table 6) and hence was classified under the sugar pattern- C. The temperature of melting (Tm) as calculated from the hypochromicity curve was 82.6^0C (Fig 8) and the G:C content of the genomic DNA was estimated to be 70.03% (Table 6). From the above results, the isolate FO-3 was identified to be belonging to the genus *Streptomyces* (Table 6).

DISCUSSION

Isolation of actinomycetes

In the present study, high number of actinomycete isolates were obtained from Forest area followed by Krishna river bank and Sugarcane farm (Table 3). Goodfellow and Simpson (1987) reported that actinomycetes are found abundantly in cultivated and uncultivated soils in various regions throughout the world. Actinomycetes were isolated by many workers from various soil samples namely, forest soil, sugar cane field including river bank soil (Okudoh and Wallis, 2007; Bakheit and Saadabi, 2014; Patel *et al*., 2014).

Detection and Determination of antagonistic activity of actinomycetes

In the present study, samples from Forest area yielded a high percentage (10.00%) of antagonistic actinomycetes inhibitory to the sensitive *E. coli* strain (Table 4). when compared to that of Krishna river bank (7.14%) (Table 4). However, Okudoh and Wallis (2007), reported very low percentage (5.28%) of actinomycetes with inhibitory activity, isolated from forest soil samples. This may be due to the differences in the concentration of organic matter present in the soil samples of different forest areas. The reasons for high incidence of actinomycetes in general and antagonistic actinomycetes in specific, in the soil samples from Forest area in the present study, may be due to the presence of high organic load and hence, high Carbon to Nitrogen (C:N) ratio. Therefore, these soil samples favor the growth of actinomycetes as well as other bacteria also, leading to high competition between actinomycetes and other bacterial species for nutrients & space. In such micro-environments with high bacterial load, only the antagonistic actinomycetes thrive in high numbers both in terms of biodiversity as well as biomass by secreting highly diverse classes of anti-microbial compounds and inhibiting the growth of other microbes (Walker and Colwell, 1975).7.14% of inhibitory actinomycetes were isolated from soil samples of the Krishna river bank in the present study (Table 4). In contrast to the results of the present study, Okudoh and Wallis (2007) observed that none of the actinomycetes isolated from the riverside soil samples were inhibitory. In both cases, the absence or low percentage of occurrence of antagonistic actinomycetes may be due to the fact that these micro-environments might have low concentration of organic matter and hence, lesser bacterial population, leading to less competition between bacterial flora for nutrients and space. This situation might not trigger the secondary metabolite pathways in actinomycetes, responsible for the secretion of anti-microbial compounds which make the actinomycetes antagonistic.

None of the actinomycete isolates from the Sugarcane farm soil samples were found to be inhibitory, in the present study. However, Okudoh and Wallis (2007) noted in their study that 1.88% of the actinomycetes isolated from the sugarcane field were antagonistic. In both cases, the reasons for absence or very low percentage of incidence of antagonistic actinomycetes from the Sugar cane farm may be due to the application of large quantitites of inorganic fertilizers during agriculture practices, especially in sugarcane cultivation (Yadav, 2009) which has a detrimental effect on the soil microflora, leading to a significant reduction in their population (Barabasz *et al*., 2002). This might lead to very less competition for space and nutrients among the soil microbes and might not trigger the secondary metabolite pathways responsible for antagonistic activity of the actinomycetes In the present study, 6.02% of the total actinomycete isolates from different soil samples were observed to be antagonistic. However, contrary to the results of the present study, lower percentage of incidence of antagonistic actinomycetes (3.11%) were reported by Okudoh and Wallis (2007). However, in their study, Velayudham and Murugan (2012) observed a very high percentage of incidence (97.22%) of the antagonistic actinomycetes. These differences in the percentage of incidence of antagonistic actinomycetes in different studies may be attributed to the differences in concentration of organic matter leading to variations in level of competition between actinomycetes and other microbial flora for nutrients and space.

In the present study, 80.00% of the actinomycete isolates were inhibitory to the human pathogenic yeast, *Candida albicans* and 20.00% of the actinomycete isolates were antagonistic to *Staphylococcus aureus* (Table 5)(Fig. 2). However, when compared to the results of the present study, a low percentage of actinomycete isolates (55.56%) were

inhibitory to *Candida albicans* and a high percentage of actinomycete isolates (47.22%) were inhibitory to *Staphylococcus aureus* (Velayudham and Murugan, 2012). However, comparatively very low percentage of actinomycete isolates, 44.03% and 9.7%, antagonistic to *Staphylococcus aureus* and *Candida albicans*, respectively were reported by Patel *et al*.(2014). All the actinomycete isolates tested (100%) exhibited antagonism at varying levels against any one of the test pathogens in the present study (Table 5). In a study, Patel *et al.*(2014) observed that 79% of the actinomycete isolates had inhibitory activity against one or more pathogens.

Identification of antagonistic actinomycetes

The actinomycete isolate FO-3 which exhibited prominent inhibitory activity against all the test pathogens in the present study, was subjected to chemotaxonomic schemes of identification and was identified to be belonging to the genus *Streptomyces* spp. (Table 6). However, it was reported that 76% of the actinomycete isolates were identified to be belonging to the genus *Streptomyces* (Bakheit and Saadabi, 2014). In another study by Rakshanya *et al.* (2011), it was observed that 60% of the antagonistic actinomycetes isolates were belonging to *Streptomyces sp.* However, Elamvazhuthi and Subramanian (2013), reported that, 100% of their antagonistic actinomycetes were belonging to *Streptomyces sp*. Soils rich in organic matter with high carbon support higher biomass of *Strepomyces spp.* (Lee and Hwang, 2002; Bonjar, 2004). From the results of the present study it can be clearly inferred that soil samples are the great sources of actinomycetes and that actinomycetes are the greatest source of diverse anti-microbial compounds. Large number of antagonistic actinomycetes can be isolated in particular, from the soil samples of ecosystems, which are rich in organic matter with a high Carbon to Nitrogen ratio.

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Table 1: Composition of AAM (Antibiotic Assay Medium)

Media Component	Quantity (g/l)
Peptic digest of Animal tissue	6.0
Yeast extract	3.0
Beef extract	1.5
Agar	15.0
D/w	Make up to 1L
pH	7.9±0.2

Table 2: Composition of SCA (Starch Casein Agar)

Media Component	Quantity (g/l)
Soluble starch	10.0
Vitamin free casamino acids	0.3
Calcium Carbonate $CaCO_3$	0.02
Fe3SO4.7H2O	0.01
KNO3	2.0
MgSO4.7H2O	0.05
NaCl	2.0
Agar	18.0
D/w	Make upto 1L
pH	7.1±0.1

Table 3: Actinomycetes isolated from soil samples of different sampling stations

Sampling Stations	Number of Soil Samples	Number of Actinomycete Isolates
Forest Area	15	30
Krishna River Bank	15	28
Sugarcane Farm	15	25
Total	45	83

Table 4: Antagonistic Actinomycetes isolated from various soil samples of different sampling stations

Sampling Stations	Number of Actinomycete Isolates	Number of antagonistic Actinomycete Isolates
Forest Area	30	3(10.00%)
Krishna River Bank	28	2 (7.14%)
Sugarcane Farm	25	0 (0%)
Total	83	5 (6.02%)

Table 5: Zone of growth inhibition of actinomycete isolates against various human test pathogens as detected by cross-streak assay

Actinomycete Isolate	Inhibition of Test Pathogens (zone expressed in mm)								
	Tp-1	Tp-2	Tp-3	Tp-4	Tp-5	Tp-6	Tp-7	Tp-8	Tp-9
FO-3	28	NGM	30	31	28	NGM	29	18	28.5
FO-4	R	NGM	24	R	8	NGM	24	28	R
FO-9	R	NGM	R	27	R	NGM	7	R	R
KR-2	R	NGM	R	28	R	NGM	28	R	R
KR-25	R	NGM	R	25	14	NGM	24	R	7

Tp – Test Pathogen, NGM- No Growth on the Medium AAM, R- Resistant

Test Pathgoens: Tp-1, *Staphylococcus aureus*; Tp-2, *Vibrio parahaemolyticus* ; Tp-3, *Salmonella typhi*; Tp-4, *Candida albicans*; Tp-5, *Salmonella paratyphi A*; Tp-6, *Shigella flexneri*; Tp-7, *Shigella sonnei*; Tp-8, *Salmonella typhimurium*; Tp-9, *Klebsiella pneumoniae*

Table 6: Tests used for the identification of the actinomycete isolate FO-3

Test/Analysis			Result
Light Microscopy		Gram Reaction	Gram +ve
		Acid-Fast Staining	Non acid-fast
		Cellular Nature	Filamentous, Asepatate hyphae with hyphal width -0.5 - 2 µ Aerial hyphae- bear spores in spirals
Biochemical Tests		Casein decomposition	+
		Xanthine decomposition	+
		Urea decomposition	+
		Acid from Xylose	+
		Acid from Lactose	+
TLC Analysis	Cell wall amino acid	LL-DAP	Present Cell wall chemotype-I
		Meso-DAP	Absent
		DD-DAP	Absent
	Whole cell sugar pattern		Sugar pattern - C No diagnostic sugar present
G:C content of genomic DNA			70.03%

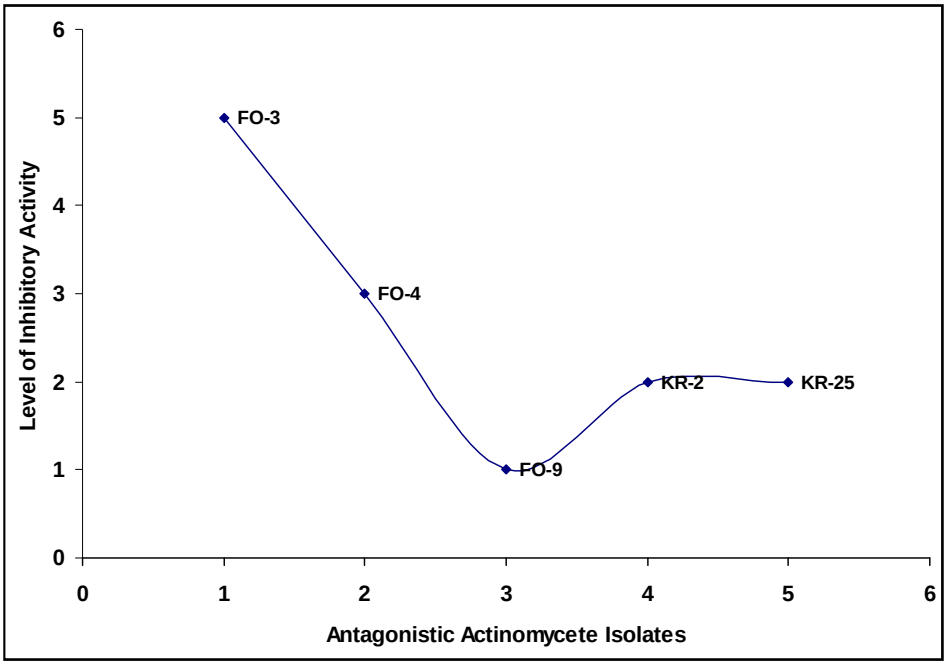

Figure 1: Inhibitory activity of actinomycete isolates against the sensitive strain of *Escherichia coli* (MTCC 739) detected and measured on a 5-point scale
5-Point Scale : 5- Highest level of inhibitory activity; 4- Higher inhibitory activity; 3- Moderate level of inhibitory activity; 2 -Lower level of inhibitory activity 1- Lowest inhibitory activity; 0- No inhibitory activity

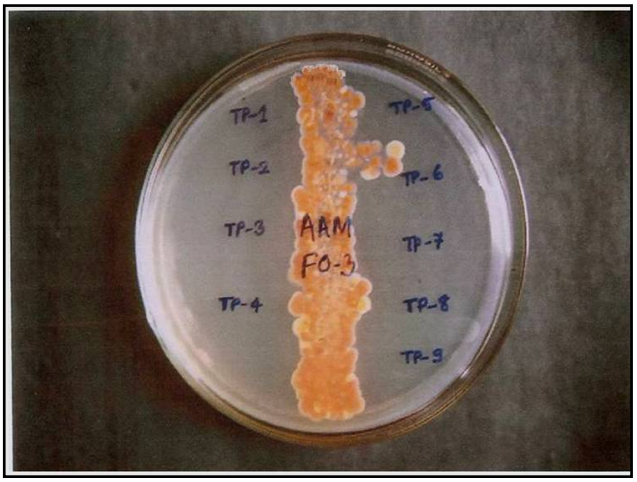

Figure 2 : Inhibitory profile of antagonistic actinomycetes against human test pathogens by cross-streak assay; Test Pathgoens: Tp-1, *Staphylococcus aureus*; Tp-2, *Vibrio parahaemolyticus* ; Tp-3, *Salmonella typhi*; Tp-4, *Candida albicans*; Tp-5, *Salmonella paratyphi A*; Tp-6, *Shigella flexneri*; Tp-7, *Shigella sonnei*; Tp-8, *Salmonella typhimurium*; Tp-9, *Klebsiella pneumonia*

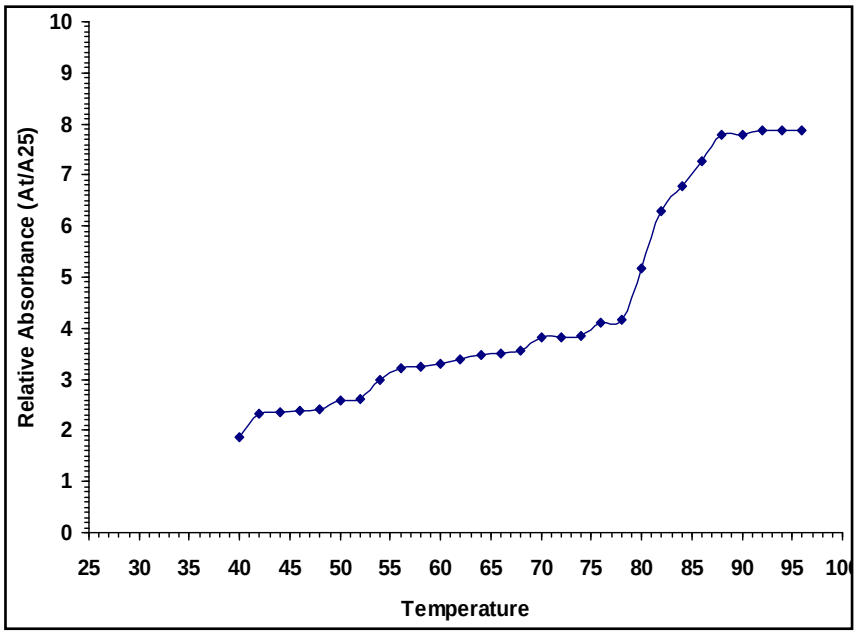

Figure 3: Hyperchromicity curve of chromosomal DNA of the antagonistic actinomycete isolate FO-3

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RESEARCH ARTICLE

Effect of Salinity on Hatching Succession of Brine Shrimp (*Artemia franciscana* Kellong, 1906): a Laboratory Experiment

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ABSTRACT

Artemia nauplii are ideal live feed for fin and shellfish larvae. However, their utilization in aquaculture hatcheries is impeded by their less culture productivity. Improvement in hatching succession of *Artemia* cyst is likely to lead to enhanced productivity. Therefore, the aim of this study is to evaluate the hatching ability of *Artemia* cyst in respect to salinity. Present research findings inferred that the hatching succession of *Artemia* cyst (*A. franciscana*) maintained at various salinity regions from 5ppt to 75ppt at the constant temperature 30°C. Our result reveals that the elevated hatching of 91.2 % and 90.5 % was obtained at 35 and 30 ppt salinity respectively with 48 hrs of incubation. However no hatching was noticed at 75 ppt salinity.

Key words: *Artemia franciscana*, salinity, cyst, hatching ability, *Artemia* nauplii

INTRODUCTION

The brine shrimp *Artemia* nauplii is need for newly born fish larvae as live food (Sorgeloos et al., 2001). *Artemia* is an excellent food source for all aquatic larvae (Hoa, 2002). *Artemia* cyst consumption increased exponentially in response to the booming shrimp and fish hatchery industries (Lavens and Sorgeloos, 2000). *Artemia* is a cosmopolitan halophilic microcrustacean and the most conspicuous inhabitant of hypersaline lakes, salt ponds, lagoons, and man-made saltworks. The genus has a worldwide distribution and is composed of well described bisexual species and parthenogenetic lineages with diverse ploidy levels (Gajardo and Beardmore, 2012; Scalone and Rabet, 2013).

Artemia is considered as an irreplaceable live feed for larval rearing of finfish and shellfish species (Sorgeloos et al., 2001; Bengtson et al., 1991; Sorgeloos et al., 1998). The demand of *Artemia* has increased with the rapid development of aquaculture industries; still the *Artemia* cysts harvested from the traditional harvesting ground of Great Salt Lake in Utha (USA), and also decline the cyst yield due to unfavorable biotope condition (Stephens, 1998; Lavens & Sorgeloos, 2000). The shortage of *Artemia* cyst has intensified the exploration of alternative resource of *Artemia* population identified in conditional Asia (Triantaphyllidis et al., 1994, 1997a., 1998; Xin et al., 1994). The natural populations of *Artemia* usually inhabit in hypersaline environment including man-made solar saltworks (Bowen et al., 1985, 1998). *Artemia* spp. plays a dominant role in the ecosystems of hypersaline waters, and often they are the only animals in these extreme biotopes (Anufriievaa et al., 2014).Vanhaecke and Sorgeloos (1989) stated that hatching percentage and growth performance of *Artemia* affected by temperature and salinity. Thoeye et al., (1987) have studied that the importance of temperature and salinity on the growth of *A. franciscana*. *Artemia* can survive the maximum temperature of 36^{o}C (Hoa, 2002). The production yield of *Artemia* is higher during the optimum level of temperature and salinity (Vos and Tansutapanit, 1979).

MATERIALS AND METHODS

One gram of *Artemia franciscana* cyst (GSL-Great Salt Lake) was chlorinated for disinfection, and added to each one liter of filtered saline water in conical cylinder filled with various salinities viz. 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 ppt salinity. The constant temperature of 30^{o}C, pH 8, and 2000 lux light was maintained and provided with vigorous aeration for cyst hatching. The experiment was extended for 48 hours and observed the hatchingability of the *Artemia* cyst at every three hours of experiment according to Sorgeloos et al. (2000 & 2001).

RESULTS AND DISCUSSION

The hatching ability of *A. franciscana* cyst observed for 48 hrs duration with different salinities from 5ppt to 75ppt with the constant temperature of 30^{o}C. It is inferred that the cyst hatching ability varied based on the salinity concentration, 5ppt salinity shows the 10.5% hatching within 48 hrs, while the least hatching of 2.85% was obtained at 27th hrs of experiment (Fig.1). *Artemia* hatching ability in 10ppt of salinity was 12.3% at 24th hrs and minimum hatching was 3.1% (Fig.2). The maximum hatching succession of 15.5% was found at 15ppt of salinity while the least hatching (4.5%) were recorded at 24th hrs (Fig.3). Hatching percentage in 20ppt salinity was 19.1% and the lowest hatching rate of 2.5% was observed at 21st hrs (Fig.4). The lowest hatching percentage of 3.9 % was observed at 25ppt salinity on18th hrs while the highest hatching was noticed as 35.7% (Fig. 5). At 30ppt salinity the hatching succession was low (5.8%) on 15th hrs while high hatching of 90.5 was observed on 24th hrs. (Fig.6). At 35ppt salinity hatching was found low (5.6 %) on 9th hour of experiment while the maximum hatching (91.2 %) was noticed on 24th hrs (Fig.7).

At 40ppt salinity *Artemia* cyst hatching was found lowest (5.9%) at 15th hrs experiment whereas the maximum hatching percentage of 88.5 was noticed (Fig.8) at 15th hr. At 45ppt salinity, low hatching (6.2%) was found at 21st hrs (Fig.9). Hatching ability at 50 ppt salinity was found low (3.5%) at 27th hrs while the maximum hatching was procured at 45th hrs (Fig.10). In case of 55ppt salinity, the least hatching of 5.3 % was noticed on 30th hrs while the elevated hatching (15.6%) was achieved on 48th hrs.As case of 60ppt salinity the least hatching (2.5%) was found on 33rd hrs whereas the highest hatching of 9.6 % was observed at 48th hrs (Fig.12). At 65ppt salinity, hatching was low (2.3%) at 39th hrs and maximum hatching (5.6%) was found at 48th hrs of experiment (Fig.13). Lowest hatching of 1.5% was found at 45th hrs in 70ppt of salinity and highest hatching (2.3%) was observed at 48th hrs (Fig.14). As case of 75ppt salinity the hatching was found initiated on According to the experimental results the maximum hatching percentage was observed at the salinity range between 30 to 35ppt within 24hrs, whereas the *Artemia* cyst hatching ability was observed the salinity range 5ppt>10ppt>15ppt>20ppt> 25ppt>30ppt>35pp<40ppt <45ppt<50ppt<55ppt <60ppt<65ppt<70ppt. While the *Artemia* cyst unable to hatch the salinity range below 5ppt and above 70ppt (Fig.15).

The increasing salinity is typically positively correlated with cyst density due to osmoregulation (Hansen et al., 2012). Egg hatching varied between the temperature and salinity (Hansen et al., 2010; Uye and Fleminger. 1976). As per the environmental condition the hatching of eggs moderated based on the heat and salinity resistant (Guisande and Harries, 1995; Marcus and fuller, 1986; Couch et al., 2001).Salinity is an important water quality parameter for good mechanism of osmoregulation (Davenport and Healy, 2006). Emimanifar et al (2014) described that the diversity of *Artemia franciscana* in Great Salt Lake (USA) was higher at hyper salinity water and its density and diversity varied based on the salinity. Luis Vargas-Chacoff et al (2009) stated that the different condition of salinity and temperature, change the regular activity of the *Artemia*. In our experiment highest hatching was noticed at the salinity between 20 and 35 ppt as agreed earlier by Sorgeloos (1980). Ahmed et al (1977) have also noticed the highest hatching of *Artemia* cyst at 20ppt of salinity at 48 hrs of incubation. However the present study showed that the optimum hatching of *Artemia* cyst obtained at 30 to 35ppt of salinity within 24hrs incubation.

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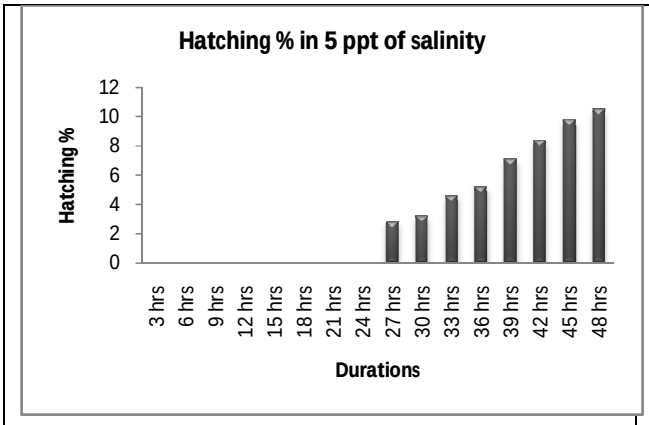

Fig.1. Hatching succession in 5 ppt of salinity

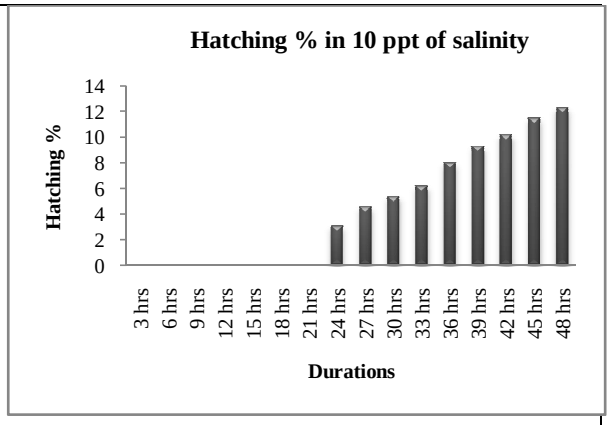

Fig.2. Hatching succession in 10 ppt of salinity

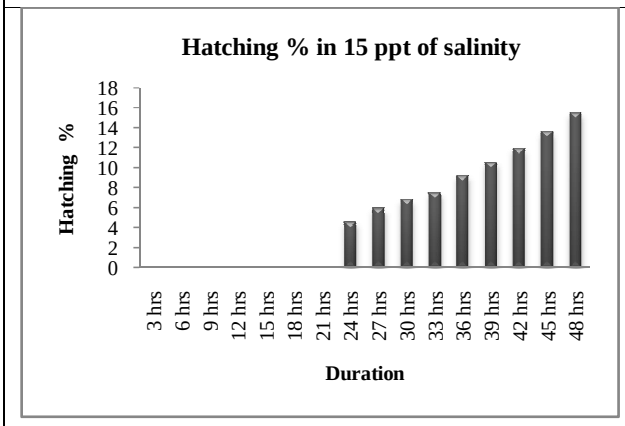

Fig.3. Hatching succession in 15 ppt of salinity

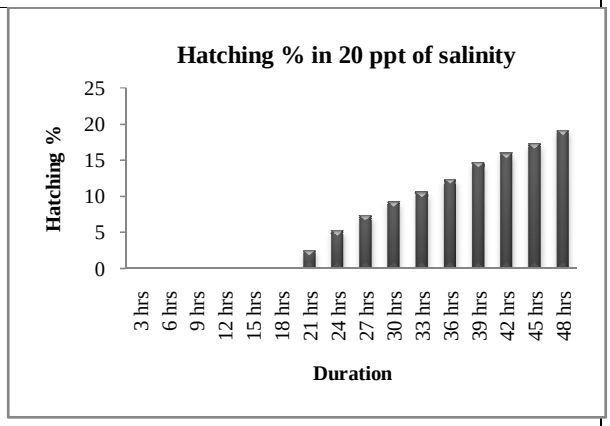

Fig.4. Hatching succession in 20 ppt of salinity

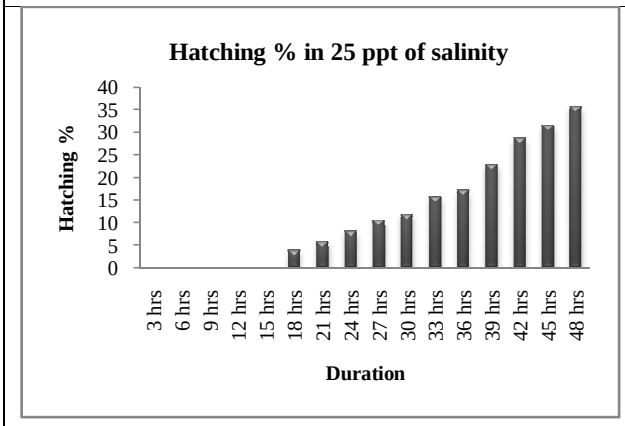

Fig.5. Hatching succession in 25 ppt of salinity

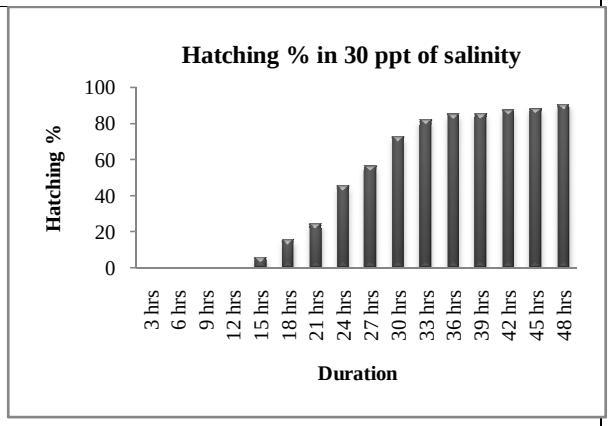

Fig.6. Hatching succession in 30 ppt of salinity

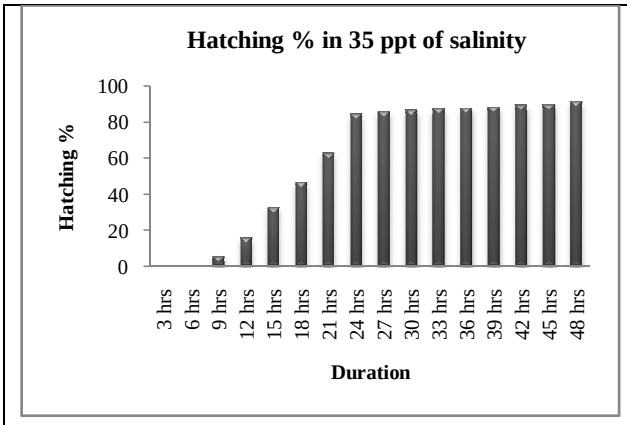

Fig.7. Hatching succession in 35 ppt of salinity

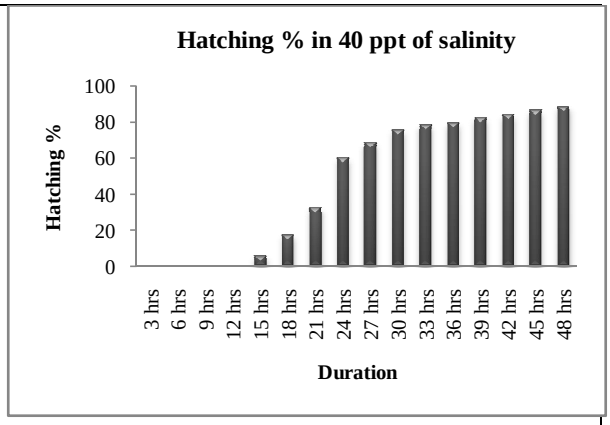

Fig.8. Hatching succession in 40 ppt of salinity

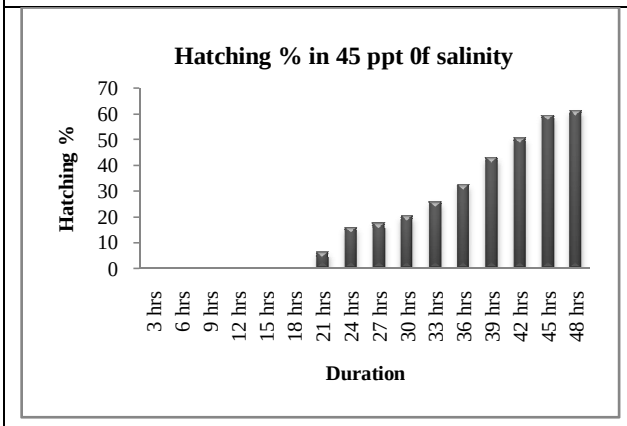

Fig.9. Hatching succession in 45 ppt of salinity

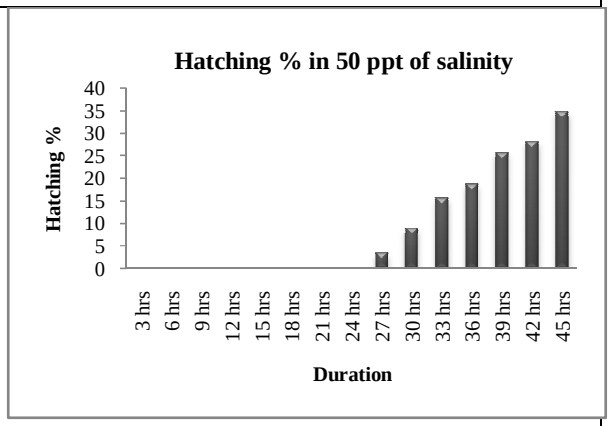

Fig.10. Hatching succession in 50 ppt of salinity

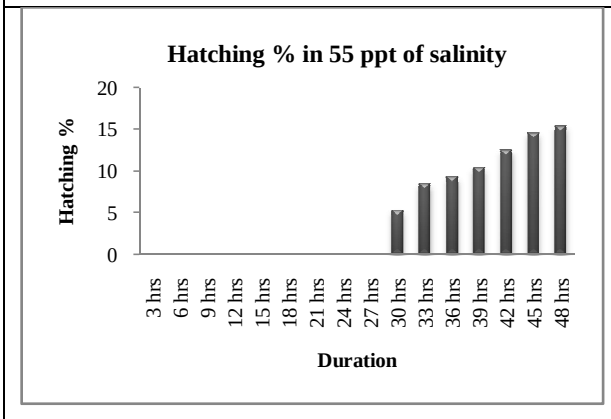

Fig.11. Hatching succession in 55 ppt of salinity

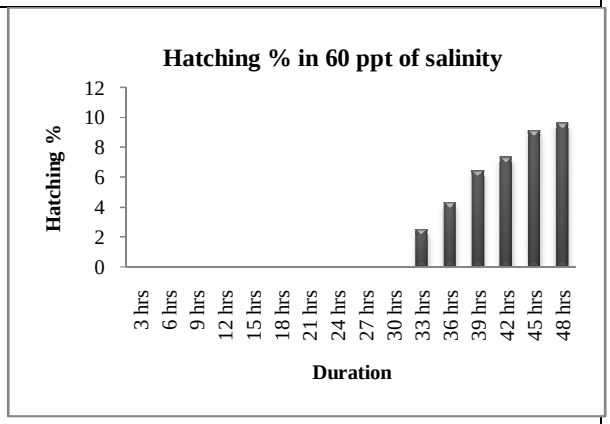

Fig.12. Hatching succession in 60 ppt of salinity

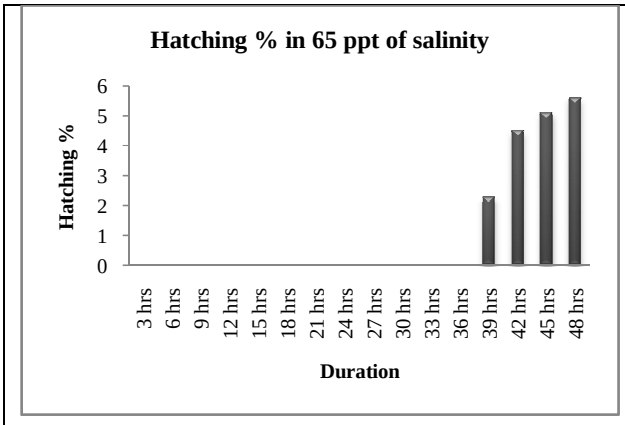

Fig.13. Hatching succession in 65 ppt of salinity

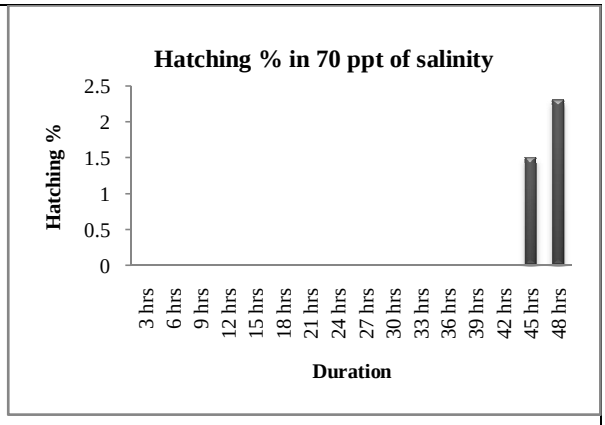

Fig.14. Hatching succession in 70 ppt of salinity

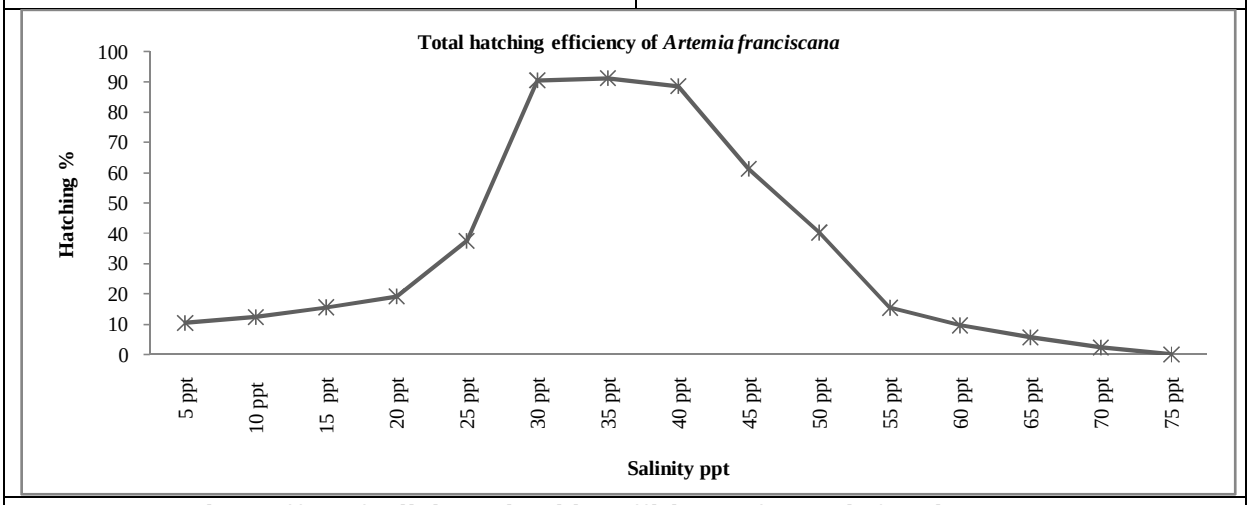

Fig.15.Effect of salinity on hatching efficiency of *Artemia franciscana* cyst

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RESEARCH ARTICLE

An Alternative Therapy of an Insulin Sensitizer Drug to the PCOS Induced Rats with a Common Perennial Vine

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ABSTRACT

Polycystic ovarian syndrome is a functional disorder of indistinct etiology. The symptoms are irregular menstrual cycle, acne, alopecia, hirsuitism, hormonal imbalance, obesity type 2 diabetes, cardiovascular disease and finally show the way to infertility. The main theme of this study is to induce PCOS in female Wistar rat models and treating the rat with an insulin sensitizer drug for long term. *Pergularia daemia* is a perennial vine belongs to Apocynaceae family found in the road sides were given to the animal to suppress the glucose level which is one of the symptom for PCOS. Reduction of the lipids and glucose is an extraordinary result found at the end of the study. Thus we conclude that a *Pergularia daemia* is an alternative effective natural herb than the allopathic medicine – metformin in treating the symptoms of PCOS in rats and this may be an effective medicine to be used in the human in future.

Key Words: Metformin, *Pergularia daemia*, polycystic ovary syndrome, Testosterone propionate, hyperandrogenism.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogenous disorder affecting 5- 10% of women of their reproductive age (1, 2). The etiology remains unclear. It chiefly affects the reproductive, metabolic and endocrine system of the body where it is the most common cause of anovulatory infertility (3, 4). It is also known as Stein and Leventhal syndrome. In PCOS, there is presence of multiple cysts which is caused by detain of follicle development at an immature stage.

Even though the mechanism behind anovulation is not distinct, it is very clear that the genetic and environmental factors play an important role in the development of this disorder (5, 7). Hyperinsulinaemia play a role in the pathogenesis of PCOS by promoting abnormal androgen production and disturbing the folliculogenesis and menstrual cyclicity (8, 9). The major features of this syndrome are acne, obesity, hirsutism, high level of androgen and infertility. The present treatment for PCOS includes various allopathic drugs like nafarelin (10), clomiphene (11), spironolactone (12) and metormin (13). Among which, metformin is commonly used drug for PCOS. It is the first insulin sensitizing drug to be used for this syndrome. It helps in the decrease of serum free testosterone, increase sex hormone binding globulin and improves ovulation in PCOS women (14).*Pergularia daemia,* commonly known as "*Veliparuthi*" in Tamil and "Uttaravaruni" in Sanskrit. In traditional medicine, the plant is used as anthelmentic, laxative, antipyretic, expectorant, infantile diarrhoea, and malarial fevers (15, 16, 17). The aerial part of the plant has many pharmacological activities like hepatoprotective (18), anti-diabetic (19), analgesic and anti-inflammatory (20). Due to the side effects of the allopathic medicine, plant therapy can be implemented in the treatment of PCOS. The plant therapy has no side effects and it heals the problem in a natural way. Thus the present study aims to evaluate the ability of metformin and *Pergularia daemia* in the treatment of PCOS induced in albino Wistar rats.

MATERIALS AND METHODS

Female Wistar rats (*Rattus norvegicus*) were obtained from the King's Institute, Chennai. The albino rats with the body weight range from 150- 180gms. The animals were acclimatized to the laboratory condition at a temperature of about 22 ± 3^0c, photoperiod of 12h/12h light/ dark cycle and humidity of 45-50% and fed with pellets. The pellets were purchased from Sai Durga enterprise, Chennai. After acclimatization, the animals were used for experimental purpose. Experimental procedure was approved by the Institutional Ethical committee for research. (437/C/CPCSEA) (Ref. 03/2013).

Experimental Design

The study was conducted on 20 female albino Wistar rats, divided into four groups. The control group (Group I) that received only 1% aqueous solution of Carboxy Methyl Cellulose (Sigma-aldrich,USA) once daily p.o. The three treatment groups rats were administered with letrozole (Sigma-aldrich, USA) at a concentration of 1.0 mg/Kg p.o dissolved in 1% of CMC (2.0 ml/Kg) once daily. The treatment period was 21 days (Kafali *et al.,* 2004). Along with the normal control group, letrozole induced PCOS rats were divided into three treatment groups. The first PCOS induced rats (Group II) serves as a polycystic ovarian syndrome model. The second PCOS induced rats (Group III) were administered with 2mg/100g metformin (Sigma-aldrich, USA) for 15 days. The Group –IV, were administered with 0.5 ml of methanolic leaf extract of *Pergularia daemia* for 7 days. At the end of the each experimental period the animals were sacrificed. The blood was collected and it is centrifuged at 3000 rpm to separate the serum for the estimation of various biochemical parameters.

RESULTS

One of the diagnostic criteria for PCOS is by estimating the biochemical parameters. The High density lipoprotein (HDL) level is represented in the FIG. 1. The HDL level is decreased in the PCOS induced group than to the control group whereas in the metfromin and plant treated group, there is a increase in the HDL level in which the plant treated group is much more effective in increasing the HDL levels. FIG. 2 shows the level of Low density lipoprotein (LDL) in which the LDL level in the PCOS induced group is increased but in the metformin treated group the level has been brought down and in the plant group the level is much more equal to the normal control group. According to the FIG. 3 there is an increase in the total cholesterol level in the PCOS induced group but the level is brought down in the metformin treated group to a certain extent which is less when compared to the plant treated group. In FIG. 4 and FIG. 5, there is a rise in the triglyceride and glucose level respectively in the PCOS induced groups but

these levels has been lowered in the treatment groups where the plant treated group is more or less equal to the control group than to the metformin treated group.

DISCUSSION

The main characteristics of PCOS are insulin resistance, high androgen level and obesity (22). Hence PCOS is a heterogenous disorder, it is very difficult to find out the underlying mechanisms behind it. In the present study, we investigated the above biochemical parameters using PCOS model, we can see that there are alterations in various parameters like HDL, LDL, total cholesterol, triglycerides and glucose. The main cause of obesity in PCOS women is due to the presence of high lipid profile. There is an increase in the LDL level whereas the HDL starts to decline in the PCOS induced groups. The triglyceride and glucose level increased rapidly in the PCOS induced group but the level was brought to normal in the metformin and plant treated group. Metformin being an insulin sensitizer drug, it helps in the condition such as hyperandrogenism and hyperinsulinaemia due to the glucose utilization in the tissues. It also inhibits the hepatic glucose production and directly inhibits ovarian steroidogenesis. The plant (*Pergularia daemia*) treated group also responded well. It has the ability to bring back various parameters to the normal condition. Thus the plant possess potential efficacy in the treatment of PCOS.

CONCLUSION

PCOS is the most common endocrine disorder in females mainly in their reproductive age. There is no generalized treatment for PCOS. The symptoms will vary from each person to the other and thus it is very difficult to create a perfect treatment. Alternative therapy includes yoga, exercise, life style modifications, acupuncture, acupressure and following a healthy diet. The allopathic treatments are widely used but as time goes on it shows some side effects but in phytotherapy there is no side effects and the recovery time is less and effective. Thus the plant *Pergularia daemia* has the prospective effectiveness in the treatment of PCOS than to the allopathic drug metformin.

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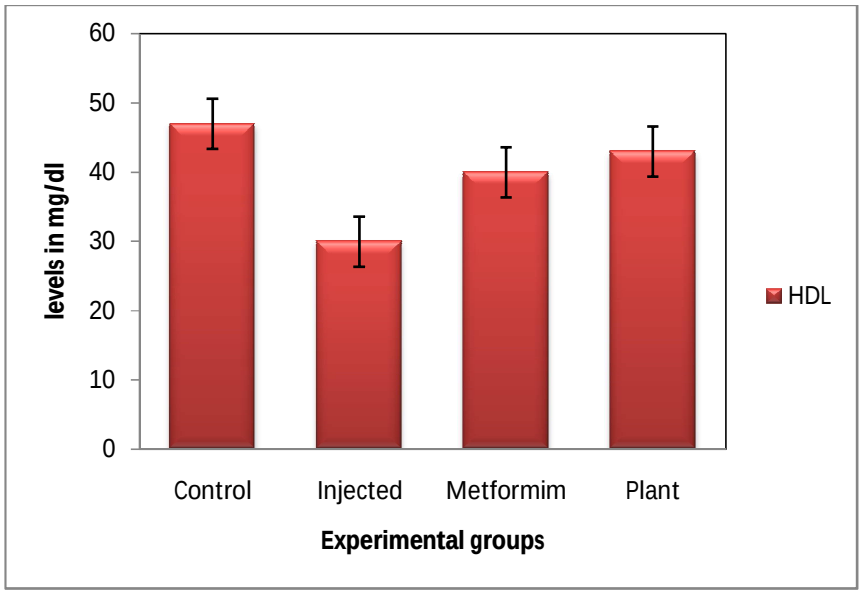

Fig. 1. Level of Hdl

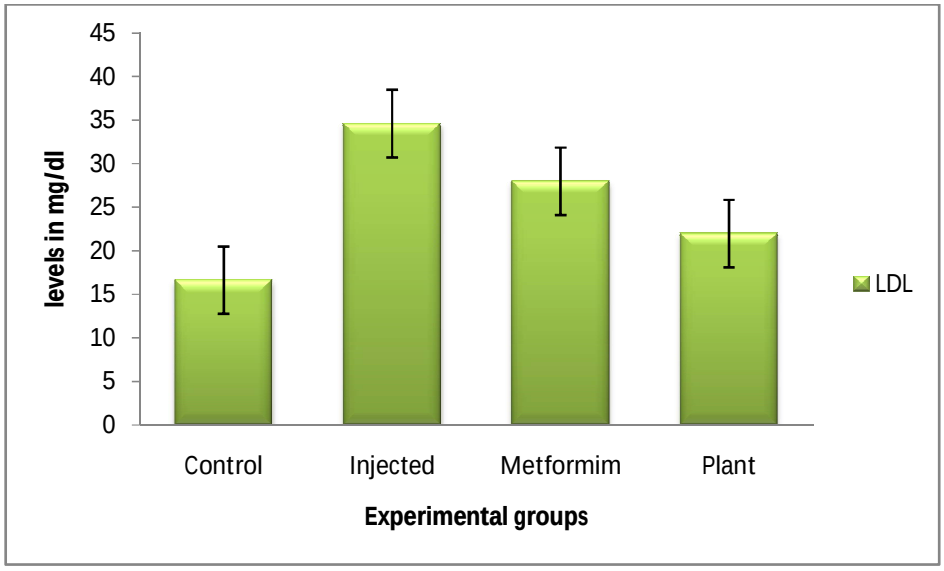

Fig. 2 Level of Ldl

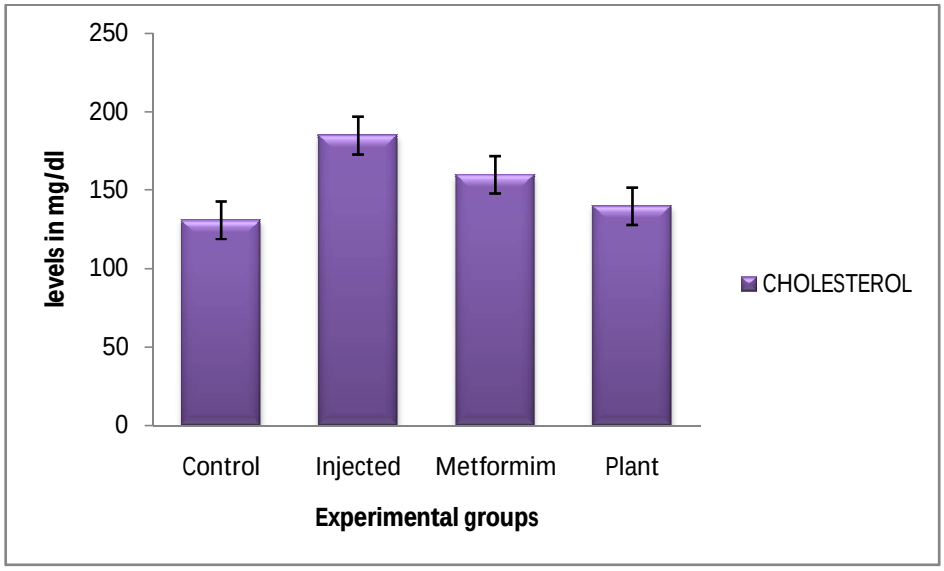

Fig. 3 Level of Total Cholesterol

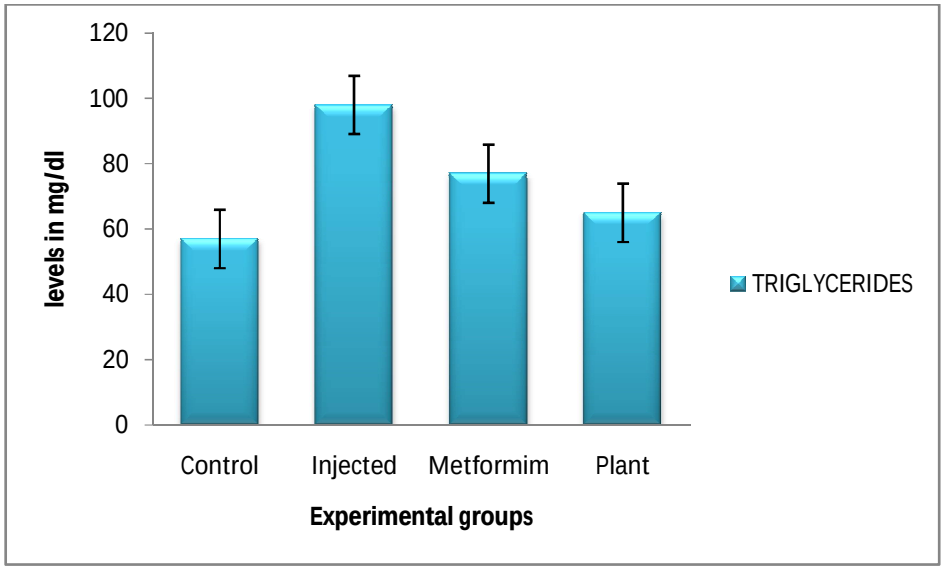

Fig. 4 Level of Triglycerides

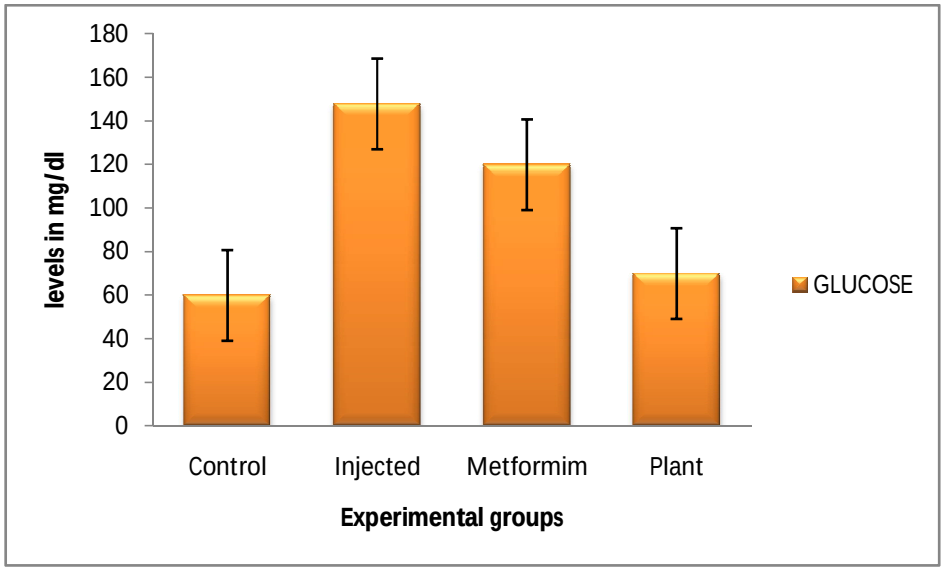

Fig. 5 Level of Glucose

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RESEARCH ARTICLE

Soil Fertility Status of Sugarcane (*Saccharum officinarum* L.) Growing Soils of Theni District, Tamil Nadu

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ABSTRACT

Soil fertility plays an important role in sustaining crop productivity of an area under favourable edaphic factors and as information on the nutritional status of soils can go a long way in developing economically viable alternatives for management of deficient nutrients in the soil. In order to increase sugarcane productivity a systematic soil fertility assessment survey was taken up in the major sugarcane growing blocks of Theni district. The cane yielding zones of the district was classified into three categories viz., low yielding (less than 75 t ha^{-1}), medium (75 to 100 t ha^{-1}) and high yielding (more than 100 t ha^{-1}). Keeping this in mind the present study was carried out to assess the fertility status of nitrogen, phosphorus and potassium in sugarcane growing soils of Theni district. The results showed that low N status (235 kg ha^{-1}) moderate P and K status (20.5 and 138 kg ha^{-1} respectively), deficient sulphur (13.9 kg ha^{-1}) indicating the soil resource base degradation and depletion of fertility status in low yielding zone. It is concluded that attention has to be given to increase nutrient status through balanced fertilization to maximize sugarcane productivity in low yielding zone.

Key Words: Soil fertility, Sugarcane Productivity, Theni district.

INTRODUCTION

In India, sugarcane is the second largest crop cultivated by 35 million farmers in 5 Mha of land with an annual production of 350 million tonnes of cane. Based on the recent projections, the country would need to produce 415

million tonnes of sugarcane from an area of 4.5 Mhawith a sugar recovery of 11 percent to meet the per capita requirement of 35 kg sweetness per year by 2020 A.D (Chand, 2007). This will deplete 0.90, 0.24 and 1.26 0 million tonnes of N, P and K from the soil which needs to be replenished (Gopalasundaram*et al*. 2012). Tamilnadu is one of the leading sugarcane producing states of the country with an average productivity of 105 t ha^{-1}. About 30 million tonnes of cane is produced annually from an area of 2.86 lakh hectares. Theni district which is situated in the western zone of Tamilnadu is a predominant sugarcane growing region covering an area of about 4427 hectares under cane cultivation with an average annual production of 4.67 lakh tonnes. In more recent times, a considerable gap between the potential and actual cane yields has been realized in different parts of Tamilnadu and specifically in Theni district where the average yield (70 t/ha) is lower than many other parts of the state. Indeed, the plateau or decreasing yield levels and declining factor productivity have been the concerns in achieving the potential yield targets. Further, the decline in nutrient status was said to be more severe in the 0-15 and 15-30 cm soil horizons (Gopalasundaram*et al*., 2012).Hence, the study resembles that a random soil survey on sugarcane growing soils of Theni district in Tamilnadu showed that the soil quality degradation caused by long term monoculture of sugarcane, imbalanced fertilization and variations among the farmers in adoption of nutrient management practices is the major limitation in achieving the maximum cane productivity.

MATERIALS AND METHODS

To assess the availability of nutrients, the sampling area has been selected based on blocks under three categories of theni district viz., low yielding (<75 t / ha), medium yielding (75- 100 t/ ha) and high yielding (> 100 t / ha) which are also indicated as low, medium and high soil quality categories for zones. About 15 surface samples (0-15 cm) and 15 subsurface samples (15-30 cm) @ 3 samples per block in three replications were collected from all the five blocks (Table 1) covering the low, medium and high yielding zones amounting to 225 number of soil samples were analyzed in detail for nutrient status of the sugarcane growing regions of Theni district. A known weight of the soil was treated with excess of alkaline potassium permanganate and distilled in the presence of sodium hydroxide. The released ammonia was absorbed in 2% boric acid and the ammonium borate was titrated against standard $H_2SO_4$for the determination of available N by alkaline permanganate method proposed by Subbiah and Asija (1956). The available Phosphorus in the soil was extracted by using 0.5 M sodium bicarbonate and the intensity of blue colour developed by ascorbic acid method was measured colorimetrically using a red filter at 660 nm. (Olsen *et al*., 1954). The available potassium in the soil was extracted by using Neutral Normal NH_4OAc and the concentration of K ions in the solution was determined using flame photometer (Stanford and English, 1949).

RESULTS AND DISCUSSION

Available Nitrogen

The available nitrogen status in the surface (0-15 cm) and sub-surface (15-30 cm) samples of the low, medium and high yielding sugarcane soils of Theni district is presented in Table 1. The available nitrogen content in surface soils of low yielding zone ranged between 169 to 282 kg ha^{-1} with a mean value of 230 kg ha^{-1}. The available N content of medium yielding zone recorded a mean available N content of 267 kg ha^{-1} with 67 percent of the soils falling under the category of low N status of less than 280 kg ha^{-1}.In general the available nitrogen content of sub surface soil samples were lesser than the surface samples and varied from 135 to 273 kg ha^{-1}, 172 to 292 kg ha^{-1} and 228 to 348 kg ha^{-1} with a mean value of 216, 245 and 287 kg ha^{-1} in low, medium and high yielding sugarcane growing soils of Theni district respectively. The available nitrogen status in the subsurface soils (15-30 cm) in all the cane yielding zones was lower compared to the surface soils which might be due to the decreasing trend of organic carbon with depth. Similar findings were also reported by Selvaraj and Naidu (2012).

Available Phosphorus

The available phosphorus status in the surface (0-15 cm) and sub-surface(15-30 cm) samples of the low, medium and high yielding sugarcane soils of Theni district is presented in Table 2. The available phosphorus content varied from 11.0 to 36.0 kg ha^{-1} with a mean value of 20.5 kg ha^{-1} in low yielding zones. The surface soils of medium yielding zones ranged between 10.5 to 78.4 kg ha^{-1} with a mean value of 32.4 kg ha^{-1}. The medium and high soil quality zones recorded higher status of available P in 73 and 93 percent of the samples respectively. The high P status of the soil may be due to continuous application of phosphatic fertilizer (DAP), bio-compost and rock phosphate enriched compost. Similar observation has been made by Muneshwar Singh *et al*., (2008) during his study on the effect of integrated nutrient management on sugarcane yield in sandy loam soils of Chidambaram taluk, Tamilnadu. The available phosphorus content of the sub surface soils ranged from 9.4 to 33.6 kg ha^{-1}, 11.2 to 56 kg ha^{-1} and 20.5 to 89.6 kg ha^{-1} with a mean value of 18.4, 29.7 and 41.4 kg ha^{-1} respectively. About 73 percent soil samples from medium yielding zone and about 93 percentof soil samples in high yielding zone recorded higher available P status of more than 22 kg ha^{-1}

Available Potassium

The available potassium status in the surface (0-15 cm) and sub-surface (15-30 cm) samples of the low, medium and high yielding sugarcane soils of Theni district is given in Table 3. The available potassium content of surface soils in low yielding zone ranging from 67 to 215 kg ha^{-1} is generally reported to be medium status and only 33 percent of soil samples were tested to be low in available K. The available K content of the soils in medium yielding zone ranged between 96 and 386 kg ha-1. The mean available K status in the low and medium yielding zones was 138 and 200 kg ha^{-1} respectively indicating moderate status of K in majority of the soil samples. The sugar factory recommendation of K2O in Theni district for sugarcane crop is 150 kg ha^{-1} and adoption of this schedule by majority of the growers besides the inherent supply of K from these light red soils have resulted in moderate K status in these soils. This is in confirmation with the findings of Verma*et al* (2005).The lesser mean available K status in the sub surface soils were 140, 239 and 426 kg ha^{-1} in low, medium and high soil quality zones respectively which might be due to intense weathering and upward translocation of potassium from lower depths along with capillary rise of groundwater. Similar results were reported in soils of Chittoor district in Andhra Pradesh.

CONCLUSION

It is concluded from the present investigation that the sugarcane growing soils showed low N status (235 kg ha^{-1}) and moderate K status (138 kg ha^{-1}) in low yielding zones due to mining of nutrients by crops aggravated by imbalanced fertilization causing lower productivity of cane grown in succession. Balanced fertilization has to be practiced for sustained and enhanced productivity of sugarcane in years to come.

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Table 1. Available Nitrogen status (kg ha^{-1}) of various Soil Quality (SQ) categories under sugarcane in Theni district of Tamil Nadu

Sample No.	Surface soil (0-15 cm)			Subsurface soil (15-30 cm)		
	Low SQ	Medium SQ	High SQ	Low SQ	Medium SQ	High SQ
1	254	304	392	226	292	348
2	235	317	348	223	286	348
3	267	298	339	213	289	336
4	254	257	464	228	241	282
5	204	292	301	197	260	320
6	226	248	286	216	237	270
7	229	254	273	208	238	252
8	204	238	318	198	220	267
9	282	254	339	273	245	312
10	238	332	288	254	267	271
11	232	257	383	241	251	310
12	267	267	263	235	260	259
13	198	201	284	201	210	235
14	194	213	296	188	204	263
15	169	185	235	135	172	228
Max	**282**	**332**	**464**	**273**	**292**	**348**
Min	**169**	**185**	**235**	**135**	**172**	**228**
Mean	**230**	**261**	**321**	**216**	**245**	**287**
SD	31.6	42.1	58.9	31.99	33.45	39.45
CV	13.7	16.1	18.4	14.8	13.7	13.8

Table 2. Available Phosphorus status (kg ha^{-1}) of various Soil Quality (SQ) categories under sugarcane in Theni district of Tamil Nadu

Sample No.	Surface soil (0-15 cm)			Subsurface soil (15-30 cm)		
	Low SQ	Medium SQ	High SQ	Low SQ	Medium SQ	High SQ
1	16.0	11.2	30.5	11.2	44.8	67.2
2	11.2	31.4	14.0	11.2	56	78.4
3	14.6	22.4	33.6	11.2	51	89.6
4	22.4	11.2	44.8	11.2	22.4	24
5	16.0	10.5	56.0	22.4	22.4	56
6	11.0	14.0	46.6	22.4	22.4	52.5
7	27.4	28.0	28.0	33.6	38	56
8	19.0	23.6	22.4	33.6	33.6	20.5
9	22.4	33.6	33.6	33.6	33.6	21
10	11.2	67.2	38.0	22.4	21	22.4
11	22.4	78.4	43.0	22.4	22.4	24
12	30.4	60.8	60.5	9.6	22.4	33.6
13	36.0	31.0	36.0	11.2	11.2	22.4
14	23.6	22.4	30.0	9.4	22.4	22.5
15	23.6	40.0	33.6	11.2	22.4	30.8
Max	**36.0**	**78.4**	**60.5**	**33.6**	**56**	**89.6**
Min	**11.0**	**10.5**	**14.0**	**9.4**	**11.2**	**20.5**
Mean	**20.5**	**32.4**	**36.7**	**18.4**	**29.7**	**41.4**
SD	7.35	21.06	12.11	9.36	12.72	23.33
CV	35.9	65.0	33.0	51.0	43.0	56.0

Table 3. Available Potassium status (kg ha^{-1}) of various Soil Quality (SQ) categories under sugarcane in Theni district of Tamil Nadu

Sample No.	Surface soil (0-15 cm)			Subsurface soil (15-30 cm)		
	Low SQ	Medium SQ	High SQ	Low SQ	Medium SQ	High SQ
1	137	262	390	146	269	399
2	119	251	245	114	586	259
3	166	187	265	165	189	659
4	160	305	298	171	324	512
5	131	219	530	133	431	547
6	215	240	382	209	252	388
7	174	231	715	170	228	1004
8	195	242	546	207	243	582
9	210	386	695	226	390	1210
10	131	143	198	116	132	197
11	108	128	240	105	110	116
12	102	110	217	99	122	183

13	67	102	112	88	116	124
14	77	96	188	86	94	100
15	84	97	280	65	95	109
Max	**215**	**386**	**715**	**226**	**586**	**1210**
Min	**67**	**96**	**112**	**65**	**94**	**100**
Mean	**138**	**200**	**353**	**140**	**239**	**426**
SD	47.3	86.4	186.5	49.76	143.73	336.35
CV	34.2	43.2	52.8	33.1	44.6	52.8

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RESEARCH PAPER

Effect of Culture Filtrate of *Sclerotinia sclerotiorum* on Seed Germination and Seedling Vigour of Indian Mustard

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ABSTRACT

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is an important oilseed crop of family *Crucifereae* (*Brassicaceae*). Amongst the fungal pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal organism of Sclerotinia rot (SR) is the most ubiquitous, omnivorous, soil-borne and destructive plant pathogen, inciting disease on more than 500 plant species. The culture filtrates of *S. sclerotiorum* prepared on Potato Dextrose Broth (PDB). The effect of culture filtrate concentrations of *S. sclerotiorum* were observed on seed germination, seedling vigour (radicle and plumule length). The inhibitory effect of culture filtrate was observed very low at 25 % concentration. At 100 % concentration the germination and seedling vigour were found least. Variety varuna resulted minimum seed germination, plumule length, and radical length and vigour index at all concentrations of culture filtrate.

Key words : Culture filtrate concentrations, *Sclerotinia sclerotiorum,*Seed germination, Seedling vigour , Indian mustard

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is an important oilseed crop. The largest cultivation of *Brassica* crops is done for edible vegetable oil production. They also play a vital role in world's agricultural economy and are recognized for their long history of cultivation and varied uses. Mustard is the second most important oilseed crop of India after groundnut in terms of area and production. Amongst the fungal pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal organism of Sclerotinia rot (SR) is the most ubiquitous, omnivorous, soil-borne and destructive plant pathogen, inciting disease on more than 500 plant species (Saharan and Mehta, 2008 and Sharma, 2014). *S. sclerotiorum* is a necrotropic pathogen, it damage to the plant tissue followed by cell death and development of soft

rot or white mould (Purdy, 1979). In, India Sclerotinia rot is threat to cultivation of oilseed *Brassica* in Rajasthan, Punjab and Haryana. 72.8 to 80 per cent disease incidence was recorded in same districts of Haryana and Punjab (Kang and Chahal, 2000 and Sharma et al., 2001). Yadav et al. (2013) reported 17.4 per cent diseases incidence from six districts of Rajasthan. Culture filtrate from different pathogenic fungi has been reported to reduce seed germination and seedling vigour. Reduction of seed germination and seedling vigour of cumin was observed in different concentrations of culture filtrate of *Fusarium equiseti* (Suthar et al., 2014). Filtrate from mycelial cultures of *Verticillum alboatrum* was found to inhibit cell growth and reduced the viability of alfalfa (*Medicago sativa*) seeds (Frame et al., 1991). The present study was therefore, undertaken to determine the effect of culture filtrates concentrations of *S. sclerotiorum* on seed germination and seedling vigour of Indian mustard varieties.

MATERIAL AND METHODS

Sclerotinia rot infected plants of Indian mustard were collected from farmer's field and isolations were made on potato dextrose agar (PDA) medium from black sclerotia present inside the diseased stem as well as from individual stem rot lesion as per procedure and purified by hyphal tip method. Small bit (5 mm dia.) from seven days old culture of pathogen was inoculated in 250 ml Erlenmeyer flasks having 50 ml of Potato Dextrose broth (PDB) and incubated at 25 ± 1°C for 20 days in BOD incubator. Fungal mycelium was separated by passing fungal culture through muslin cloth and then through sterilized Whatman No. 1 filter paper and heated at 100 °C for 2 minutes to inactivate enzymes. (Watpade and Mehta, 2013). Filtrate and sterilized water were used to make 0, 25, 50, 75 and 100 per cent of culture filtrate solution. Seeds of Indian mustard varieties namely Arawali, Laxmi, NRCDR-2, RRN-505 and Varuna, were surface sterilized with sodium hypochlorite (0.1%) and 25 seeds were placed in sterilized thrice blotter paper containing Petri plate, each watered with 10 ml of culture filtrate except 0 % and incubated in BOD chamber at 25 ± 1° C and 80 % relative humidity. Percentage of seed germination was recorded after 3 days and radicle length and plumule length was recorded after 7 days of incubation. The experimental design was completely randomized (CRD) with four replications.Vigour index was calculated by the following formula suggested by Abdual Baki and Anderson (1973). Vigour index = Germination per cent x (Radicle length + Plumule length)

RESULTS AND DISCUSSION

The culture filtrates of pathogen inhibited the seed germination as well as subsequent seedling vigour (Table 1). The inhibitory effect of culture filtrate was observed maximum at 100 % v/v concentration. It is clear from the results that toxicity of culture filtrate increases with increase in concentration. NRCDR-2 variety exhibited highest seed germination (95.25 %), maximum plumule length (72.90 mm) radical length (99.80 mm) and vigour index (16449.6) at 0 % concentration of culture filtrate. NRCDR-2 variety exhibited highest seed germination (76.25 %), maximum plumule length (19.10 mm) radical length (33.40 mm) and vigour index (4003.12) at 100 % concentration of culture filtrate, whereas variety Varuna resulted minimum seed germination (46.50 %), plumule length (8.80 mm), radical length (15.20 mm) and vigour index (1116) at 100 % concentration of culture filtrate. Toxic metabolites in the culture filtrates of *S. sclerotiorum* cause reduction in seed germination and radical and plumule length. 100 per cent concentration of filtrate was found to be more prominent. This shows that *S. sclerotiorum* produces toxic metabolites in the media in which they are grown. Pathogenic fungi may often damage their host plants by producing phytotoxins, which cause various symptoms including necrosis, chlorosis, wilting, water soaking and eventually death of plants. Similar work done by Suthar et al. (2014) on *Fusarium oxysporum* f. sp. *cumini* culture filtrate. They tested effect of culture filtrate at different concentrations (0 %, 10 %, 20 %, 30 % and 50 %) on seed germination and seedling vigour of two cumin varieties (GC-4 and GC- D). The reduction in seed germination, root length and shoot length was observed with increasing concentration of culture filtrate and also inhibitory effect was observed at 50 % v/v concentration. Bairwa et al. (2014) also reported Varuna as susceptible cultivar in their studies of biochemical and enzymatic basis of resistance against *S. sclerotiorum*.

ACKNOWLEDGEMENTS

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Table : 1Effect of different concentrations of culture filtrate on seed germination and seedling vigour of Indian mustard varieties (*in vitro*)

	Concentrations of culture filtrate*																			
	0%				25%				50%				75%				100%			
ustard riety	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index	Germination (%)	Plumule length (mm)	Radicle length (mm)	Vigour index
ruvali	91.00	72.50	95.40	15278.9	89.00	85.30	96.90	12655.8	81.75	41.60	56.20	7995.15	76.75	24.20	41.30	5027.12	65.00	16.10	30.10	3049.2
	(72.54)				(70.63)				(64.71)				(61.17)				(54.33)			
rawi	93.00	60.30	93.50	14303.4	78.50	87.10	87.90	11382.5	71.25	28.10	51.20	8650.12	62.50	19.90	31.20	3150.75	53.75	12.50	19.90	1741.50
	(74.66)				(63.08)				(57.58)				(52.24)				(47.15)			
KDDR-1	96.25	72.90	99.80	16449.6	91.75	87.30	90.70	12579.0	85.35	46.70	61.10	9915.38	81.00	29.90	47.50	6259.4	76.25	19.10	33.40	4068.12
	(77.41)				(73.32)				(67.58)				(64.16)				(60.82)			
RH-905	94.25	68.60	95.10	15346.9	85.00	85.00	86.50	12027.5	76.00	36.10	52.30	6942.4	71.75	21.90	37.20	4240.47	63.75	13.60	26.20	2537.25
	(76.13)				(68.27)				(60.67)				(57.89)				(52.95)			
ean	88.00	56.10	81.70	12126.4	74.50	44.30	69.90	8845.15	65.25	21.10	39.40	3947.62	55.00	14.80	23.30	2095.5	46.50	8.30	16.20	1116.0
	(69.73)				(59.67)				(53.88)				(47.87)				(42.99)			
±SE	1.88	1.41	1.97		2.08	1.25	2.07		1.77	0.70	0.93		1.47	0.43	0.69		1.36	0.23	0.46	
D(p=0.05)	5.75	4.39	6.07		6.43	3.87	6.37		5.46	2.17	2.86		4.54	1.34	2.14		4.17	0.71	1.39	

* Average of four replications
Figures given in parentheses are angular transformed values

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RESEARCH ARTICLE

The Scientific Documentation of Diversity of Butterflies in Bishop Heber College Campus, Trichy District, Tamil Nadu.

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ABSTRACT

The Bishop Heber College located in Trichy district of Tamil Nadu, India, is a scattered agricultural patches present in the vicinity. The college campus includes a gardens, grass land and trees (3 acres), which cover a quarter (25 acres) of the total area. A detailed survey of butterflies was conducted from June 2011 - December 2013. The prime objective of this present study is scientific documentation of diversity of Butterflies in Bishop Heber College Campus, Trichy District, Tamil Nadu. Two year survey of 80 butterflies species belonging to five families were recorded from the Bishop Heber College campus. The highest number of butterflies was recorded belonging to the family Nymphalidae (24 species) followed by Pieridae (19 species), Papilionidae (7 species),Hesperiidae (10 species) and Lycaenidae (20 species). The highest number of butterfly species was recorded in the Vasantham garden, PG blocks, and community college areas. Observed species were grouped in five categories on basis of number of species. The butterflies were categorized as Very Common (VC) 30% (24 species), Common (C) 25% (20 species), Uncommon (UC) 24% (19 species), Occasional (O) 12 % (10 species) and Rare (R) 9% (7 species). Generally a large number of butterflies were seen in the months of October, November, December and January (Post - monsoon) but thereafter declined in early summer (March). Few butterflies like Nilgiri clouded yellow, White-Banded Royal, Double-Tufted Royal and Peacock Royal are very rare butterflies that were observed only once during the study period.

Kew words: Lepidoptera, College campus, Habitats, Status, Abundance,

INTRODUCTION

Insects comprise more than half of earth's diversity of species. Butterflies are lovely, colourful scaled wing insects belonging to the order Lepidoptera: Rhopalocera of class Insecta. Butterflies are widely appreciated for their aesthetic value which was considered as an important ecological indicator. (Chakara varthy et al.,1997). Butterflies provide economic and ecological benefits to the human society. Healthy biological communities depend on butterflies as pollinators, seed dispersers, herbivores, predators and prey There are about 1, 40,000 species found all over the world. As larvae, butterflies are typically host specific and often show a "Botanical instinct" (closely related butterfly species choose closely related plants). They are also sensitive to temperature, weather conditions and habitat quality to serve as good indicators of environmental change (Sparrow et al., 1994 and Habrial, 1992). Environmental conditions play a key role in defining the function and distribution for plants and animals. There is an intimate association between butterflies and plants as their lives are exceptionally interlinked (Feltwell, 1986) to different patterns in their distribution depending on the availability of their food plants and suitable environment. Therefore seasonal patterns play an important role for butterflies and its host plants.

MATERIALS AND METHODS

Six locations were selected which were monitored every day from June 2011 - December 2013. Observations were made through transects each of 500 m length with 20- 40 m on either side. Some species which are difficult to identify, were caught by hand net and released after identification to their habitat. Every day ten transects were covered from 07:00-13:00hr and 14:00-17:00hr. Abundance of butterflies in different habitats were recorded. Mostly photograph and video documents was done. Species identification was made using various field guides and other available literature.Map showing the locations of the study area of Bishop Heber College, Trichy, Tamil Nadu

Study Area

The Bishop Heber College located in Trichy district of Tamil Nadu, India, is scattered agricultural patches are present in the vicinity. The college campus includes gardens, grass lands and trees (3 acres), which covers a quarter (25 acres) of the total area. The entire campus is very rich in ornamental and wild plants providing sites for butterfly nectaring and egg laying. The short duration of the study precluded a complete documentation of the butterfly fauna of the area. College campus has a rich and diverse butterfly fauna because of the availability of wide range of habitats. Butterfly species density was assessed quantitatively across different habitats. The entire campus was divided into six different habitats, which were divided on the basis of vegetation, such as **Vasantham Garden**, (Grass land, Trees, herbs, shrubs, and flowering plants) **PG Block** (Trees, herbs, shrubs, and flowering plants) **Hostel Areas** (Grass land, Trees, herbs and shrubs) **A ground** (Grass land, Trees) **B ground** (Trees, wild herbs and shrubs) and **Community college** (herbs, shrubs, and flowering plants). The sampling was carried out at different habitat during June 2011-2013.

RESULTS

During a two year survey 80 species of butterflies belonging to five families was recorded from the Bishop Heber College campus. The highest number of butterflies was recorded belonging to the family Nymphalidae (24 species) followed by Pieridae (19 species), Papilionidae (7 species), Hesperiidae (10 species) and Lycaenidae (20 species).This study revealed that Nymphalidae was most dominating family with highest number of species in all the six sites. The highest number of butterfly species was recorded in the Vasantham garden, PG blocks, and community college areas. Observed species were grouped in five categories on basis of number of species in the field. The butterflies were categorized as Very Common (VC) 30% (24 species), Common (C) 25% (20 species), Uncommon (UC) 24% (19 species), Occasional (O) 12 % (10 species) and Rare (R) 9% (7 species). Generally a large number of butterflies were

seen in the months of October, November, December and January (Post- monsoon) but thereafter declined in early summer (March). Nilgiri clouded yellow, White-Banded Royal, Double-Tufted Royal and Peacock Royal are very rare butterflies that visited the campus only once during the study period. Most butterfly species were observed from the monsoon (hot/wet season) and post – monsoon to early winter (cool/wet season).

DISCUSSION

In our observation on the butterfly species diversity and availability of larval host plant from June 2011 - December 2013, six habitats of Bishop Heber College were selected. A total of 80 species of butterflies were recorded under five families. Among which, 4 species Nilgiri clouded yellow (PIERIDAE) White-Banded Royal, Double-Tufted Royal and Peacock Royal (LYCAENIDAE) are newly recorded from this area. Among the 50 species of butterflies, *Borbo cinnara, Baoris farri,* (*HESPERIIDAE,) Graphium doson, Graphium Agamemnon, Papilio polytes, Atrophaneura aristolochiae, Atrophaneura hector, Papilio demoleus(PAPILIONIDAE) Eurema hecabe, Eurema brigitta, Eurema blanda, Eurema laeta, Catopsilia Pomona, Catopsilia pyranthe, Jamides bochus, Pseuudozizeeria maha, Chilades parr (PIERIDAE), Danaus genutia, Danaus chrysippus, Euploea core, Melanitis, Byblia ilithyia, Acraea violae, Hypolimnas bolina* and *Hypolimnas misippus(NYMPHALIDAE)* occurred throughout our study period (August - January). Most of the butterfly species were recorded in Vasantham garden and Community college. Mora than 130 species of host plant were also recorded in the six habitats.

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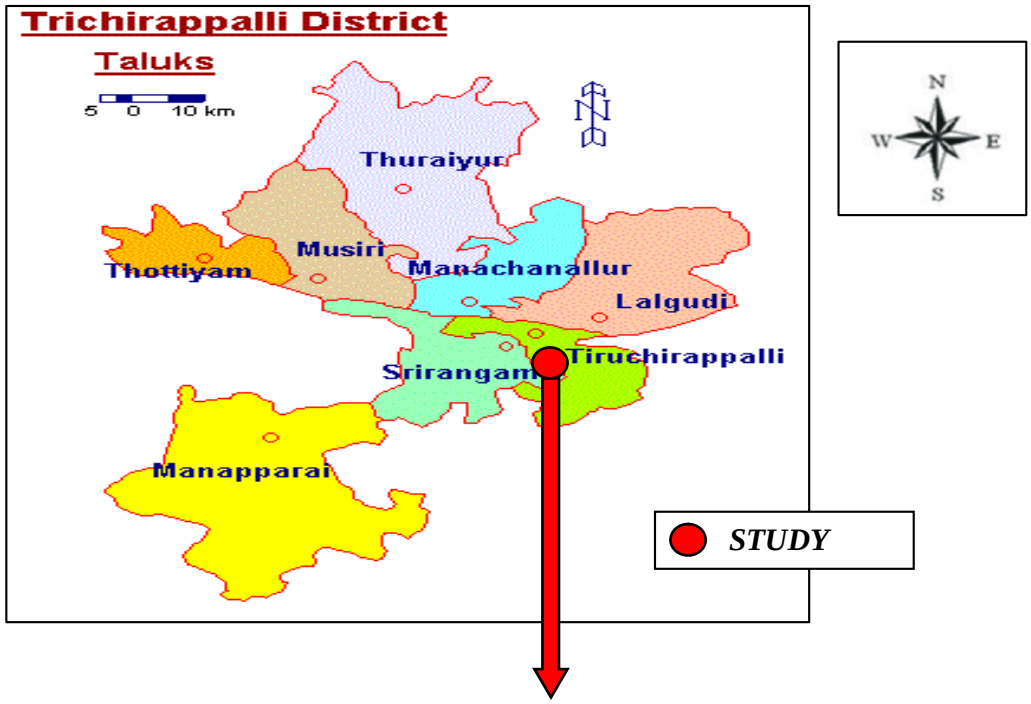
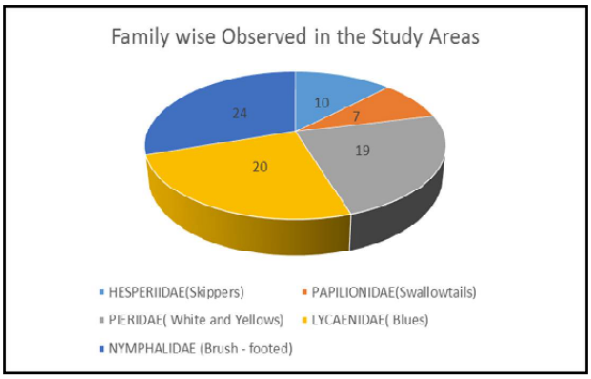
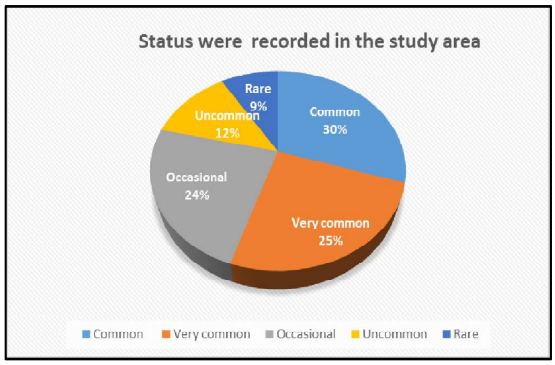


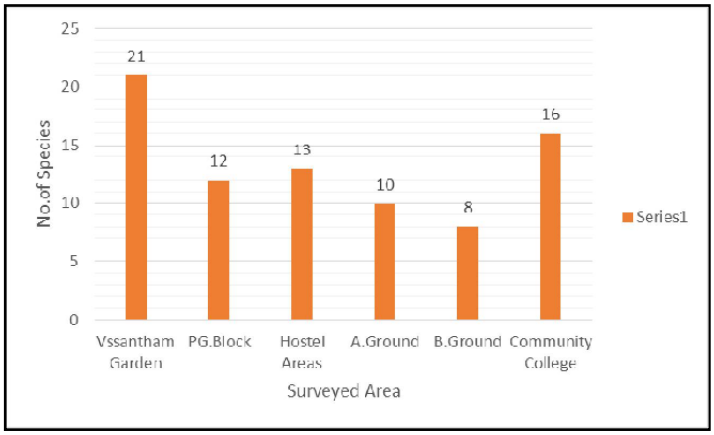
Fig.1.Study Area Map

Fig.2.Study Area





Graph 1. Comparison of Butterfly species richness across various sites



Nesarajan and Horne Iona Averal

Common banded awl
Hasora chromus

Brown awl
Bavaria exclamationis

African marbled skipper
Gomalia elma

Bevan's swift
Pseudoborbo bevani

Ceylon swift
Parnara bada

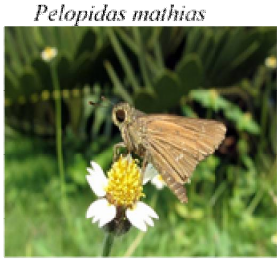
Grass demon
Udaspes folus

Indian palm pob
Suastus gremius

Paint brush swift
Baoris farri

Rice swift
Borbo cinnara

Small branded swift
Pelopidas mathias

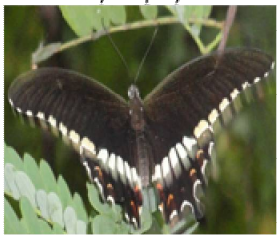


Common jay
Graphium doson

Tailed jay
Graphium agamemnon

Blue Mormon
Papilio polymnestor

Common Mormon
Papilio polytes



Common rose
Atrophaneura aristolochiae

Crimson rose
Atrophaneura hector

Lime butterfly
Papilio demoleus

Common grass yellow
Eurema hecabe

Small grass yellow
Eurema brigitta

Three spot grass yellow
Eurema blanda

Nesarajan and Horne Iona Averal

Spotless grass yellow
Eurema laeta

Common emigrant
Catopsilia pomona

Mottled emigrant
Catopsilia pyranthe

Nilgiri clouded yellow
Colias nilagiriensis

Small salmon arab
Colotis amata

Crimson tip
Colotis danae

Small orange tip
Colotis etrida

Plain orange tip
Colotis eucharis

White orange tip
Ixias marianne

Yellow orange tip
Ixias pyrene

Great orange tip
Hebomoia glaucippe

Dark wanderer
Pareronia ceylanica

Common albatross
Appias albina

White striped albatross
Appias libythea

Common gull
Cepora nerissa

Common jezebel
Delias eucharis

Indian sunbeam
Curetis thesis

White-Banded royal
Dacalana cotys

Double-Tufted Royal
Dacalana vidura

Pecock royal
Tajuria cippus

Common pierrot
Castalius rosimon

Striped pierrot
Tarucus nara

Dark cerulean
Jamides bochus

Common cerulean
Jamides celeno

Forget-me-not
Catochrysops strabo

Pale grass blue
Pseudozizeeria maha

Lesser grass blue
Zizina otis

Dark grass blue
Zizeeria karsandra

Tiny grass blue
Zizula hylax

Zebra blue
Leptotes plinius

African babul blue
Azanus jesous

Gram blue
Euchrysops cnejus

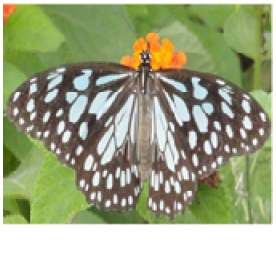
Lime blue
Chilades lajus

Eastern grass blue
Chilades putli

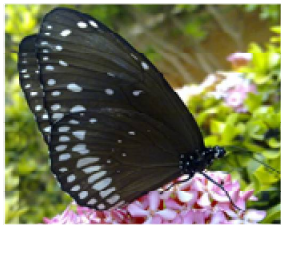
Indian cupid
Everes lacturnus

Small cupid
Chilades parr

Glassy tiger
Parantica aglea

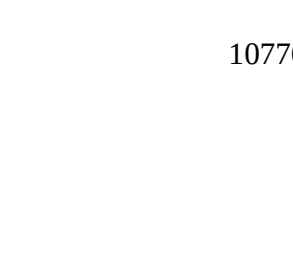


Common indian crow
Euploea core



Black rajah
Charaxes solons

Common eveing brown
Melanitis



Nesarajan and Horne Iona Averal

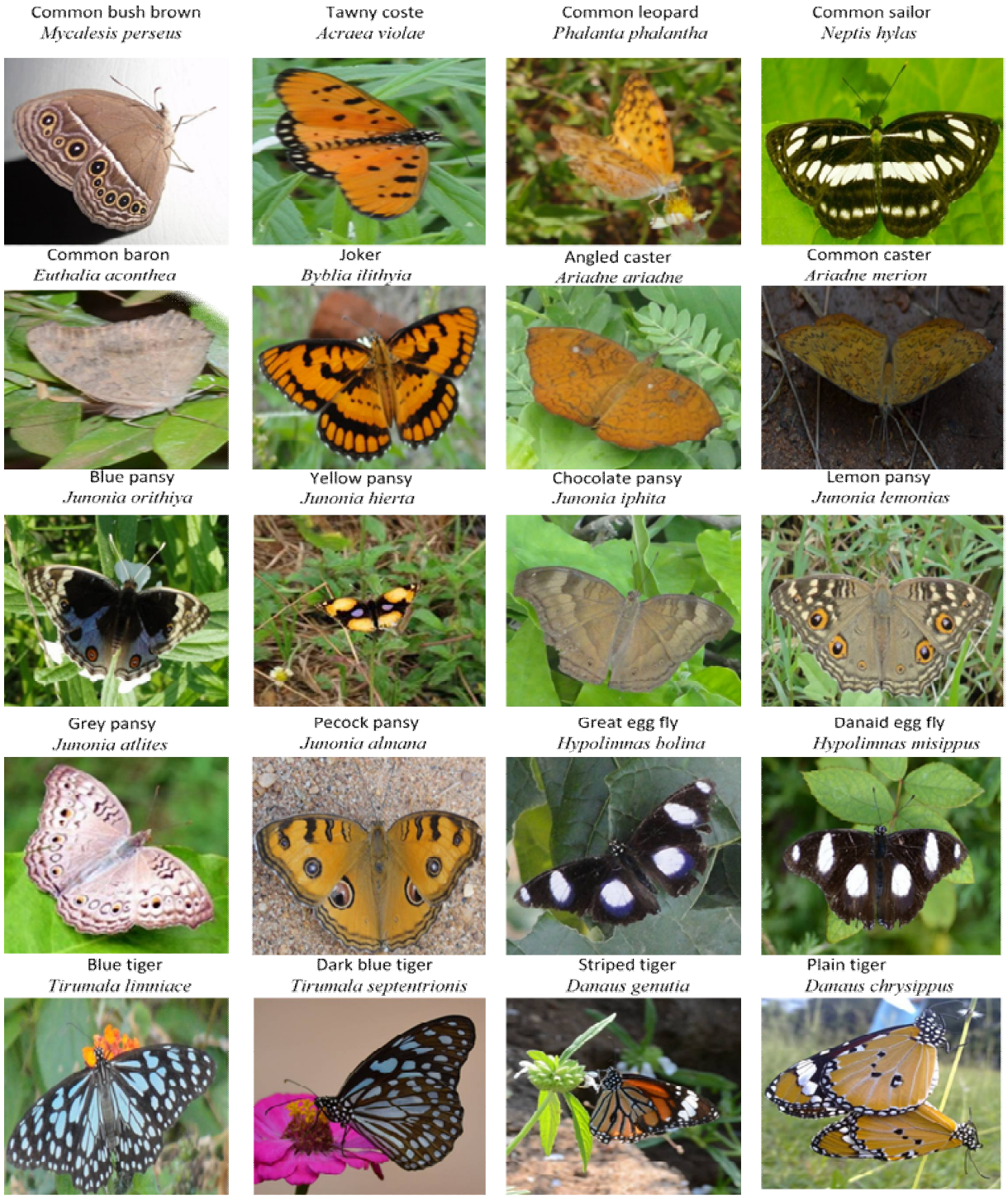


Fig. 3. Biodiversity of Butterflies

Nesarajan and Horne Iona Averal

S.No	Familywise	Scientific name	Common name	Location						Status
				VG	PG	HA	A	B	CC	
		FAMILY:HESPERIIDAE(Skippers)								
	Sub family : Coeliadinae									
1	1	***Hasora chromus***	Common banded awl	✓	✓	✓	✓	✓	✓	C
2	2	***Badamia exclamationis***	Brown awl	✓	×	✓	✓	✓	✓	O
		Sub family : Pyrginae								
3	3	***Gomalia elma***	African marbled skipper	×	×	✓	×	×	×	R
Sub family : Hesperiinae										
4	4	***Pseudoborbo bevani***	Bevan's swift	✓	×	×	×	×	✓	C
5	5	***Parnara bada***	Ceylon swift	✓	✓	×	×	×	✓	R
6	6	***Udaspes folus***	Grass demon	✓	×	✓	×	×	×	R
7	7	***Suastus gremius***	Indian palm pob	✓	✓	×	×	×	×	C
8	8	***Baoris farri***	Paint brush swift	×	✓	✓	×	×	×	C
9	9	***Pelopidas mathias***	Small branded swift	✓	×	×	×	×	✓	O
10	10	***Borbo cinnara***	Rice swift	✓	✓	✓	✓	✓	✓	C
		FAMILY:PAPILIONIDAE(Swallowtails)								
		Sub family : Papilioninae								
11	1	***Graphium doson***	Common jay	✓	✓	✓	✓	×	✓	VC
12	2	***Graphium agamemnon***	Tailed jay	✓	✓	✓	✓	×	✓	VC
13	3	***Papilio polymnestor***	Blue Mormon	✓	×	✓	×	×	×	C
14	4	***Papilio polytes***	Common Mormon	✓	✓	✓	✓	✓	✓	VC
15	5	***Atrophaneura aristolochiae***	Common rose	✓	✓	✓	✓	✓	✓	VC
16	6	***Atrophaneura hector***	Crimson rose	✓	✓	✓	✓	✓	✓	C
17	7	***Papilio demoleus***	Lime butterfly	✓	✓	✓	✓	×	✓	C
		FAMILY:PIERIDAE(White and Yellows)								
		Sub family : Coliadinae - Yellows								
18	1	***Eurema hecabe***	Common grass yellow	✓	✓	✓	✓	✓	✓	VC
19	2	***Eurema brigitta***	Small grass yellow	✓	✓	✓	✓	×	×	VC
20	3	***Eurema blanda***	Three spotgrass yellow	✓	✓	✓	✓	✓	✓	VC
21	4	***Eurema laeta***	Spotless grass yellow	✓	×	×	✓	×	×	VC
22	5	***Catopsilia pomona***	Common emigrant	✓	✓	✓	✓	✓	✓	VC
23	6	***Catopsilia pyranthe***	Mottled emigrant	✓	✓	✓	✓	✓	✓	VC
24	7	***Colias nilagiriensis***	Nilgiri clouded yellow	✓	×	×	×	×	×	VR
		Sub family : Pierinae - Whites								
25	8	***Colotis amata***	Small salmon arab	✓	×	×	×	×	×	O
26	9	***Colotis danae***	Crimson tip	×	✓	×	×	×	×	O
27	10	***Colotis etrida***	Small orange tip	×	×	×	✓	✓	×	C
28	11	***Colotis eucharis***	Plain orange tip	✓	×	×	×	×	×	C
29	12	***Ixias marianne***	White orang tip	✓	×	×	×	×	×	O
30	13	***Ixias pyrene***	Yellow orang tip	×	×	×	✓	×	×	O
31	14	***Hebomoia glaucippe***	Great orange tip	×	✓	×	×	×	×	C
32	15	***Pareronia ceylanica***	Dark wanderer	×	×	✓	×	×	×	C
33	16	***Appias albina***	Common albatross	✓	×	✓	×	×	×	O
34	17	***Appias libythea***	Western striped albatross	✓	×	✓	×	×	×	O
35	18	***cepora nerissa***	Common gull	✓	×	×	×	×	×	O
36	19	***Delias eucharis*s**	Common jezebel	✓	✓	✓	✓	✓	✓	C
		FAMILY:LYCAENIDAE(Blues)								
		Sub family : Curetinae Sunbeam								
37	1	***Curetis thesis***	Indian sunbeam	✓	✓	×	×	×	✓	VC

37	1	***Curetis thesis***	Indian sunbeam	✓	✓	×	×	×	✓	VC
0		***Sub family : Theclinae***								
38	2	***Dacalana cotys***	White-Banded Royal	×	×	×	×	×	✓	VR
39	3	***Dacalana vidura***	Double-Tufted Royal	×	×	×	×	×	✓	VR
40	4	***Tajuria cippus***	Peacock Royal	×	×	×	×	×	✓	VR
		Sub family : Polyommatinae								
41	5	***Castalius rosimon***	Common pierrot	×	×	✓	×	×	×	R
42	6	***Tarucus nara***	Striped pierrot	✓	✓	✓	×	×	×	O
43	7	***Jamides bochus***	Dark cerulean	✓	×	✓	×	×	×	C
44	8	***Jamides celeno***	Common cerulean	×	✓	✓	×	×	×	C
45	9	***Catochrysops strabo***	Forget – me – not	✓	✓	✓	✓	✓	✓	VC
46	10	***Pseuudozizeeria maha***	Pale grass blue	✓	✓	✓	✓	✓	✓	VC
47	11	***Zizina otis***	Lesser grass blue	✓	✓	✓	✓	✓	✓	VC
48	12	***Zizeeria karsandra***	Dark grass blue	✓	✓	✓	✓	✓	✓	VC
49	13	***Zizula hylax***	Tiny grass blue	✓	✓	✓	✓	✓	✓	C
50	14	***Leptotes plinius***	Zebra blue	✓	×	×	✓	×	×	C
51	15	***Azanus jesous***	African babul blue	×	×	✓	×	×	×	R
52	16	***Euchrysops cnejus***	Gram blue	×	×	×	×	×	✓	R
53	17	***Chilades lajus***	Lime blue	✓	✓	✓	✓	×	×	C
54	18	***Chilades putli***	Eastern grass jewel	✓	✓	✓	✓	✓	✓	VC
55	19	***Everes lacturnus***	Indian cupid	✓	✓	✓	✓	✓	✓	C
56	20	***Chilades parr***	Small cupid	✓	✓	✓	✓	✓	✓	C
		FAMILY:NYMPHALIDAE (Brush - footed)								
		Sub family : Danainae - Milkweed								
57	1	***Tirumala limniace***	Blue tiger	✓	✓	✓	✓	✓	✓	VC
58	2	***Tirumala septentrionis***	Dark blue tiger	✓	×	×	×	✓	×	C
59	3	***Danaus genutia***	Striped tiger	✓	✓	✓	×	×	×	VC
60	4	***Danaus chrysippus***	Plain tiger	✓	✓	✓	✓	✓	✓	VC
61	5	***Parantica aglea***	Glassy tiger	✓	×	×	×	×	✓	O
62	6	***Euploea core***	Common Indian crow	✓	✓	✓	✓	✓	✓	VC
		Sub family : Charaxinae								
63	7	***Charaxes solons***	Black rajah	✓	✓	×	×	×	×	R
		Sub family : Satyrinae - Browns								
64	8	***Melanitis***	Common evening brown	✓	✓	×	✓	×	×	VC
65	9	***Mycalesis perseus***	Common bush brown	✓	×	×	×	✓	×	C
		Sub family : Heliconiinae								
66	10	***Acraea violae***	Tawny coster	✓	✓	✓	✓	✓	✓	C
67	11	***Phalanta phalantha***	Common leopard	✓	×	×	×	×	×	R
		Sub family : Limenitinae								
68	12	***Neptis hylas***	Common sailor	×	✓	×	×	×	×	O
69	13	***Euthalia aconthea***	Common baron	✓	✓	✓	×	×	×	O
		Sub family : Biblidinae - Jokers & Castors								
70	14	***Byblia ilithyia***	Joker	✓	✓	✓	✓	✓	✓	C
71	15	***Ariadne ariadne***	Angled castor	✓	✓	×	×	✓	×	C
72	16	***Ariadne merione***	Common castor	✓	✓	✓	✓	✓	✓	C
		Sub family : Biblidinae - Nymphalinae								
73	17	***Junonia orithiya***	Blue pansy	✓	×	×	×	×	✓	VC
74	18	***Junonia hierta***	Yellow pansy	✓	×	×	×	×	×	VC
75	19	***Junonia iphita***	Chocolate pansy	✓	✓	×	×	×	×	C
76	20	***Junonia lemonias***	Lemon pansy	✓	✓	✓	✓	✓	✓	VC
77	21	***Junonia atlites***	Grey pansy	×	✓	×	✓	×	×	O
78	22	***Junonia almana***	Peacock pansy	✓	✓	✓	✓	✓	✓	VC
79	23	***Hypolimnas bolina***	Great egg fly	✓	✓	✓	✓	✓	✓	VC
80	24	***Hypolimnas misippus***	Danaid egg fly	✓	✓	✓	✓	×	✓	VC

Note:; Vasantham Garden, PG Block, Hostel Areas , A ground, B ground, Community college C- Common; VC- Very Common;O - Occasional; UC - Uncommon; R- Rare; # New Record;

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RESEARCH ARTICLE

Influence of Resistance Training on Health and Some Selected Physical Variables among College Males

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ABSTRACT

Resistance training (RT) is also known as strength or weight training. RT is very beneficial to all athletes. (Behringer, 2010) reveals that the resistance training serves as the dynamic force for a healthy life and is the key role for improving athletic performance in various sports. The purpose of this study was to find out the influence of resistance training on health and selected physical variables among college males. A group (n=30) subjects were selected for this study; their ages were between 19-22 years. The test considered for health (body composition and resting pulse rate) and selected physical variables (sit –ups test, push-ups test) to find out the performance from pre to post test. Resistance training program was employed for 12 weeks; training program was consisted with 10 resistance exercises performing only on the machines based on FITT principle, 2 days of training in a week. For analyzing the data mean, SD and t-test were considered with the help of statistica software. The significance level was adjusted at 0.05 level.The analyzing of data reveals that the mean and S.D with regard to body mass index (BMI) from pre to post test were (28.68, 7.06) and (27.43, 6.56). Resting pulse rate with mean and S.D were (69.20, 5.50) & (63.96, 4.92). The mean and S.D with regard to sit-ups test were; (19.30, 4.23) & (24.47, 5.02); regard to push-ups test with mean & S.D were (16.23, 7.36) & (21.93, 7.45.It is concluded that the influence of resistance training on body mass index, resting pulse rate, sit-ups and push-ups had shows significant performance from pre to post test among the participants.

Key words: Health, Training, Performance, Strength.

INTRODUCTION

Resistance training (RT) is also known as strength or weight training. RT is very beneficial to all athletes. Presently the demand of resistance training is very high globally due to its remarkable benefits with regard to health and fitness. Strength training programs performed below steady environment are excellent for improving muscle force and power (ACSM, 2009; Kraemer and et al, 2004). (Behringer, 2010) reveals that the resistance training serves as the dynamic force for a healthy life and is the key role for improving athletic performance in various sports. Circuit weight training among the different strength training modalities is very effective in enhancing performance in untrained men, (Harber et al., 2004). Strength training is a method for using resistance to muscular contraction to build the strength, anaerobic endurance, and size of skeletal muscles. If properly performed and executed, strength training can provide significant functional benefits and improvement in overall health and well-being, including increased bone, muscle, tendon and ligament strength and toughness, improved joint function, reduced potential for injury, increased bone density, increased metabolism, improved cardiac function, and elevated HDL, improves good cholesterol. Training commonly uses the technique of progressively increasing the force output of the muscle through incremental weight increases and uses a variety of exercises and types of equipment to target specific muscle groups. Strength training is primarily an anaerobic activity, although some proponents have adapted it to provide the benefits of aerobic exercise through circuit training.

Resistance training should be an important component of all fitness programs from more for strength and power athletes to more for individuals who exercise for the health benefits. Of course, athletes in sports requiring strength and power, such as weight lifting, bodybuilding and sprinting most emphasize resistance training. However many other athletes also benefits from strength training, especially those in sports requiring a high level of muscular endurance (Kumar, 2004).Resistance training is exercise designed specifically to increase muscular strength and endurance through increased workload demand and may include the use of free weights, machine weights, elastic tubing/stretch bands, hydraulic machines or body weight (e.g. push-ups, chin-ups) (Stratton et al., 2004). Recent studies had shows that supervised resistance training programs do not appear to have any adverse effects in children and adolescents (Malina, 2006) and in fact may improve cardiovascular fitness, body composition, bone mineral density and blood lipid profiles (Benson, Torode, & Fiatarone Singh, 2008b; Faigenbaum, 2000; Malina, 2006).Resistance training has long been considered an important activity for adults and the latest physical activity recommendations for youth, adults, and older adults now include guidelines for resistance training (U.S. Department of Health & Human Services, 2008).The purpose of this study was to find out the influence of resistance training on health and selected physical variables among college males.

MATERIALS AND METHODS

Selection of subjects

Thirty subjects were selected for this study from the various sections of college undergoing physical education classes at King Fahd University of Petroleum & Minerals, Saudi Arabia during the year 2013-14. The age of the participants was between 19-22 years. The reason of this study was explained and doubts were addressed to the participants.

Experimental Design

The participants (N=30) were selected for this study randomly. The resistance training program was employed for 12 weeks, 45 minutes of training per session, two days of training program in a week. The resistance training exercises which was employed on the participants was, (A1: sitting calf raises, A2: Standing leg raises, A3; Adductors, A4: abductors, A5: leg extensions, B1: high pulley, B2: incline chest press, B3: sitting shoulder press, B4: sitting triceps

extensions, and B5: Preacher curls). The resistance training program which was employed on the participants is presented in the below table-1.

Procedure of testing

The selected test with regard to health is consisted of two test; Body composition (body mass index, BMI) and resting pulse rate (1 min) employed on the participants. The test which consider for physical variables were sit-ups and push-ups test for 30 seconds respectively and the scores were recorded during pre and post test. A Pre and post test was conducted before and after the 12 weeks training program. The training was given at the Gymnasium at stadium, King Fahd University of Petroleum & Minerals, Saudi Arabia. All the scores for pre and post test were recorded for analyzing the data.

Statistical Analysis

To compare the mean differences between pre to post test, mean, standard deviation and t-tests were computed by means of Statistica Software. A significance level at 0.05 level was adjusted.

RESULTS AND DISCUSSION

The analyzing of data for selected variables i.e. (body mass index, resting pulse rate, sit-ups test and push-ups test), performance from pre to post test among subjects is presented in the table -3 by the help of statistical tools i.e. mean, standard deviation and t test.

The analyzing of data reveals that the mean and Standard deviation with regard to body mass index (BMI) from pre to post test were (28.68, 7.06) and (27.43, 6.56) respectively. Resting pulse rate from pre to post test with mean and Standard deviation were (69.20, 5.50) and (63.96, 4.92) respectively. The mean and Standard deviation with regard to sit-ups test from pre to post test were (19.30, 4.23) and (24.47, 5.02) respectively. From pre to post test with regard to push-ups performance with mean and Standard deviation were (16.23, 7.36) & (21.93, 7.45) respectively.

DISCUSSION

The results of this study suggested that twelve weeks of resistance training program have a significant effect in enhancing health and selected physical variables among males. This is evident from the earlier studies that the resistance training increases the health and also it depends on the intensity of the training schedule. This study is agreement with the findings of this study. (Beni, 2012) investigated that the effect of resistance training for eight weeks had shows reduction in total fat percent. Resistance training has an not direct effect on body weight and fat loss through increasing Resting Metabolic rate (RMR) and enhancing fat oxidation. Increasing or maintaining muscle mass or Fat Free Mass (FFM), increasing serum catecholamine levels, and enhancing post-exercise utilization of energy are all factors that play a role in the ability of resistance exercise to increase RMR. Because resistance training maintains or increases muscle mass even during severe caloric restriction. (RMR) will remain elevated or will be maintained during weight loss. In addition to increased RMR the rate of fat oxidation is affected both acutely and chronically (Jefferey L and et al, 2002). The selected physical variables like strength had also showed significant improvement during the ten weeks of resistance training program. It was revealed that the children and youth had shows significant improvement regard to strength and power (Faigenbaum et al, 1996; Falk B, & Tenenbaum, 1996). In the present study the selected college male's scores were very low in the pre test pertaining to all the selected health and physical variables. In the post test the participants had shows an improved performance in enhancing in all the selected variables. This is evident that the resistance training program, two days of training per week, 45 minutes per session, for twelve weeks is also useful in enhancing health and physical performance.

CONCLUSION

It is concluded that the influence of resistance training on health i.e. body mass index, resting pulse rate, had shows significant performance from pre to post test among the participants. It is also concluded that the influence of resistance training on selected physical variables i.e. sit-ups and push-ups had shows improved performance from pre to post test among the participants, which is significant and encouraging.

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Table-1: Resistance Training program

Week : 1	Intensity:20%, of body weight	Sets 2	Reps 25	Rest between the sets : 1 min
Week : 2,3	Intensity : 20% and 40%	Sets 2	Reps 20	Rest :1 min
Week : 4,5,6	Intensity : 30% and 60%	Sets 2	Reps 15	Rest :2 min
Week : 7,8,9	Intensity (20 %, 40%, 80%)	Sets 3	Reps 15,12,10	Rest :3 min
Wk,10.11,12	Intensity (20%, 60%, 100%)	Sets 3	Reps15,12,6	Rest 3 min

Table-2: Showing the details of the selected variables for the pre and post test

Sl.no	Variables	Test (Pre and post)
1	Body composition	To find out body mass index (BMI)
2	Resting pulse rate (1 min)	To find out the resting pulse rate (score)
3	Sit-ups (30 sec)	To find out the muscular endurance
4	Push –ups (30 sec)	To find out the strength endurance

Table-3: Showing mean, standard deviation and t-value of the selected variables.

Variables	Test	Group (N=30)		
		Mean	S.D	p-value
BMI	Pre	28.68	7.06	0.0000
	Post	27.43	6.56	
Resting pulse rate	Pre	69.20	5.50	0.0000
	Post	63.96	4.92	
Sit-ups test (30 seconds)	Pre	19.30	4.23	0.0000
	Post	24.47	5.02	
Push up test (30 seconds)	Pre	16.23	7.36	0.0000
	Post	21.93	7.45	

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RESEARCH ARTICLE

Biochemical Composition and Characterization of Edible Tissues of Penaeidean Shrimps, Nagapattinam Coast, Tamil Nadu, India

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ABSTRACT

Crustacean seafoods including shrimps were collected during winter monsoon from Nagapattinam coast for analyzing the nutritional quality of the edible tissues for human consumption by calculating the amount (%) of protein, lipid, carbohydrate, ash and moisture on moisture free sample (n=3). Proximate analysis showed the protein content (%) higher than other components between the species and *Fenneropenaeus indicus* showed the increased level of protein (75.3%). *Penaeus monodon* and *Penaeus semisulcatus* showed protein level as 44.2% and 45.4% respectively and the species showed 11%, 12% and 14% of lipid content respectively with the moisture % as in *P. monodon* (80.6 %), *P. semisulcatus* (45.4%) and *F. indicus* (54.6 %). The composition of lipids and protein were inversely proportional to the moisture content. The carbohydrate % ranged between 21-29% followed by the ash content 5-6.7 % between the species. FTIR analysis was performed for spectrum confirmation using Perkin Elmer spectrophotometer system to detect the characteristic peaks and their functional groups. It confirmed the presence of alcohols, alkyl halides, carboxylic acids, aromatics and alkynes in the muscle tissue which showed their nutritional quality having essential amino acids and fatty acids.

Key Words: Shrimp, Protein, Lipid, Ash, FTIR.

INTRODUCTION

India is diversified with varied aquatic species along the coastline of about 8119 km; a continental shelf of 0.5 million sq.km and an Exclusive Economic Zone (EEZ) of 2.02 million sq.km. Tamil Nadu with a long coastline of 1,076 km ranks second among all the maritime states in India in terms of coastal length and fourth in terms of marine fish production in India. The Indian decapod crustaceans reveal that 117 species of prawns inhabit the marine areas which fall under the domain of commercial fishing and the number of penaeoid species now found in Indian waters is 122, which forms 34.9% of the world species showing high diversity of species (Karuppasamy *et al*., 2013). Seafoods are considered as a healthier food due to the presence of essential nutrients in them that are unique to the marine foods than in meat and poultry. Also it has an excellent cardio protective character.

The quality of any species is determined by their biochemical composition and it is of great concern. Since the coastal regions on the wild shrimps have limited information on the species composition and their role in biochemical composition which are more important for human health and development. Penaeid shrimps have become the economically important species. Very little information is available on the quantitative occurrence of major biochemical constituents. Among all World Health Foods, shrimp ranks as our 4th best source of high-quality protein. Hence Shrimp meat is an excellent source of high quality protein and essential amino acids and essential fats like eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22: 6n-3, DHA) acids.The proximate composition of shrimps and other crustaceans has found to be varied due to the seasonal factors, climatic factors, geographic factors, habitat, developmental stage, sex and sexual maturation. The importance of chemical analysis in marine fishes and prawns is to express the food value in terms of energy units (Ravichandran, 2009). Thus the present study aims in providing that the marine shrimps (*Penaeus monodon, Penaeus semisulcatus* and *Fenneropenaeus indicus*) collected from the Nagapattinam coast as a stable, sustainable and predictable food supply to the growing population as a higher protein source with the evaluation of their biochemical composition.

MATERIALS AND METHODS

The three penaeid species of shrimps *(P. monodon, P. semisulcatus* and *F. indicus*) were collected during winter monsoon from the Nagapattinam coast, Tamil Nadu, India (Lat 10 □ 45' 34 " N; 79 □ 51' 1" E) (Fig. 1). Samples were washed with water to remove any adhering contamination, drained under folds of filter paper. Samples were then put in crushed ice in insulated containers and brought to the laboratory for preservation prior to analysis.

Taxonomic Identification

Sex identification was made through stereo-microscopic inspection of the first and second pairs of pleopods and the species obtained were mostly females than males. Eight morphometric measurements were made on each specimen species. They were Total length (TL), carapace length (CL), carapace height (CH), diagonal carapace length (DCL), first segment width (FSW), first segment length (FSL), sixth abdominal length (SISL) (Table.1). Shrimps ranging from 5 to 6 cm carapace length (CL) were identified to genus level according to the characters.

Estimation of Biochemical Composition

The defrosted shrimps were separated into the exoskeleton (head and outer body shell) i.e., shell and the endoskeleton (i.e. flesh). The endoskeletons were oven dried at 95-105 □ C and ground into fine powder (moisture free) which was used to analyse the proximate composition of the shrimps. In the present study, biochemical composition viz. moisture, protein, carbohydrate, lipid and ash content were analysed by standard methods. The estimation of Moisture (%) content by hot-air oven method (Folch *et al*., 1957), protein (%) by the method of Lowry *et al*., 1951, carbohydrate (%) by Dubois *et al*., 1956 and ash (%) by AOAC, 1995.

FTIR Analysis

FT-IR spectroscopy of solid sample of chitin and chitosan from *M. mogiensis* shell was relied on an AVATAR 330 Spectrometer. Sample (10 μg) was mixed with 100 μg of dried Potassium Bromide (KBr) and compressed to prepare a salt discs (10 mm diameter) for reading the spectrum. The spectrum was obtained and compared with the standard (Mantsch and Chapman, 1996).

RESULTS

Taxonomic Identification

The genera classification proposed by Perez Farfante and Kensley (1997) have been adopted for classification of genus Penaeus and Fenneropenaeus. The WoRMS Register (World Register of Marine Species), ITIS Standard Search and the carideorum catalogues also have been referred and for the conformation of the families, genera and species. The penaeid shrimps selected were *Penaeus monodon* (Fabricius, 1798), *Penaeus semisulcatus* (De Haan, 1844) and *Fenneropenaeus indicus* (H. Milne Edwards, 1837). The selected species were morphometrically measured using the standard scales (Table.1).

Proximate Analysis

The proximate composition of the penaeid shrimps viz. protein, carbohydrate, lipid, moisture and ash were analysed using the standard methodologies and shown in the table. 2. The overall % of different biochemical components varied between the species. Protein content (%) when comparing between the penaeid species *P. monodon*, *P. semisulcatus* and *F. indicus* showed 44.2%, 45.4% and 54.6% (Fig. 2) and the highest protein level was observed in the white shrimp, *Fenneropenaeus indicus* (54.6%) respectively. Also the species showed the % of moisture in the muscle of *P. monodon*, *P. semisulcatus* and *F. indicus* (80.6%, 77.8% and 75.3%) of dry weight respectively (Fig. 3) and higher percentage was seen in *P. monodon* (80.6%). Lipid content (%) showed comparatively higher concentration in *P. semisulcatus* (14%) when comparing *P. monodon* (11%) and *F. indicus* (12%) (Fig.4). From the study, carbohydrate concentration (%) in the three shrimps, *P. monodon*, *P. semisulcatus* and *F. indicus* were 21.9%, 29.3% and 28% respectively and a higher level of 29.3% was observed in *Penaeus semisulcatus* (Fig.5). The ash determined using muffle furnace showed in *P. monodon*, *P. semisulcatus* and *F. indicus* were 6%, 6.7% and 5.2% respectively. The highest ash content was seen in *P. semisulcatus* (6.7%) comparing the other two species (Fig. 6).

Characterization of the tissue of shrimps (Fourier Transform-Infra Red (FT-IR) Spectral Analysis)

FTIR of the biological sample (tissue) shows distinct IR frequencies correspondingly their functional groups (Table. 3) and the spectra (Fig. 7, 8 and 9). The major peaks depicted in the figures shows the strong band at frequencies 1082, 1234, 1398, 1450 and 1654 cm^{-1} showing their corresponding functional groups in *P. monodon* followed by *P. semisulcatus* having their strong band frequency ranges at 1082, 1232, 1397, 1456, 1542 and 1654 cm^{-1} respectively. *F. indicus* showed strong bands at frequencies 1082, 1236, 1398, 1541 and 1651 cm^{-1} respectively. From the FTIR frequency table, the sample showed the presence of the major functional groups for the presence of amino acids, fatty acids.

DISCUSSION

Nutrition is about the relationship between food and good health. We require nutrients, the various components that make up food for normal growth and development.In this study we imply that shrimps as one of the highly nutritious food when comparing meat and poultry. Crustaceans comprise nearly about 20% of the Indian Aquatic

Food Production.The global estimate of crustacean species diversity is 1,50,000 of which 2,934 species of crustacean that have been reported so far. The marine species contributes nearly about 94.85%. Among the crustaceans, shrimps are the major commodities in which 55 species of commercial shrimps are recorded (www.mpeda.com).The present study were carried out to determine the chemical composition of the body component ie., the muscle from the three shallow water penaeid shrimp species, *P. monodon, P. semisulcatus* and *F. indicus* collected from the landing centre of Nagapattinam. The results were discussed that the protein was found as the major constituent in the muscles of all the selected shrimps. The average dry weight of the species was correspondingly implies its muscle content constituting the components like carbohydrate, protein, lipids and fatty acids exempting the moisture which varies greatly between species.

The protein content was found to vary between 44-54% of the dry weight of the three species. The lowest protein (44%) was found in *P. monodon*, which is having a higher moisture content of about 80%. The higher protein content (54%) was observed in *F. indicus* of the dry weight (Fig. 3) which is indirectly proportional to the lower moisture content of about 75%. 45% of protein and correspondingly the moisture content of about 77% imply that the moisture content reduces the protein concentration in the muscle of the species. Since the proteins are lipo-protein in nature and they are highly hydrophobic. Crustaceans digest and absorb lipids from their feed and transport it to appropriate cells for utilization and store in the muscle cells. In this study the protein shows higher level in low water containing tissues ie., in *F. indicus*. Protein was found as the major constituent in the muscle of shrimps. The same difference in the proximate composition in the edible muscle was reported for *Fenneropenaeus pennicillatus*, *Fenneropenaeus merguiensis,* pink shrimp (Rosa and Nunes, 2003), black tiger shrimp and white shrimp (Sriket *et al*., 2007 and Karuppasamy *et al*., 2013). The lipid content varied between 10-14% of the dry weight. The moisture content influenced the total lipid concentration. The hydrophobic lipid content is lesser in more moisture (80%) containing tissue ie., in *P. monodon* (11%). The highest lipid content was observed in *P. semisulcatus* (14%) (Fig. 5) having more moisture and the lowest lipid content were studied in *Fenneropenaeus indicus* when comparing the other shrimps in this study.

The reports on the yield and the chemical composition of body components of some species of shrimps were reported (Gopakumar, 1993) and the moisture varied 79.3-83.6% which correlates the results from the present study of about 75.3- 81%. According to the reported study, protein in meat varied from 13.6-15.4%, which is lower to the obtained result and the observed varying moisture content in shrimps depends on the species and size. Carbohydrate necessary for the metabolic activities providing energy to the species ranged from 17% to 29%. The higher carbohydrate concentrates in *P. semisulcatus* and reduced in *F. indicus* and *P. monodon* (Fig. 6).The ash concentrates more in *P. semisulcatus* (6.7%) when comparing *P. monodon* and *F. indicus* (6% and 5.2%) (Fig. 7) and it depends on the body weight of the species. Thus the proximate composition of shrimp muscles are dependent on the factors like species, growth stage, feed and season (Karakoltidis *et al*., 1995, Sikorski *et al*., 1990 a,b).The decrease in water content resulted in the relative increase in protein, fat and ash content that is highly correlating the present study. Thus results obtained from the present study and earlier reports suggest that the biochemical composition of shrimp muscle varies according to species and size. Research increasingly shows a close connection between what we eat and risk of developing several health problems. While our genes, to some extent determine our state of health, type, variety, preparation methods and portions of food we eat play a far more significant role. FTIR analysis for spectrum confirmation using Perkin Elmer spectrophotometer system to detect the characteristic peaks and their functional groups confirmed the presence of alcohols, alkyl halides, carboxylic acids, aromatics and alkynes (Table 3) in the muscle tissue which showed their nutritional quality of showing essential amino acids and fatty acids that are to be quantified in the future study.

The species selected for determining proximate composition of the three commercially important species reveals an initiative that *F. indicus* is highly proteinaceous when comparing the other two and directly correlates their nutritional efficiency. Future analysis on the fatty and amino acids, carotenoid concentration proves their successful intake as a “protein and pigment enriched food” to the society depriving of nutrition. Also further seasonal analysis

of the three species at different geographical locations implies future research evidences in the field of nutrition and health. The present study summarises that the selected penaeid shrimps showed variation in their biochemical constituent that depends on the age, species, environment, nutrition and morphometry. These factors are all inter related and correlates their nutritional potential. The study also concludes that *F indicus* contributes high protein content and it varied depending on the moisture content. Thus its high protein content, act as a nutritious food and a detailed fatty acid profile on the species is further required so their specialization can be focused on the medicinal aspects for curing diseases and syndromes.Thus the Crustacean species from the present study possess bioactive proteins and lipids which could make them serve as alternative food resources for the Nation and even as better aid to the pharmaceutical industry as their processing of crustacean wastes are used in producing natural polymers like chitin and chitosan. These results supported highly that along with the commercial species these species can also be a good supportive source of food to the society.

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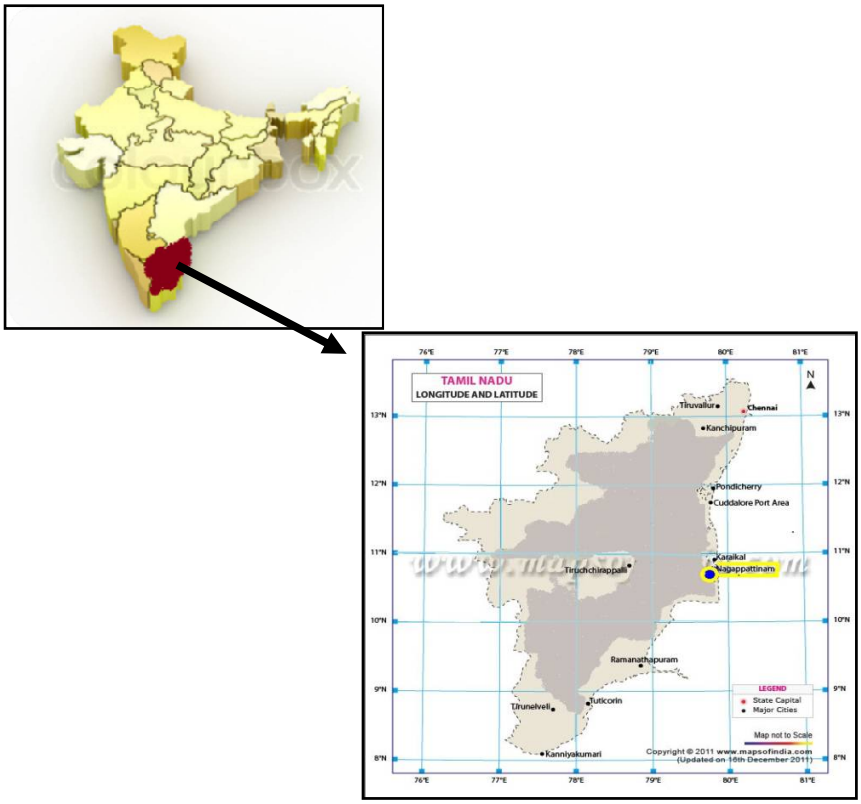

Fig. 1 Map showing the Nagapattinam coast

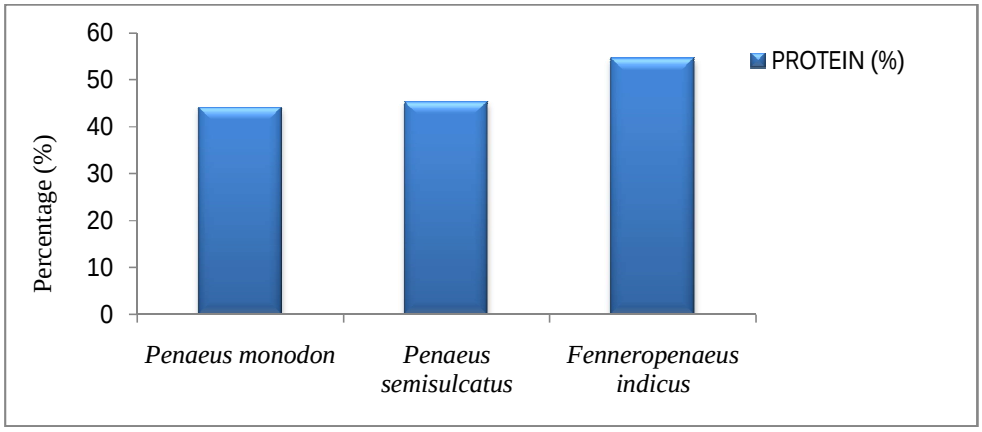

Fig. 2 Comparison of protein content (%) in different Penaeid species

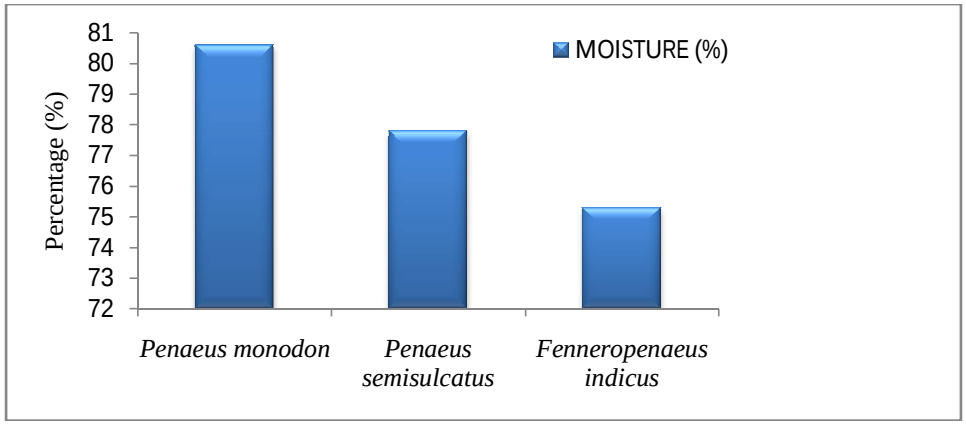

Fig. 3 Comparison of moisture (%) in different Penaeid species

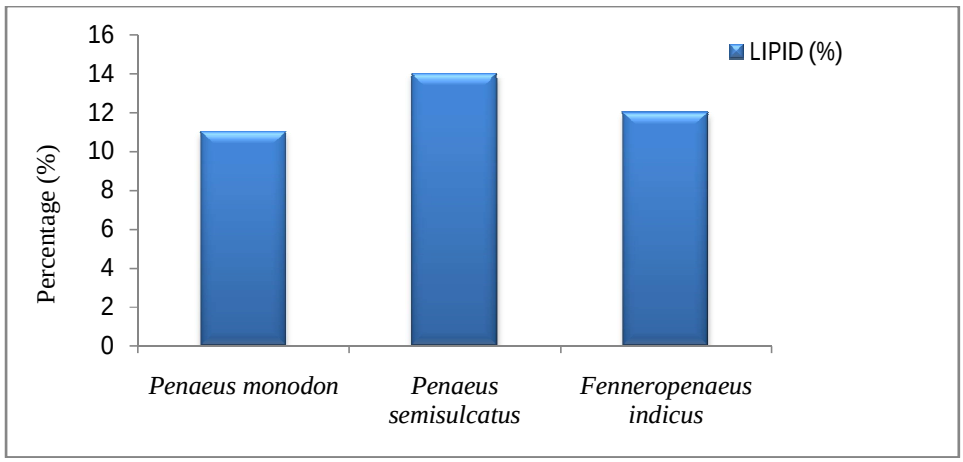

Fig. 4 Comparison of lipid content (%) in different Penaeid species

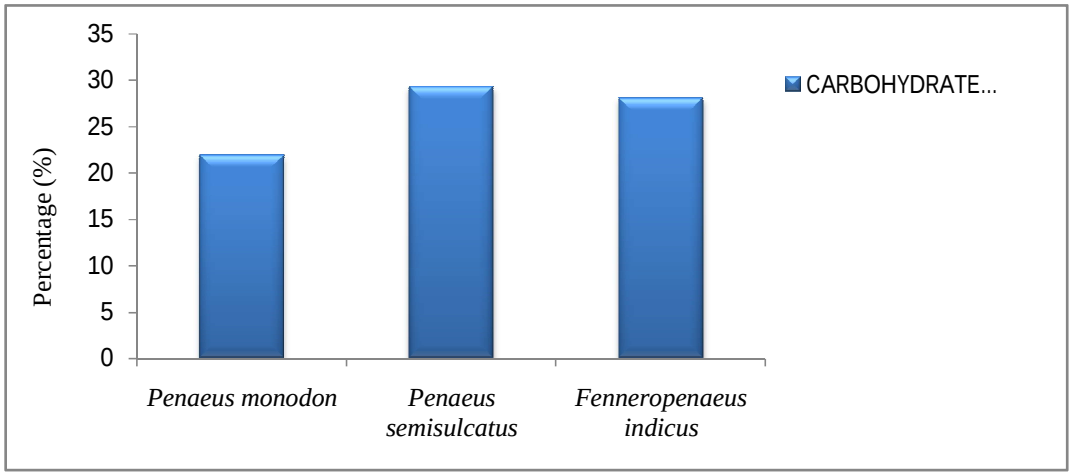

Fig. 5 Comparison of carbohydrate (%) in different Penaeid species

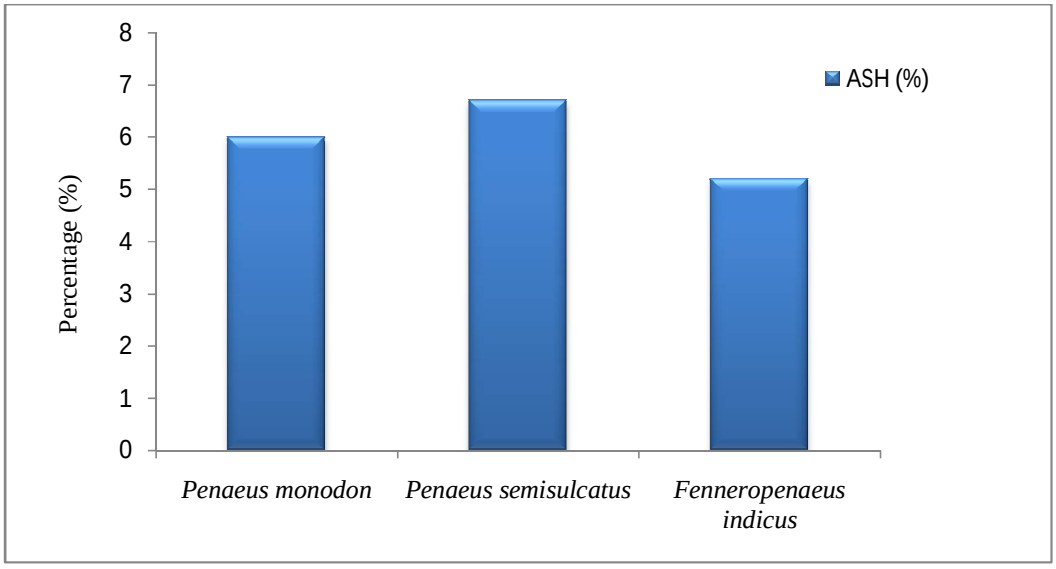

Fig. 6 Comparison of ash (%) in different Penaeid species

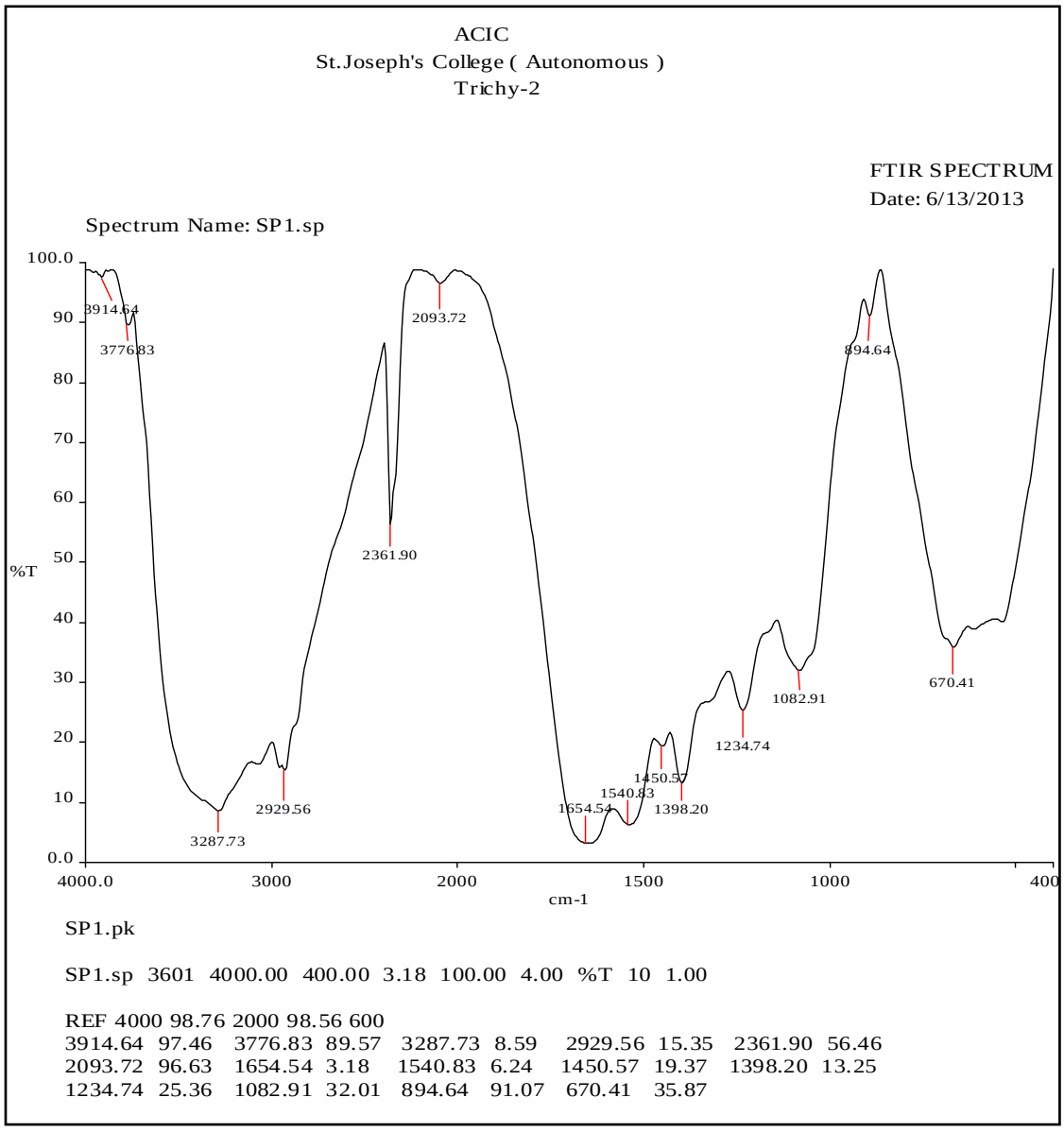

Fig. 7 FTIR study in *P. monodon*

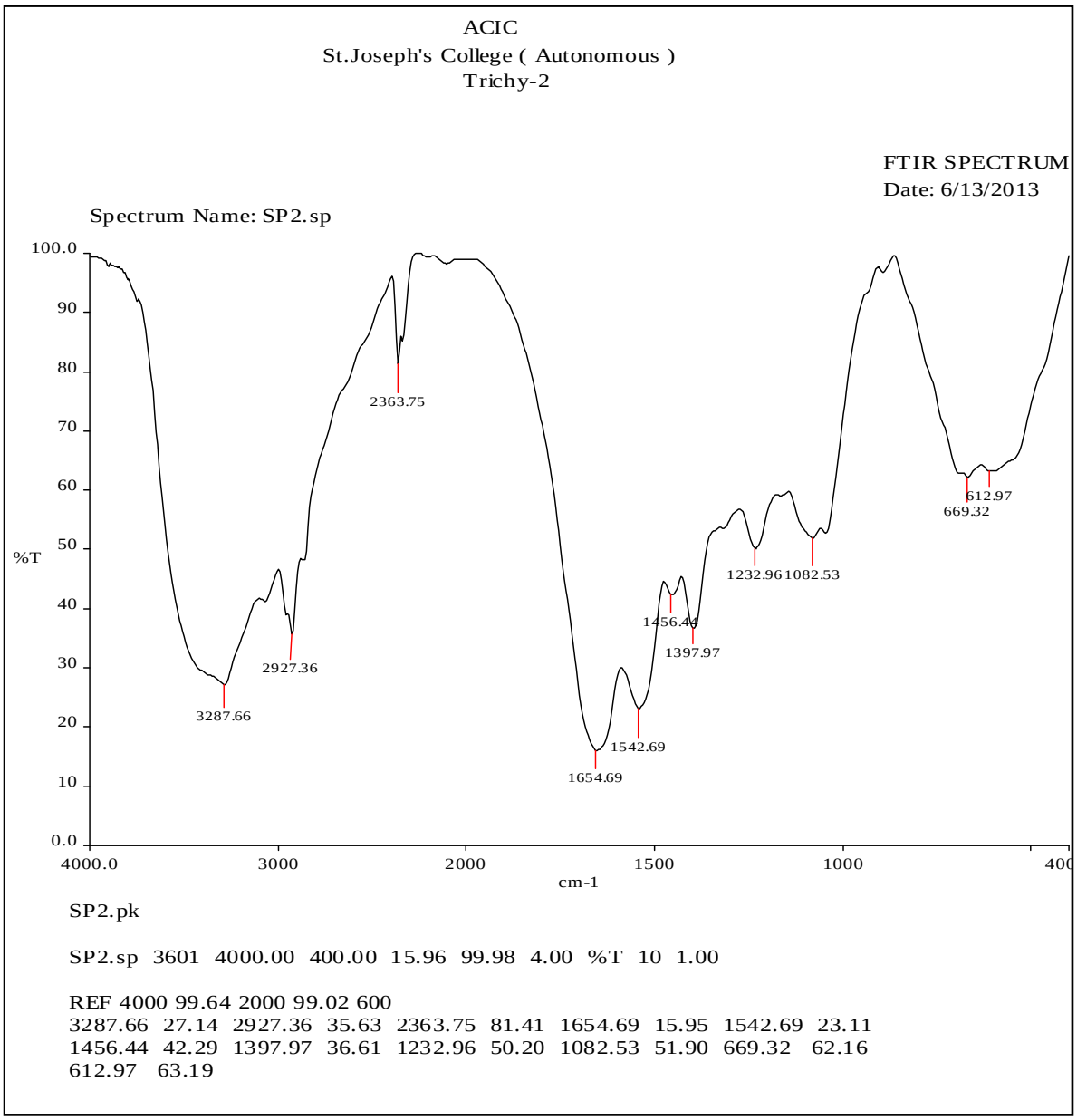

Fig. 8 FTIR study in *P. semisulcatus*

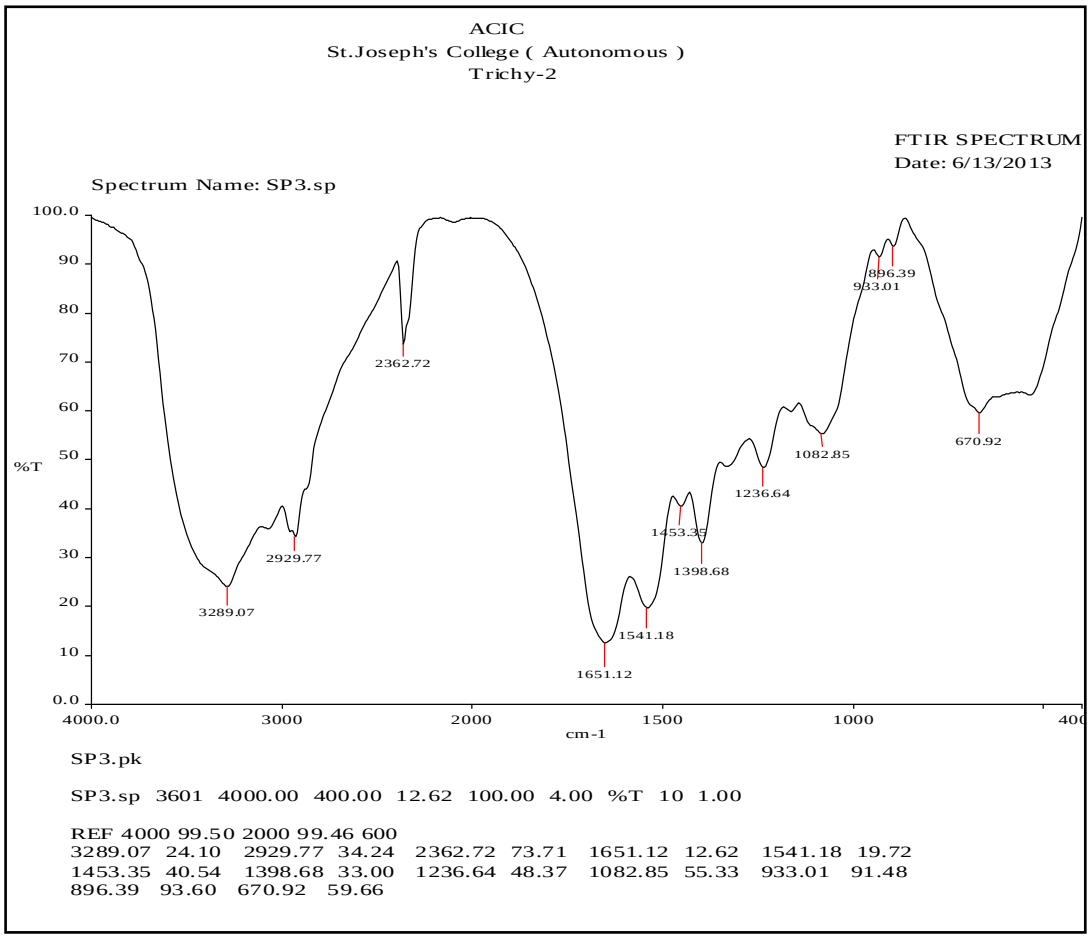

Fig. 9 FTIR study in *F. indicus*

Table.1 Morphometric measurement of the penaeid shrimps

Morphometric Measurement	Species		
	Penaeus monodon	***Penaeus semisulcatus***	***Fenneropenaeus indicus***
Total length (TL) cm	11.8-12.5	10.5-11.0	13.8-14.0
Body length (BL) cm	10.3-10.7	9.1- 13.2	12-13.4
Carapace length (CL) cm	5.0-6.3	4.0-4.9	5.5-5.8
Carapace height (CH) cm	1.6-1.65	1.9-2.5	2.4-3.1

Diagonal carapace length (DCL) cm	3.8-4.0	3.2-3.3	3.5-3.7
First segment width (FSW) cm	1.3-1.5	1.7-1.9	2.5-2.7
First segment length (FSL) cm	1.3-1.4	1.0-1.9	1.4-1.7
Sixth abdominal segment length (SISL) cm	1.5-1.9	1.5-1.8	1.6-1.9
Weight (g)	10.0-10.5	5.0-8.3	15.0-18.0

Table.2 Proximate composition in Penaeid Shrimps

S. No.	Species	Protein (%)	Carbohydrate (%)	Lipid (%)	Moisture (%)	Ash (%)
1.	*Penaeus monodon*	44.2	21.9	11	80.6	6.0
2.	*Penaeus semisulcatus*	45.4	29.3	14	77.8	6.7
3.	*Fenneropenaeus indicus*	54.6	28	12	75.3	5.2

Table. 3 FTIR Frequencies obtained in the Shrimp tissue

S. No.	Compound type	IR Frequency Range (cm^{-1})	Tissue (Meat)		
			Penaeus monodon (cm^{-1})	***Penaeus semisulcatus*** (cm^{-1})	***Fenneropenaeus indicus*** (cm^{-1})
1	Protein (Amide I)	1690 -1600	1654	1654	1651
2	Protein (Amide II)	1575 -1480	1540	1542	1541
3	C-O stretch (Alcohols, Ethers, Carboxylic acids, Esters)	1260 -1000	1082 1234	1082 1232	1082 1236
4	C=O stretch (Aldehydes, Ketones, Carboxylic acids, Esters)	1760 -1670	1654	1654	1651
5	C-H Aliphatic stretch Lipid (-CH_2, - CH_3)	3000 -2850	2929	2927	2929
6	C - H Alkynes stretch	3333 -3267	3287	3287	3289

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RESEARCH ARTICLE

A Comparative Study of some Selected Fitness Variables between Between Male Bodybuilders and Power Lifters of Telangana

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ABSTRACT

The purpose of this present study was to compare the selected fitness variables between Bodybuilders and power Lifters of Telangana. A group of Sixty (60) males participated at the state level, Bodybuilders (N=30) and power lifters (N=30) ranging between 18 to 24 years were selected randomly from the Telangana, India for this study. To compare the mean differences between the state level bodybuilders and power lifters, mean, S.D and t-tests were computed using Statistica Software. Body composition (percentage of body fat), Flexibility (hip & trunk flexibility), muscular strength (bench press 1RM), and muscular endurance (push-ups test for 30 sec) were found to be statistically significant. The mean and S.D of the bodybuilders and power lifters for percent of body fat were (16.4, 2.3) and (19.7, 1.8). With regard to sit and reach test the Mean and S.D between the bodybuilders and weight lifters were (23.90, 4.26) and (17.53, 4.79) Mean and S.D between the groups regard to 1RM bench press were (77.7, 15.0) and (85.0, 17). Mean and S.D between the bodybuilders and weight lifters regard to sit-ups test were (24.5, 2.3) and (17.1, 3.8). It is concluded that both the groups differ significantly with regard to body composition, flexibility, muscular strength and endurance. Furthermore it is also concluded that the power lifters had shows greater performance than their counter part with regard to muscular strength and bodybuilders had shows better performance with respect to body composition, flexibility, and muscular endurance.

Key words: Fitness, Bodybuilding, power lifting, Athletes

INTRODUCTION

Winning in a sport is not a luck or chance or gift, one has to work hard systematically and understand his strength and weaknesses to achieve his target. Body composition can be measured in several ways, through caliper to measure the thickness of subcutaneous fat in multiple places on the body. (Haslam and James 2005), Body Mass Index (BMI) is closely related to percentage body fat and total body fat. (Jeukendrup, A, 2005), Generally the BMI is used as a means of correlation between groups related by general mass and can serve as a indistinct means of estimating adiposity. (Romero-Corral & et al 2008) Body composition for athletes is often better calculated using measures of body fat, as determined by such techniques as skin fold measurements or underwater weighing and the limitations of manual measurement have also led to new, alternative methods to measure obesity, such as the body volume index. Flexibility is the range of motion around a joint, good flexibility in the joints helps in preventing injuries in all stages of life (Johnson & Nelson, 1998). Muscular strength is the ability of the muscle to exert force during activity (Mathews, 1985) .Muscular endurance is the ability of the muscle or group of muscles to overcome or to act resistance for longer duration under conditions of fatigue or tiredness (H. Singh, 1991). The physique or body composition, including the size, shape of the muscle, plays an important role in the sports. Mesomorphic body type is well suits to bodybuilding and endomorphic body type is suitable for power lifters. Body composition makes an important contribution to an individual's level of physical fitness performance, particularly in bodybuilding, bodybuilders with low fat percentage dominates the show and can win the highest level of bodybuilding competitions. Power lifters are not concerned about the percentage of body fat; they are only concerned in lifting the weights i.e. power. Body composition can be measured in several ways, through skin fold caliper to measure the thickness of subcutaneous fat in multiple places on the body. These measurements are then used to estimate total body fat with a margin of error of approximately four % points (Voorhees, 2007). Flexibility is the ability of an individual to move the body and its parts through as wide a range of motion as possible without undue strain to the articulations and muscle attachments. A high level of flexibility helps in saving energy during vigorous movement because of the full range of moment of the joint and muscles, the individual may be less prone to injury. Flexibility is the range of motion around a joint, high flexibility helps in lowering the injuries in all stages of life (Uppal, 2004). It is a very important component of sports performance that can be significantly improved if approached correctly. Muscular strength is the ability of the muscle to exert force during activity, and is very important for power lifters and weight lifters to improve their performance. Bodybuilders also improve maximum muscular strength in off-season program to achieve more mass and strength to re-shape the muscles in onn-season training program. Muscular endurance is also plays an important role in the performance of individuals in various sports and games. Muscular endurance is an important fitness component and helps individuals in performing high performance.. Muscular endurance is the ability of the muscles to continue to perform without fatigue (Hardayal Singh, 1991).

MATERIALS AND METHODS

In this study, a sample of sixty male subjects (thirty bodybuilders and thirty power lifters) who had participated at the state level competition from Hyderabad during the year 2013-2014 were randomly selected as subjects. The age was ranged from 18-24 years. To compare the mean difference between the bodybuilders and power lifters with regard to body composition (% of body fat) were considered, for hip & trunk flexibility (Sit & reach test) was employed, muscular strength was assessed by (parallel bench press 1max rep), and with regard to muscular endurance (sit-ups test for 30 seconds) was considered. Body composition was assessed by taking the skin fold measurement at four sites namely biceps, triceps, subscapular, and suprailiac (Durnin & Womersley, 1974). The Lange Skin fold Caliper was used to assess percentage body fat. Muscular strength test was assessed by parallel bench press test 1RM and the score were recorded by the tester. The Sit and Reach Test was used to measure flexibility. Sit-ups test was employed to measure muscular endurance of the participants. 't'-test was employed with the help of statistica software . The level of significance was at 0 .05.

RESULTS

Table-1: Body composition Results

The mean, Standard deviation and t-test of the percent body fat between bodybuilders and weight lifters. Mean and S.D between the bodybuilders and power lifters were (16.4, 2.3) and (19.7, 1.8) respectively. The data clearly shows that the bodybuilders are having less fat percent than the power lifters, which is significant at ($p<0.05$).

Table-2: Flexibility (hip & trunk) Results

The mean, standard deviation and t-test of the flexibility performance between bodybuilders and weight lifters. Mean and standard deviation between the bodybuilders and power lifters are (23.9, 4.3) and (17.5, 4.8) respectively. The data clearly shows that the bodybuilders are having greater flexibility than the power lifters, which is significant at ($p<0.05$).

Table-3: Muscular strength Results

The mean, standard deviation and t-test of the muscular strength between bodybuilders and power lifters. Mean and Standard deviation between the bodybuilders and power lifters were (77.7, 15.0) and (85.0, 17) respectively. The data clearly shows that the power lifters are par excellent in muscular strength (parallel bench press 1RM) compare to the body builders, which is significant at ($p<0.05$).

Table-4: Muscular Endurance Results

The mean standard deviation and t-test of the muscular endurance performance between bodybuilders and weight lifters. Mean and standard deviation between the bodybuilders and power lifters were (24.5, 2.3) and (17.1, 3.8) respectively. The data clearly speaks that the bodybuilders had shows greater performance with regard to muscular endurance (sit-ups test for 30 seconds) compare to the power lifters group, which is significant at ($p<0.05$).

DISCUSSION

From the results of the study, the above tables showed that there was a significant difference in body composition between the bodybuilders and power lifters. Regard to the flexibility (hip& trunk flexibility) between bodybuilders and power lifters the data speak greater performance from the bodybuilders. In case of flexibility which is an important for the bodybuilders and power lifters respectively. Bodybuilders need lot of strength, muscular endurance, and cardio-vascular endurance apart from flexibility, to compete against opponents on stage by displaying their muscles to the judges for the comparison. Power lifters also need lot of power, strength, speed, muscular endurance apart from flexibility to compete in their event. When exercising squats, bench press and dead lifts the power lifters need lot of skills, flexibility, power, speed, muscular endurance to perform well. From the analysis, it revealed that there is a significant difference on body composition (percentage of body fat) between bodybuilders and power lifters. With regard to muscular strength both the groups differ significantly and power lifter had shows greater performance in (bench press exercise 1RM). Power lifters train with very heavy weights to improve power and strength to compete in various tournaments. Bodybuilders also emphasized and include bench press exercise in their schedule to improve pectoral muscles. Lastly both the groups regard to muscular endurance (sit-ups for 30 sec) differ significantly. The bodybuilders had shown greater performance compare to their counter parts power lifters. Bodybuilders regularly includes sit-ups exercises in their schedule, this is one of the reason that they had perform well in sit-ups test. Abdominals are the essence of bodybuilding game, that's the reason all the

bodybuilders gives lot of importance for abdominals. For power lifters abdominals are not merely necessary as bodybuilders they are only concern in lifting maximum weights.

The following earlier studies are in support with this study

(Samir Kumar & et al, 2014), revealed that the significant relationship was found among basketball group and tug of war group with regard to the selected motor skill related fitness component. (P.Srinivas, 2014), investigated a study and find a significant differences on selected motor fitness variables, speed, balance and endurance among the state level basketball, football and volleyball players. (H.H.Patil, 2014), the finding of this study showed that the male Kho-Kho Players were significantly better than the Kabbadi players with regard to cardio-vascular efficiency. (EB.Srikanth, 2014), It was investigated that the basketball players were having better explosive strength and handball players are having good agility.

CONCLUSION

It is concluded that there is a significant difference in body composition of bodybuilders and power lifters. The trunk & hip flexibility of bodybuilders and power lifter differ significantly. With regard to muscular strength both the groups differ significantly. Furthermore with regard to muscular endurance between both the groups showed significant difference. Moreover in one of the variable i.e. muscular strength the power lifters had shows greater performance in compare with bodybuilders group. Interestingly bodybuilders had shows greater performance with regard to body composition, flexibility, and muscular endurance.

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Table -1: The below tables from 1 to 3 shows the analysis of data.

Sl. no.	Percent Body Fat	No. of Subjects	Mean	SD	't' Value	P -value
1	Bodybuilders	30	16.4	2.3	9.51	0.00
2	Power lifters	30	19.7	1.8		

't'-test for dependent samples marked difference are significant at p< 0.05

Table -2: Flexibility (hip & trunk) Results

Sl.no	Flexibility	No. of Subjects	Mean	SD	't' Value	p-value
1	Bodybuilders	30	23.9	4.3	7.11	0.000
2	Power lifters	30	17.5	4.8		

't'-test for dependent samples marked difference are significant at p< 0.05

Table -3: Muscular strength Results

Sl. no.	Muscular strength	No. of Subjects	Mean	SD	't' Value	p-value
1	Bodybuilders	30	77.7	15.0	6.7	0.000
2	Power lifters	30	85.0	17		

't'-test for dependent samples marked difference are significant at p< 0.05

Table -4: Muscular Endurance Results

Sl. no	Sit-ups test	No. of Subjects	Mean	SD	't' Value	p-value
1	Bodybuilders	30	24.5	2.3	9.01	0.000
2	power lifters	30	17.1	3.8		

't'-test for dependent samples marked difference are significant at p< 0.05

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RESEARCH ARTICLE

Anticariogenic Effect of Silver Nanoparticles synthesized using *Premna latifolia* Leaves

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ABSTRACT

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrates metabolism by *Streptococcus mutans*, a cariogenic bacterium. The silver nanoparticles were synthesized using *Premna latifolia* leaves and the nanoparticles were characterized using UV–Vis spectroscopy and FESEM studies. The synthesized silver nanoparticles were investigated to evaluate the antibacterial activity against *Streptococcus mutans*. Silver nanoparticles showed maximum anticariogenic activity in comparison to standard drug. The results obtained show that silver nanoparticles synthesized using *Premna latifolia* leaves might be used in oral formulations to treat dental caries.

Keywords: *Premna latifolia, Streptococcus mutans*, Nanoparticles, Dental Caries

INTRODUCTION

Dental caries are a major problem worldwide. *Streptococcus mutans* is a primary cause, having the ability to adhere to tooth surfaces while producing acid and surviving in acid conditions (Pooja *et al*., 2012; Jenkinson and Lamont, 2005; Wefel, 1985). Left untreated, dental caries will gradually lead to tooth loss, with ensuing chewing difficulties and ultimately, variety of health problem (Hamada and Slade, 1980). Dental caries prevention is preferable to treatment,

because treatment might come too late to avoid the loss of the tooth. Conventional preventive methods such as the use of alcohol or antibiotics, e.g. chlorhexidine, erythromycin, ampicillin and penicillin, have proven effective in preventing dental caries (Järvinen, 1993). However, excessive use of these chemicals has been reported to change the oral and intestinal flora, and can cause other problems such as vomiting, tooth staining or oral cancer (Park *et al*., 2003). Importantly, antibacterial agents can also promote the development of resistant bacterial strains (Chung *et al*., 2006). For these reasons, alternative methods such as the use of medicinal plants are of increasing interest. Various compounds in plants that are produced for self-protection could support each other in inhibiting bacterial growth, while also reducing the chances of the development of resistant bacterial strains (Chaiya *et al*., 2013). However, few studies on these plants have been conducted. Several plants have demonstrated antibacterial activity, but their effects have not yet been proven against cariogenic bacteria. The genus *Premna* is a widely distributed, medicinally important member of the family *Verbenaceae,* many species of which are extensively used in traditional medicine for the treatment of various disorders (Rao *et al*., 1985). *P. latifolia* Roxb. is a small decidious tree, widely distributed both in tropical and sub-tropical areas. Leaves are aromatic when crushed and diuretic when given internally and are applied externally in dropsy. A good volume of phytochemical work has been done on *Premna* species and several new terpenoid compounds have been isolated (Suresh *et al*., 2011). Methanolic extract of *P. latifolia* leaves exhibited remarkable anticariogenic activity against *Streptococcus mutans, Streptococcus sanguis, Streptococcus sobrinus, Streptococcus mitis, Streptococcus oralis* isolated dental caries patients (M Rajathi D Modilal *et al*.,2015). Silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine (Frattini *et al.,* 2005). In the present investigation silver nanoparticles were synthesized using *Premna latifolia* extracts and its antimicrobial activity is evaluated against human cariogenic bacteria *Streptococcus mutans*.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material *Premna latifolia* was collected from in and around Tiruchirappalli, Tamilnadu, India. The plant was authenticated in the department of Botany, St. Josephs College and it has been deposited in herbarium.

Evaluation and synthesis of silver nanoparticles (Chauhan and Upadhyay *et al.,*2012)

Preparation of extract

10 g of *P.latifolia* fresh leaves were weighed and added to 100 ml of distilled water and allowed it to boil, the filtrate was filtered through Whatmann filter paper, the extract was used for further analysis.

Biosynthesis of Silver nanoparticles

1Mm fresh silver nitrate solution was prepared in a Brown bottle; 10 ml *of P.latifolia* leaf extract was added into 90 ml of silver nitrate and incubated at room temperature for 2 h.

UV-Vis spectra analysis

The bioreduction of Ag^+ in aqueous solution at different temperature interval was monitored by UV-Vis spectroscopy.1ml of sample was taken in Eppendroff's tube, centrifuge it at 10,000 rpm for 10 min, discard the supernatant, wash the pellet two times at 7000 rpm for 2 min and then dilute the pellet with 1ml of D.W for further analysis.

Field Emission Scanning Electron Microscopy (FESEM)

The morphological characterizations of the samples were done using FESEM. In the FESEM an electron beam is focused into a fine probe and subsequently raster scanned over a small rectangular area. As the beam interacts with the sample it creates various signals (Secondary electrons, internal currents, photo emission, etc.), all of which can be appropriately detected(Annamalai *et al.,* 2011).

Antimicrobial activity of silver nanoparticles by well diffusion method

The silver nanoparticles (Ag NPs) synthesized from *P.latifolia* was tested for their antimicrobial activity by well diffusion method against cariogenic organism *S.mutans.* The pure cultures of organism were sub cultured on Muller–Hinton broth at 35°C on rotary shaker at 200 rpm. The strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm were made on Muller–Hinton agar plates using gel puncture. Using micropipette 25µl, 50µl, 75µl and 100µl of the sample of nanoparticles solution were poured into wells on all plates. After incubation at 35°C for 48 h, the different levels of zone of inhibition were measured.

RESULTS AND DISCUSSION

The colour changes were noted by virtual observation in extracts of *Premna latifolia* when incubated with aqueous solution of $AgNO_3$ at room temperature. It started to change its colour from watery to yellowish brown due to the reduction of silver ions, this showed the formation of silver nanoparticles (Saifuddin *et al.,*2009).Synthesized silver nanoparticles from leaf extracts of *P.latifolia* were analyzed.The UV-Vis spectroscopy method can be used to track the size evolution of silver nanoparticles based on localized surface plasmon resonance band exhibited at different wavelength. Figure 1 shows the UV-Vis spectra obtained from solution at room temperature; the spectra shows a peak at 411 nm. An absorption peak between 410-460 nm confirms the presence of silver nanoparticles (Rajan *et al*., 2013).A rapid increase in the synthesis of nanoparticles was observed with an increase in reaction time. The concentration of the extracts also plays a major role as it is responsible for the synthesis of symmetrical nanoparticles. As metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules, in green synthesis, it is believed that the natural material extract acts as reducing agent for the generation of metal nanoparticles. FESEM images of bio-synthesized $AgNP_S$ were observed. The results of Fe-SEM analysis show that the silver nanoparticles synthesized are of uniform size distributed evenly and spherical in shape. Researchers have reported the synthesis of nanoparticles from various plant sources. The reports shows that the silver nanoparticles obtained from various sources are mostly spherical in shape and of different sizes (Shankar *et al.,* 2005; Kasthuri *et al.,* 2009).

The silver nanoparticles synthesized by *P.latifolia* extract exhibited anticariogenic activity against *Streptococcus mutans.* It was found that the nanoparticles caused inhibition of *Streptococcus mutans* in a dose dependent manner. From the table it is evident that, for the four concentrations taken for each extract; the highest concentration 100 µl/ml showed the maximum zone of inhibition for the bacterial species taken in the study. Inhibition caused by standard antibiotic was lower than that of nanoparticle at the concentration of 100 µl/ml. The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria(Sondi and Salopek-Sondi, 2004). The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Morones *et al.,* 2005). Further study is needed to identify the mechanism of action of nanoparticles against cariogenic bacteria

CONCLUSION

Cariogenic disease is still a major oral health problem in most industrialized countries, affecting 60-90% of school children and the vast majority of adults. In order to overcome the disease biomedicine need to be developed, against artificial drugs; where this biomedicine is cheap and safe. Thus, silver nanoparticles synthesized using *P. latifolia* leaves has antimicrobial activity and can be used clinically to find novel antibacterial compounds against cariogenic bacteria *Streptococcus mutans*.Devi KV, Pai RS. Antiretrovirals: Need for an Effective Drug Delivery. Indian J Pharm Sci 2006;68:1-6.

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Fig. 1.Synthesis of silver nanoparticles from leaf extract incubated with silver nitrate solution

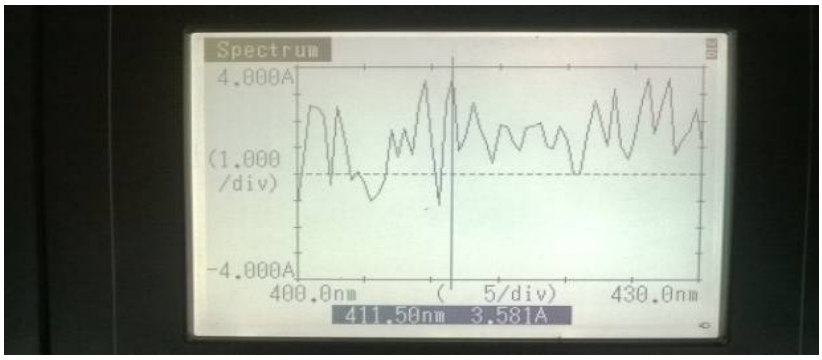


Fig.2. UV-Vis specroscopy Graph obtained for silver nanoparticles synthesized using *P.latifolia* leaves

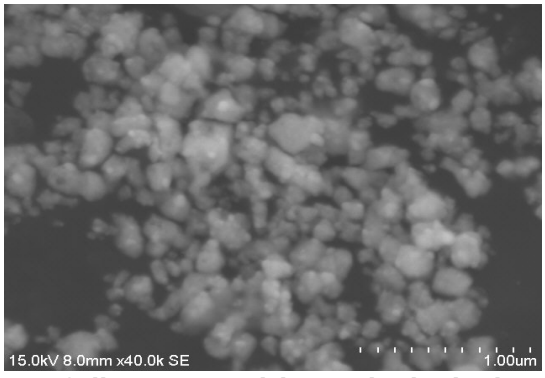
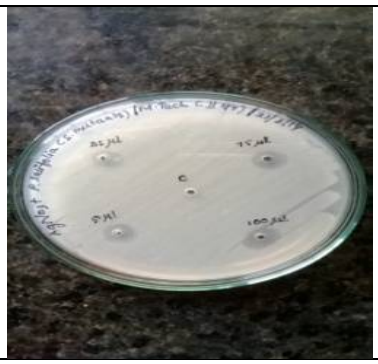


Fig.3. FESEM image of silver nanoparticles synthesized using *P.latifolia* leaves

Table 1: Antimicrobial properties of silver nanoparticles synthesized using *P.latifolia* leaves against the cariogenic bacteria *Streptococcus mutans*

Pathogens	Antimicrobial activity*				Positive control**
	Conc. Of 25 µl/ml	Conc. of 50 µl/ml	Conc. of 75 µl/ml	Conc. of 100 µl/ml	Conc of 100 µl/ml
Streptococcus mutants	11mm	14mm	16mm	22mm	21mm



*measured by the diameter of zone of inhibition in mm, Conc=Concentration,
**Ciprofloxacin is the positive control group
Zones are mean diameter of five replicates

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RESEARCH ARTICLE

Zinnia Flower (Asteraceae) as a Key Nectar Source for Butterflies in the Bishop Heber College Campus, Trichy, TamilNadu

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ABSTRACT

Zinnia is a genus of plants of the sunflower tribe within the daisy family. Zinnias are popular garden flowers because they come in a wide range of flower colours and shapes, and they can withstand evenhot summer temperatures, and are easy to grow from seeds. Zinnias are annuals, shrubs, and sub-shrubs.Most species have upright stems but some have a lax habit with spreading stems that mound over the surface of the ground. The Zinnia plant's height ranges from 15 cm to 1 meter.The floral characteristics such as the pink colour of the flower, lack of odour, short-tubed corolla with deep seated nectar having 17% –22% sugar concentration are well tailored for visitation by butterflies.The nectar is hexose-rich and contains the essential amino acids such as arginine and histidine and the non-essential amino acids such as alanine, aspartic acid, cysteine, glysine, hydroxyproline, tyrosine, glutamic acid and serine.The inflorescences with clusters of flowers provide an excellent platform for foraging by butterflies. The flowers are long-lived and attractive to butterflies. A variety of butterflies visit the flowers for nectar and in doing so, they pollinate them.Nymphalids are very diverse and utilize the flowers until exhausted.The flowers being mediun in size with a good amount of nectar compel the butterflies to do a more laborious search for nectar from a greater number of flowers. But, the clustered state of the flowers is energetically profitable for butterflies to reduce search time and also flight time to collect a good amount of nectar; such a probing behaviour is advantageous for the plant to achieve self- and cross-pollination. Therefore, the study shows that the association between *Zinnia*and butterflies is mutual and such an association is referred to as psychophilous.

This plant serves as a key nectar source for butterflies at the study site where floral nectar sources are scarce during the whole year.

Keywords: Butterflies, nectar source, psychophily, Zinnia flower.

INTRODUCTION

Butterflies are phytophagous. The ability of herbivorous insects to feed on plants has been demonstrated to be intricately linked to plant taxonomic diversity (Mitter et al. 1988) and involves competition between plants and insects (Dawkins and Krebs 1979). The dominant strategy among herbivorous insect species involves specialization on a set of closely related plants (Ehrlich and Raven 1965; Eastop 1973; Ehrlich and Murphy 1988; Ward and Spalding 1993). This makes it very difficult to discern the relative importance to the insect of chemical, visual, and mechanical stimuli from host and non-host plants (Schoonhoven et al. 1998; Hooks and Johnson 2001). However, it is generally assumed that the process of host selection in specialist insects is governed primarily by volatile chemical signals, later by visual stimuli, and finally by non-volatile chemical signals (Hern et al. 1996; Hooks and Johnson 2001). Butterflies demonstrate a hierarchy in host preferences, discriminating among plant species, among genotypes, among individuals with different phenological and physiological conditions, and even among plant parts (Wiklund 1984).Thus, the relationship between any given butterfly species and its host plant is very specific. Among all the resources required by butterflies that comprise a habitat (Dennis et al. 2003 2006; Dennis 2010), the larval host plants are the key resource, being fundamental for reproduction .Adult butterflies visit a wide variety of available flowers are considered to be opportunistic foragers (Courtney 1986). However, studies conducted to date, indicate that butterfly species show distinct flower preferences (Erhardt& Thomas 1991). Later, Kunte (2000) also stated that butterflies do not feed indiscriminately from any flower that they might find. They prefer certain floral nectars with specific chemical composition. Their visits to different flowers also depend on other factors like floral colour, shape, size, position and arrangement in the inflorescence. For a butterfly, a flower must offer a reasonable reward, yet may physically restrict access to the reward. Access may be limited by a complex flower structure demanding particular foraging skills. Faegri& van der Pijl (1979) stated that the floral features such as large, red or blue, narrow, tubular flowers with deep nectaries and often yellow rings or other markings on the petals which function as nectar guides are important for butterfly visitation. Gunathilagaraj et al. (1998) reported that butterfly flowers are often regular, tubular and sweet-smelling. Butterfly flower is typified by red, yellow or blue upright flowers that have diurnal anthesis. Opler (1983) suggested that corolla colour and shape, positioning of sexual parts, position on plant, presence of nectar guides,andfragrance play important roles in the selection of flower foragers. Baker & Baker (1982, 1983) described two categories of flowers with reference to flower-butterfly relationships. The first category is “true butterfly flowers” which are characterized by deep, narrow corolla tubes with relatively copious sucrose-rich nectar. The second category is “bee and butterfly flowers” which are characterized by short-tubed corolla with hexose-rich nectar for which Asteraceae members have been shown to be excellent examples. The findings of the present study on the floral biologyof *Zinnia* flower and its mutual association with butterflies have been examined in the light of these generalizations in order to adjudicate *Zinnia* floweras a key nectar resource for butterflies during the whole year at the study site.

MATERIALS AND METHODS

Zinnia flowersoccurring at the two sites (Vsantham garden and Community college areas) of Bishop Heber College of Tamil Nadu were used for the study during 2011 to 2013. The details of flower morphology were analysed such as flower sex, shape, size, colour, and odour, sepals, petals, stamens and ovary and growth pattern. The Zinnia flowers have a range of appearances, from a single row of petals, to a dome shape, with the colours white, chartreuse, yellow, orange, red, purple, and lilac. They are grown in fertile, humus-rich, and well-drained soil, in an area with full sun. They will reseed themselves each year. Leaves are opposite and usually stalk less (sessile), with a shape ranging from

linear to ovate, and pale to middle green in colour. Ten fresh flowers were used to measure the total volume of nectar/flower. The nectar sugar concentration was measured by using a Hand Sugar Refractometer (Erma, Japan) as per Dafni et al. (2005). Nectar analysis for sugar types was done as per the Paper Chromatography method of Harborne (1973). Nectar analysis for amino acid types was done as per the Paper Chromatography method of Baker and Baker (1973). Regular observations were made on butterflies visiting the flowers for nectar to record the number of foraging visits species-wise and then family-wise in order to assess the importance of *Zinnia* floweras a key nectar source during the study period.

RESULTS

Plant phenology and floral biology: Zinnia floweris aannuals, shrubs, and sub-shrubs species with scattered distribution in the college campus(Image 1a,b).The field surveys in the entire college area covering an extent of 2km^2 showed that the population of this species is limited to about 600 (approximately) plants only. Zinnias care popular garden flowers because they come in a wide range of flower colours and shapes, and they can withstand hot summer temperatures, and are easy to grow from seeds. The flowers are small, 1 cm long, tubular, white with a yellow tinge, odourless, regular and bisexual.The corolla is tubate tipped with five lobes, 7mm long, white and conceals nectar(Image 1c - e).The stamens are five each with dithecousanthers having versatile fixation, very small and situated below the stigma; the anthers appear star-like at the mature bud stage but take different postures after anthesis.The style is erect and terminated with spathulate bifid stigma. Nectar is produced in minute amounts which stands at 0.9 ± 0.17µl per flower and is collected at the base of corolla tube.The nectar sugar concentration ranged from 17% -22%; the sugar types include sucrose, fructoseand glucose but the last is more dominant. The nectar contains both essential and non-essential amino acids.The nectar is hexose-rich and contains the essential amino acids such as arginine and histidine and the non-essential amino acids such as alanine, aspartic acid, cysteine, glysine, hydroxyproline, tyrosine, glutamic acid and serine. The flowers remain in place for few days and fall off subsequently.

Foraging activity of butterflies: The butterflies foraging for nectar included 25 species representing Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Hesperiidae (Fig. 1, Table 1). The Papilionidaeby 5 species, Pieridaeby 4 species, Lycaenidae by 1 species, Nymphalidae by 3 species, and Hesperiidae by a single species. The papilionids were*Graphiumagamemnon* (Image 1f). The pierids were *Deliaseuchariss*(Image 1g).The nymphalids were *Euploea core* (Image 1h), The Lycaenidae were*Zizulahylax*(Image 1i),The*Hasorachromus were* Hesperiidae(Image 1j),The papilionids were*Graphiumagamemnon*(Image 1f) *Graphiumdoson*(Image 1f*)Papiliopolytes (female,male)* and*Papiliodemoleus,* The pierids were *Deliaseuchariss*(Image 1h) *Euremablanda*(Image 1h) ,*Catopsiliapyranthe*(Image 1h) *CatopsiliaPomona*(Image 1h) andThe nymphalids were *Tirumala septentrionis*(Image 1i),*Euploea core* (Image 1j), *Hypolimnasmisippus*(Image 1k), The lycaenids were *Zizulahylax*(Image 2i),. The hesperiid was *Hasorachromus*(Image 2m). Of these, the individuals of Papilionidae butterflies were more than those of other families at the flowers throughout the flowering season. The data collected on the foraging visits of butterflies of each family showed that made papilionids36%, pierids29%, nymphalids 21%, lycaenids 7%and hesperiids 7% of total visits (Fig. 2). The aggregated arrangement of flowers provides a comfortable landing place for butterflies and this arrangement also enables them to probe several flowers in each visit in succession for nectar before their departure. The clusters of paniculate inflorescences borne terminally stand out prominently and the butterflies were found to be attracted to them even from a long distance.The butterflies frequently moved between individual plants of *zinnawhich* occur scattered in the habitat, this inter-plant foraging activity was considered to be important in promoting cross-pollination.

DISCUSSION

Environmental conditions such as temperature, humidity, wind, precipitation (rainfall) plays a key role in defining the function, distribution, abundance of plants and butterflies in combination with other factors..Flowering is

influenced by the surroundings, temperature, time of exposure to sunlight, humidity, wind, rainfall and favorable circumstances for pollination, seed formation and seed dispersal. The period between each flowering season is called the Oscillatory period. This is under the control of an endogenous oscillator. Under these favorable conditions, the receptors such as phytochromes present in the leaves will send the signals to rush more nutrients, Carbohydrates, sugars, water and florigens such as Gibberellins (plant hormones) to the meristems to produce floral buds. This floral bud blooms on receiving more sugars and water. But the blooming will take place only when the Inhibitors concentration falls bellow certain levels. Burkhardt (1964) and Faegri& van der Pijl (1979) characterized butterfly-flowers as mentioned in the introduction section. Baker & Baker (1983) reported that the short-tubed flowers tend to be hexose-rich and if grouped in conspicuous inflorescences provide an excellent standing platform for foragers, and attract, both butterflies and short-tongued bees.Cruden et al. (1983) also stated that hexose-richness appears to prevail in the nectar of short-tubed flowers. These authors also documented that the nectars of most butterfly-pollinated flowers fall within the range of 15 to 25% sugar concentration. Kingsolver & Daniel (1979) suggested that the nectar sugar concentrations of 20–25% optimize the net energy gain by the butterflies.

Nectar is a potential source of amino acids for the nutrition of butterflies. Naturally, butterfly nectars areUsually, three to four essential amino acids and several non-essential amino acids are found in floral nectars (Baker & Baker 1982; 1983). Baker & Baker (1986) reported that the amino acids add taste to the floral nectar and it depends on their concentration. Their presence serves as an important cue for butterflies to make flower visits and in the process effect pollination The nectar of Zinniaprovides alanine, aspartic acid, cysteine, glysine, hydroxyproline, tyrosine, glutamic acid and serine. A later study on a temperate species Euphydryaseditha showed that amino acids in the adult diet led to heavier eggs (Murphy et al. 1983). Jervis & Boggs (2005) reported that the butterflies are agents of selection for higher nectar amino acid production. The requirement of amino acids during adult stage of the butterfly is related to the larval nutritional condition. The larval food plant has a key role in the evolution of the flower-butterfly mutualism, and demonstrates that the importance to butterfly reproduction, and of different nutrient source varies with butterfly nutritional state Zinniathein florescences of flowers provides an excellent platform for foraging by butterflies. The retention of flowers for four days appears to be an adaptive trait for the plant to enhance its attractiveness to butterflies. With these floral, structural and functional characteristics, zinnia has been found to be foraged by butterflies of all five families of Lepidoptera. The short-tubed flowers facilitate butterflies with any length of proboscis to collect nectar easily. The flowers being small in size with minute amounts of nectar compel the butterflies to do a more laborious search for nectar from a greater number of flowers. But, the clustered state of the flowers is energetically profitable for butterflies to reduce search time and also flight time to collect a good amount of nectar.Zinniaattracts more number of individuals and species of papilionidaebutterflies when compared to those of other families of butterflies suggesting that papilionidaebutterflies use this plant as an important nectar source. The psychophily is advantageous for the plant because butterflies do not collect pollen for themselves but only carry pollen on their proboscis and effect pollination while collecting nectar. Therefore, the study shows that zinnia flowering is quite attractive to butterflies and it is a keystone species for them since it provides them with nectar for the whole year. There are no other plant species in flowering which attract a diversity of butterflies to this extent during the flowering period of zinnia and hence this species plays a crucial role for the local butterflies for their nutrition in the habitats.

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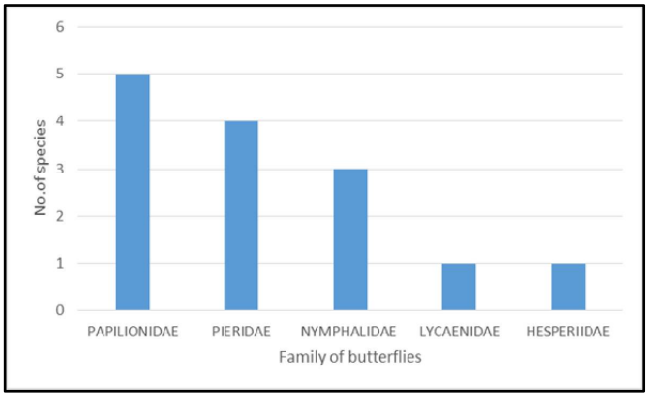

Figure 1. Family-wise number of butterfly species foraging for nectar on *Zinnia*

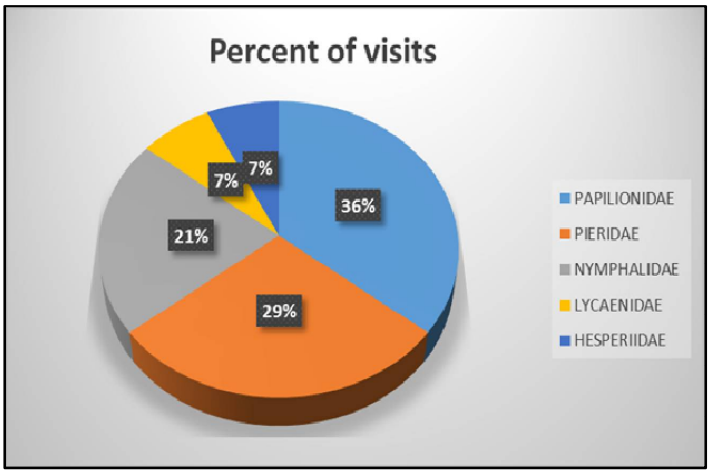

Figure 2. Family-wise percentage of foraging visits of butterflies on*Zinnia*

Table 1. List of nectar feeding butterflies on Zinnia

Scientific Name	Common name
Papilionidae	
Graphiumagamemnon	Tailed jay
Graphiumdoson	Common jay
Papiliopolytes	Common Mormon (female)
Papiliopolytes	Common Mormon (male)
Papiliodemoleus	Lime butterfly
Pieridae	
Deliaseuchariss	Common jezebel
Euremablanda	Three spot grass yellow
Catopsiliapyranthe	Mottled emigrant
Catopsiliapomona	Common emigrant
Nymphalidae	
Tirumala septentrionis	Dark blue tiger
Euploea core	Common Indian crow
Hypolimnasmisippus	Danaid egg fly
Lycaenidae	
Zizulahylax	Tiny grass blue
Hesperiidae	
Hasorachromus	Common banded awl

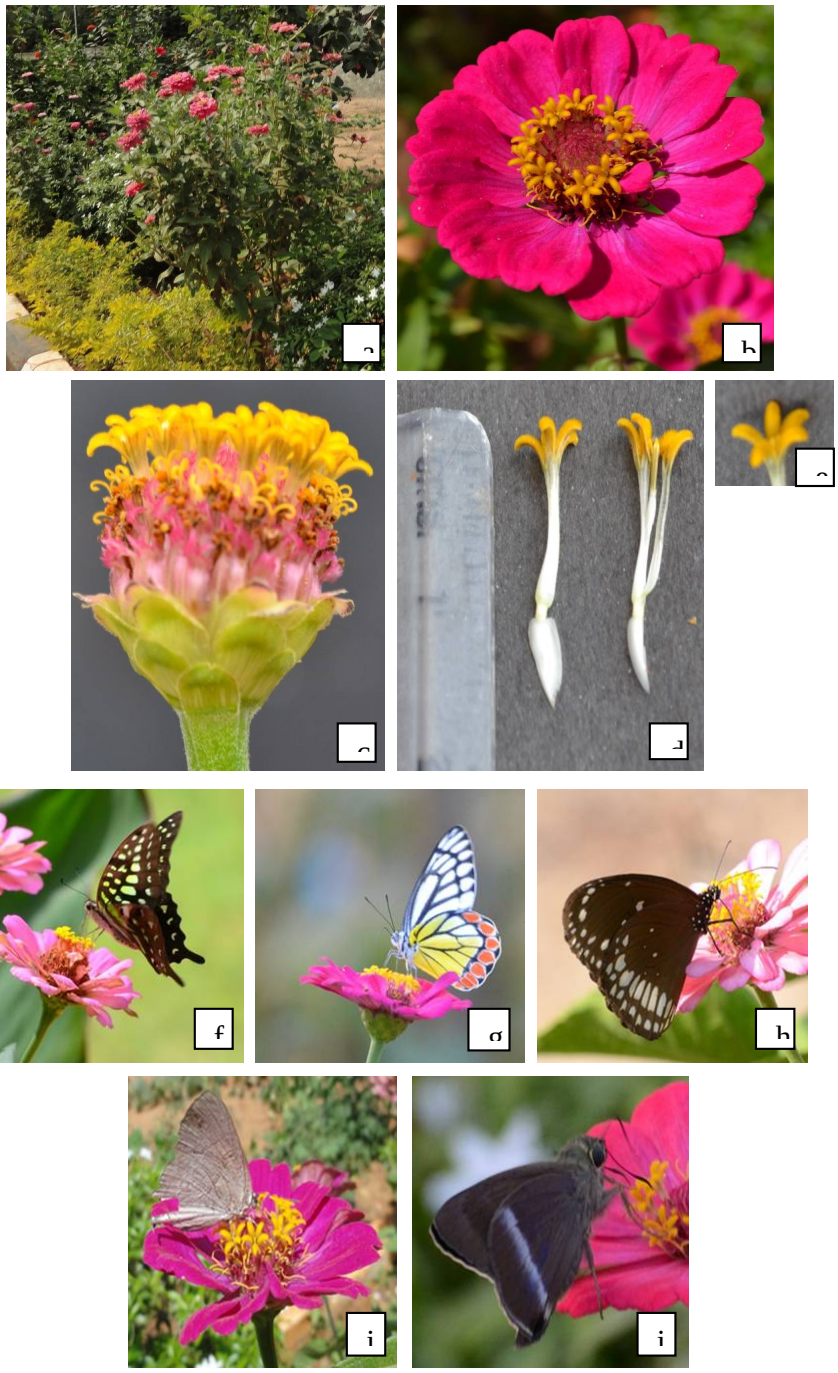

Image 1.Zinnia

a - habit; b - flower; c - corella; d & e inflorescence; f - *Graphium Agamemnon, g - Deliaseuchariss, h - Euploea core, I - Zizulahylax, j - Hasorachromus*

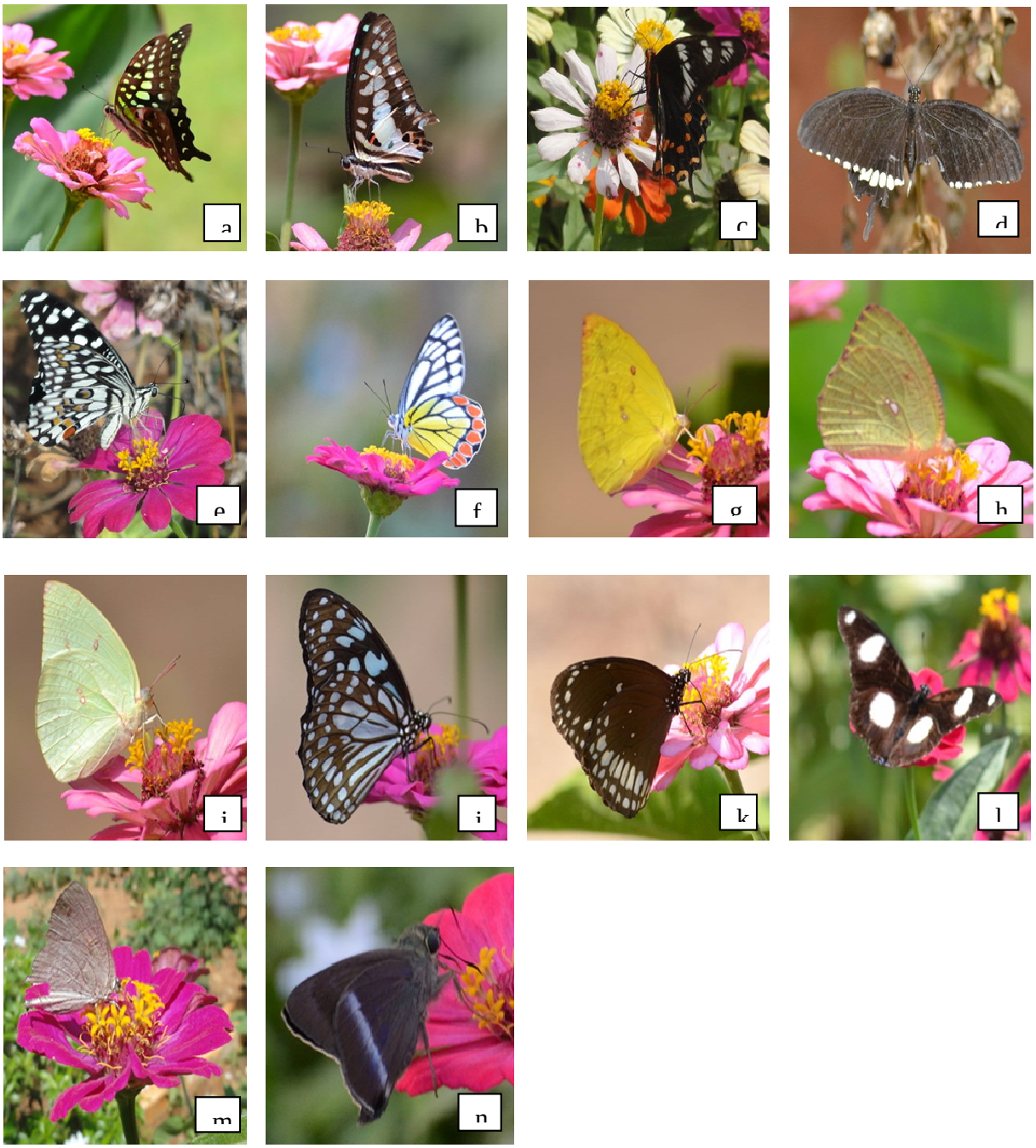

Image 2. Butterflies observed on Zinnia

a -*Graphiumagamemnon* b - *Graphiumdosonc* ,d,-*Papiliopolytes (female,male)*; e -*Papiliodemoleus,*; f -*Deliaseuchariss;* g -*Euremablanda*; h -*Catopsiliapyranthe*;i -*CatopsiliaPomona*; j -*Tirumala septentrionis*; k -*Euploeacore*;l -*Hypolimnasmisippus*; m -*Zizulahylax*; n - *Hasorachromus*

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RESEARCH ARTICLE

Synthesis and Characterization of Carotenoids from *Micrococcus luteus* and its Impacts in Human Health Concern

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ABSTRACT

Pigmentation is a common feature of bacteria of different phylogenetic and environmental origins and these bacteria are called as chromobacteria.In photosynthetic organism, carotenoids are most commonly occurring pigment associated with the photosynthetic membrane, and they help in harvesting and transferring light energy to chlorophyll and also protect the photosynthetic bacterial apparatus against photooxidation. Carotenoids pigments are widely present in *Micrococcus luteus*which isyellow in colour and these are water soluble, which diffused in to the growth medium and give pigmentation to the colonies.Carotenoids are isoprenoid lipids containing 40 carbon atoms and are detectable in the cell membranes of microorganisms. Carotenoids also play an important potential role in human health by acting as biological anti oxidant, protecting the cells and tissues from the damaging effects of free radicals and singlet oxygen.In this present work carotenoids are isolated, separated and purified by coloumn chromatography then these pigment are analysed for their structural elucidation by FTIR and their spectral peaks indicates presence of continuous conjugated carbon double bonds of carotenoids. Finally their beneficial role was examined by its antimicrobial activity. This pigment shows good bactericidal effect against *Pseudomonas aeroginosa* and *E.coli*. The fungicidal activities against Penicillin sp and Aspergillus sp. Carotenoids are potential antioxidants and have 61.2 % DPPH Radical Scavenging Activity. Hence from their properties it can be used as an alternative to the synthetic compounds in food and pharmaceutical technology or serve as lead compounds for the development of new drugs with the prospect of improving the treatment of various disorders.

Key-words: Pigments, Carotenoids, Chromatography, Antioxidant and Antimicrobial Activity.

INTRODUCTION

Colour is the most pleasing attribute of any article, colours play a vital role to sense the environment and it is present in higher organism to guide their interactions with others. The colorant refers to any chemical compound that imparts colour, while the pigment indicates normal constituents of cells or tissues giving colour. The present trend throughout the world is shifting towards the use of eco-friendly and biodegradable materials, the demand for natural pigment is increasing, so now microbial pigment is gaining its popularity among the researchers. Microorganisms are the most powerful creatures in existence and determine the life and death on this planet. Microorganisms are associated with all the foods that we eat and are responsible for the formation of certain food products by the process of fermentation. It can be used as a source of food in the form of single cell proteins and food supplements in the form of pigments, amino acids, vitamins, organic acids and enzymes. In this way the pigments from microbial sources are a good alternative. When the microbial cells are used to produce the colors the term refers to "microbial pigment". Microorganisms are known to produce a variety of pigments; therefore they are promising source of food colorants (Ali, 2011 and Ahmad*, et al*., 2012).In photosynthetic organism, carotenoids are mostly associated with the photosynthetic membrane, and they help in harvesting and transferring light energy to chlorophyll and also protect the photosynthetic bacterial apparatus against photooxidation. Carotenoids pigments are also present in a wide variety of particular genus, such as *Flavobacterium* (Holmes, *et al.,*1984) and genus such as *Micrococcus* (Kocur*et al.,* 1984). Bacteria belonging to the genus *Micrococcus* may be yellow, yellowish, green or orange as in *Micrococcus luteus.Micrococcus* is aerobic, spheres, 0.9 to 1.8 m in diameter, occurring in clumps and in irregular clusters of tetrads.It is non motile, non spore forming and Gram-positive organism.They are chemoorganotroph, strict aerobes, grow in pH ranging from 6.8 to 7.5 at temperature between 22^{o}C to 37^{o}C. They produce a yellow or yellowish green, water-insoluble carotenoids pigment. Some groups of bacteria express various pigments at different stages of growth.Pigments are produced mainly in cell bound state and their structures vary according to its organization of genes.Carotenoids are isoprenoid lipids containing 40 carbon atoms and are detectable in the cell membranes of many microorganisms. The presence of isoprene derivatives in *Micrococcus luteus* helps in synthesis of sterols, carotenoids, fatty acids and it forms platform for isolation of important enzyme in chemical and pharmaceutical industries.The structure of a carotenoid ultimately determines what potential biological functions that pigment may have.The microbial pigments plays important role as antimicrobial agent as these pigments can synthesize certain antimicrobial compounds like ethanols, hydrogen per oxide, organic acids and CO_2 during its metabolic process.Carotenoids also play an important potential role in human health by acting as biological anti oxidant, protecting the cells and tissues from the damaging effects of free radicals and singlet oxygen.These antioxidants interact and stabilize free radicals which prevent some of the damages caused by them. *Micrococcus luteus* capable of survival under stress conditions such as low temperature and starvation and do not form spores as survival structures. Other health benefits of carotenoids are related to enhancement of immune system, protection from sunburn and inhibition of the development of certain types of cancers (Nishino, 2002). A number of epidemiological studies have revealed that an increased consumption of a diet rich in Carotenoids is correlated with a diminished risk for various types of cancer, cardiovascular diseases (Sandmann, 2001).They also possess abilities to tolerate very toxic organic pollutants and metals and hence play a potent role in bioremediation. In this present study yellow pigment carotenoid was extracted from *Micrococcus luteus,* characterized and screened for its biological properties with reference to human health concern.

MATERIALS AND METHODS

Collection of bacterial strain

The bacterial culture *Micrococcus luteus* MTCC-109 (Microbial Type Culture Collection) was obtained from the Institute of Microbial Technology (IMTECH), Chandigargh.

Cultural maintenance of bacteria

The *Micrococcus luteus* were grown on nutrient agar medium supplemented with 2% of glycerol and adjusted to pH 7.2. The inoculam was maintained in nutrient broth and 24 hours culture was streaked over the surface of the medium and incubated at room temperature for one week. After incubation, there was development of yellow colour colonies. These pigmented colonies were removed by adding 70% of aqueous acetone. The cells were scraped from the agar surface using a glass rod bent at right angle. The cells were then dried at 60 $\pm^{o}$ C and weighed and stored in vials covered with aluminium foil.The morphological structureofbacterial colonies was viewed under microscope by Gram staining.

Extraction of yellow pigment from *Micrococcus luteus*

The cold methanol was effective in extracting the yellow pigment carotenoids from the bacterial cells, when the mixture was mixed with abrasive such as alundum. But in this study a convenient method of 50 ml of methanol was added to one gram of dried biomass and it was boiled in hot water bath for 10 minutes. After cooling, the cells were removed by centrifugation. The supernatant was yellow in colour and it was collected for further separation of carotenoids.The concentrated methanol extract was diluted to 90% by adding water. Then equal volume of petroleum ether was added to it and shaken in a small separatory funnel. The petroleum ether phase was removed separately and it was repeatedly washed with fresh 90% methanol to ensure the complete removal of the pigment. Then the water molecules are removed from petroleum ether phase by shaking it with saturated salt solution of anhydrous sodium sulphate for half an hour and then it was evaporated to dryness under partial vacuum. After that to this dried pigment, 50ml of 2% potassium hydroxide in methanol was added and kept at 40^{o}C for three hours. Again the methanol concentration was reduced to 90% by adding water and it was shaken in separatory funnel with equal volume of petroleum ether. Next these residues are separated by coloumn chromatography.

Purification of yellow pigments by column chromatography

The pigments were separated and it was purified by chromatographic adsorption technique. Calcium carbonate was used as adsorbents and it is activated by heating at 150°C for five hours, and then cooled to room temperature. Petroleum ether was used as the solvent of for the carotenoid separation. The adsorption column is a glass tube with 15cm long and 15mm in diameter, sealed at one end to a tube of 6mm bore and approximately 8cm in length. And it is attached to a vacuum flask. The adsorbent calcium carbonate was packed into the column. Then the petroleum ether was poured onto the column and suction was used to test for the presence of cracks in the column. 10ml of pigment residues was poured onto the adsorption column. As the solution passed through the column the pigments formed narrow band and it is collected gradually into a series of vials. Finally it was evaporated to dryness under reduced pressure and used for further analyses.

Determination of carotenoids

The amount of carotenoids from the bacteria was determined by method used by Raganna (1986).1ml of bacterial extraction was dissolved in petroleum ether and it is transferred to separating funnel, to which 3% of sodium sulphate was added and shaken to separate the coloured portion. The coloured solution was collected and final volume was made upto 25ml.The optical density was measured at 449nm and reading was compared with standard curve. The quantity of carotenoids was calculated as Concentration of carotene in solution as read from standard curve (µg) x Final volume of separated colour solutions x Dilution

$$\mu g \text{ of carotenoids} = \frac{\text{standard curve } (\mu g) \times \text{Final volume of separated colour solutions} \times \text{Dilution}}{\text{Weight of Sample}} \times 100$$

Characterization of microbial pigments

Analysis by thin layer chromatography (Harborne, 1973)

The carotenoids were subjected to thin layer chromatography (TLC) to separate the bioactive compounds. The TLC plates were prepared using silica gel G and distilled water. The spot was allowed to dry and the TLC plate was placed into the TLC chamber which was saturated with Hexane : Acetone(60:40) solvent mixture. When the solvent reached 2 cm below the top, the plates were taken out of the chamber and detected for the spots and Rf value was calculated.

High Performance Liquid Chromatography (HPLC) Analysis

Carotenoids were analyzed by HPLC equipped with two 510pumps and a spectrophotometer detector. The extract solution was analyzed by using a Shimadzu, C_{18} column water spherisorb ODS2 (4.6 mm ID X 25cm.100 % methanol) column at 25°C, using acetonitrile : water (solvent A) and ethyl acetate (solvent B) at a flow rate of 1ml/min, peaks were monitored with a HPLC- water equipped with water 2996 phase diode array detector. Gradient for separation was 0 - 100% ethyl acetate in acetonitrile /water (9:1) over 25 min with floe rate of 12ml/min.

Chemical structural analysis of purified pigments

The chemical structure of the purified pigment was analyzed by UV-Visible Spectrophotometer and Infrared Analysis.

Spectral analysis by UV- spectroscopy

A UV-Visible spectrophotometric analysis was done to detect the presence of UV-absorbing compounds in all the pigments using shimadzu (Japan) model UV- 1700 series Spectrophotometer.The spectral range for carotenoids is around 360 to 520nm.

Spectral analysis by Fourier Transfer Infrared Spectral (FTIR)

IR spectral analysis of isolated pigments was done by keeping the samples in vacuum desiccators over solid KOH for 48 hours and then IR spectral Analysis was done with 1mg sample in a Fourier transfer Infrared spectrophotometer (Shimadzu, japan).

DPPH Radical Scavenging Assay

The free radical scavenging activity of the fraction was measured in vitro by 1- Diphenyl-2-Picrylhydrazyl (DPPH Assay).About 0.3mm solution of DPPH in 100% ethanol was prepared and 1 ml was added to 3ml of the pigment fractiondissolved in ethanol at different concentration. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using UV- Visible Spectrometer (Shimadzu). The scavenging activity at different concentration was determinedand the IC50 value of the fractions was compared with that of ascorbic acid (Vitamin C) which was used as the standard. The percentage of radical scavenging activity was calculated

% RSA = Absorbance of control – Absorbance of sample/Absorbance of control x 100

Antimicrobial Activity of pigment against Pathogens

Test Microorganisms

Seven bacterial strains and the four fungal strains used in the present study were the clinical isolates obtained from Aravind Eye Hospitals, Madurai. The bacteria used were *Escherichia coli, Staphylococcus aureus, Klebsiellapneumoniae, Pseudomonas aeruginosa, Streptococcus epidermis,Salmonella typhi*and *Acenitobacter sp.* The fungal strains used were *Aspergillusniger, Alternariasp,Penicillumsp,Fusariumoxysporum.*

Antibacterial Assay

The effect of carotenoid pigment on the several bacterial strains was assayed by Agar well diffusion method and by Disc diffusion method. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Antifungal Assay

The Antifungal activity was analyzed by preparing the experimental PDA plates supplemented with 1% of fungal pigment and only PDA plates served as control dish. The antifungal properties were estimated against several fungal strains by inoculating with 2μl suspension of conidiophores in the central zone of the Petri dishes. After incubation the diameter of the cultures were measured and compared with control. Finally the incubation ratio was calculated by the formula Inhibition ratio (%) = C-E/C x 100 where 'C' is diameter of the mold in control plate and 'E' is the diameter of the mold in experimental Plate.

RESULTS

Cultural and microscopic examinations of *Micrococcus luteus*

The organism observed on nutrient agar medium revealed that colonies were circular, convex, entire, smooth, glistening, granular and yellow in colour (Figure 1).Gram Positive bacterial strain *Micrococcus luteus* was isolated and preliminary morphological observations revealed that it is a non spore forming, spheres, 0.9 to 1.8 μ m in diameter, occurring in clumps, tetrads (or) irregular tetrads and non motile in nature (Figure 2).

Extraction and purification of yellow pigment from *Micrococcus luteus*

The release of pigment from whole wet cells was done by sequential extractions. An extraction of yellow pigments was done using solvents with different polarity. The carotenoid pigment is covalently bounded to the glycoprotein complex of their cell constituents. Hence cell disruption with methyl alcohol along with acetone was very effective in pigment extraction. The extraction was repeated, until all the pigments had been extracted and this was carried out in container covered by aluminium foil to avoid light isomerization.Further the yellow pigments were purified by column chromatography. The yellow bacterial pigments were adsorbed strongly on activated calcium carbonates and there was satisfactory separation of yellow pigments, when petroleum ether was employed as solvents. The successful separation of the carotenoids depends on the selection of proper adsorbent and solvents.

Determination of carotenoids

The yield of biomass and carotenoid was significant and there was good growth of *Micrococcus luteus* on nutrient agar medium. The maximum area covered in the medium showed the highest growth giving maximum yield of

biomass 4.71 g/l. The quantification of carotenoid content was determined at 449nm and was estimated with the standard curve of carotenoid and there was 65.25µg/g of carotenoids. Hence the production of pigment depends on the essential nutrients of the medium.

Analysis by Thin layer Chromatography

The plates developed in Hexane and acetone (60:40) showed the separation of microbial pigments carotenoids in two spots, with different migration rates. The dark orange spots with Rfof 0.85 and 0.65 was developed, which corresponds to carotenoids hydrocarbons. This result was similar to the findings of Godinho&Bhosle, 2008.

Analysis by High Performance Liquid Chromatography

The HPLC profile of yellow pigment from *Micrococcus luteus* can be ascertained from Figure 3a and Figure 3b.The retention time were 1.8 min (21.8%), 2.1 min (68.7%) and 2.7 min (5.3%) for my experimental sample and the total run time was 15 mins. The entire major peaks present in standard are present in my sample which indicates that the yellow pigment belongs to carotenoid family (GodinhoandBhosle, 2008).

Spectroscopic measurements

The absorbance properties of the microbial pigments facilitate both qualitative and quantitative analysis.The yellow pigment from *Micrococcus luteus* was completely extracted in acetone and petroleum ether, the spectral analysis showed maximum absorbance at 464,534 &351 nm respectively Figure 4. The three clear peaks shows the characteristics of carotenoid compound and further it reflects its purity and compared with the standard data of other various carotenoids. This confirms that the yellow pigment is carotenoid (Aizawa and Inakuma, 2007).

Fourier Transfer Infrared Spectral (FTIR) Analysis

IR spectral analysis of three isolated pigments was done and their spectrum has the following functional groups.The FTIR Spectrum of the yellow pigment (4500-500cm^{-1}) is shown in the Figure 5 and the Table 1 represents the functional groups present in the compounds.From the IR spectrum the peaks indicates the presence of more number of hydrocarbon groups with C-H and C-C multiple bond conjugated stretching and bending and from this it is confirmed that the yellow pigment has constituents of carotenoids (Moh*et al.,* 1999).

DPPH Radical Scavenging Assay

DPPH is a stable purple colour radical that turns to yellow or yellowish brown, when it reacts with antioxidant analytes and the degree of discoloration indicates the scavenging potentials of antioxidant pigment extracts. This activity depends on the hydrogen donating ability (Brand- William, *et al*., 1995).The antioxidants activities of the microbial pigment carotenoid and their percentage of inhibition was compared with the standard Vitamin 'C' represented a scavenging effect of 72.34%. Here in this study carotenoids from *Micrococcus luteus* showed excellent antioxidant activity of 61.2%.

Antibacterial Assay

The *in vitro* antibacterial activity of the microbial pigments was tested against the several test organisms by well and disc diffusion methods and amphicillin was used as control (10µg/ml). The antibacterial activity potentials were assessed by the presence or absence of inhibition zone in diameters.In well and disc diffusion method, the microbial pigment carotenoid (yellow) from *Micrococcus luteus* exhibited highest antibacterial activity against *Pseudomonas*

aeruginosa, with inhibition zone of 19 mm and 18 mm followed by *E.coli* of 18 mm and 16 mm and *Streptococcus epidermis* of 16 mm and 15 mm respectively (Figure 6).

Antifungal Assay

Antifungal activity was estimated using four fungal strains as test organisms, cultivated on potato dextrose agar medium(control plates) and on the same medium supplemented with 1% of microbial pigments(Experimental plates).The diameter of the colonies were measured and the inhibition ratios were calculated for each fungal strain.
All the fungal test strains showed the inhibition in the presence of all three microbial pigments. The yellow pigment carotenoid from the bacteria showed maximum of 70% inhibition in *Penicilliumsp* and minimum of 33% in *Alternariasp* (Figure 7).

DISCUSSION

Carotenoids are yellow, orange and red coloured molecules found in several species of bacteria. *Micrococcus luteus* is Gram positive, non-motile and non-spore forming organism produces yellow or yellowish orange water soluble pigment.The pigment is covalently bounded to the glycoprotein complex through sugar moieties. The extractability of carotenoids from wet cells is done by exhaustive sequential extraction with solvents like acetone, methanol, diethyl ether and petroleum ether.Here in this study acetone is used which is a protein denaturizing agent and they disrupt the complex.And these carotenoids are completely soluble in ether and forms yellow colured complex (Van den Berg, *et al*., 2000).This yellow coloured pigment is purified by coloumn chromatography in which activated calcium carbonate is used as adsorbent because it strongly adsorb the bacterial carotenoid from the mixture and gives satisfactory separation of carotenoid hydrocarbon, when petroleum ether used as solvents. The production of carotenoids at optimum pH 6 and temperature 35^{o}C was 65.25 µg/g and yield was4.7 g/l. These findings were similar to those using apple pomace based medium with *Rodotorulasp*(Sadhu and Johshi, 1997).The thin layer chromatographic analysis of the pigments revealed the presence of their functional groups with the specific Rf value. The separation of these biopigments was based on the differential affinity of the compounds and their respective stationary and mobile phase.

The chromophores of carotenoids from *Micrococusluteus* exhibit yellow to red colours and they are readily soluble in hexane, Petroleum ether and toluene.The TLC analysis of these yellow pigments revealed the presence of hydroxylated, hydrocarbons and ketones compounds in the carotenoid pigment (Zhang *et al*., 2006).In current study, HPLC analysis provides a simultaneous determination of pigments in microbiota. The chromatogram of the microbial pigments at described conditions, showed different peaks with their specific retention time. The total run time was 15 min. A clean chromatogram shows negligible interference of endogenous and exogenous chromophore substance in microbial cells (Zhao*et al.,* 2004).HPLC is an extremely useful tool in studying a range of aspects of microbial pigments. Ion pairing agent injection yielded good separation of pigments (Ken *et al.,* 1998).The carotenoids resolved in to three distinct peaks and the greater number of conjugated double bonds, the higher than λ max value (Godinho and Bhosle,2008).The qualitative measurements of the pigments and colourants are specifically done by observing the λ max by UV-Visible spectrophotometry, the standard technique for quantifying the colourants that is solublized in their respective solvents. Here in this study the UV Visible spectral data of carotenoids absorbs maximally at three wavelengths (464,534 and 351nm) resulting in three clear peaks which is characteristic of carotenoid pigments. The polyene chromophore of *Micrococcus luteus* absorbs light in 400 to 550 nm range and reflects yellow to red colours. These pigments are readily soluble in polar solvents (Trutko*et al.,* 2005).FTIR is an accepted tool for the characterization of biomolecules in drug discovery research by development of high-throughput screening and combinatorial chemistry.In this present work FTIR spectroscopy operates in mid infrared region of 4000-400 cm^{-1} which has been proved to be powerful tool for quantitative analysis of microbial pigments.

The IR spectrum of carotenoids observed strong bands of conjugated alkene –CH= CH- groups which is very specific for the presence of carotenoid group and this was similar and corresponds to the results of Sujak, 2012.

Free radicals, mainly reactive oxygen species (ROS) has a noxious effect on cells and induce oxidative damage of DNA and other cellular components leading to cancer related mutations (Valko*et al.,* 2004). Antioxidants play a major role in protection of human body against damage of ROS.DPPH radical assay is extensively used to evaluate radical scavenging activities in less time than other methods. The carotenoids reacts with DPPH, which is nitrogen centred radical with characteristic absorption at 517nm and convert it to stable diamagnetic molecule 1,1, dipenyl-picryl hydrazine, due to its hydrogen donating ability at rapid rate .When this electron becomes paired off the absorption decreases stoichiometrically with respect to the number of electrons taken up. The reduction in Purple colour indicates the presence of antioxidant molecules (Abdille, *et al.,* 2005).Carotenoids from bacteria showed 61% of RSA as it has capacity to quench single oxygen radicals and can act as efficient antioxidants (El-Agamey&McGarvey, 2008).In the present study carotenoids from *Micrococcus luteus* can inhibit the growth of other microorganisms or reduce the growth in the medium by the production of their secondary metabolites. These metabolites produced indirectly when there is change in pH, osmotic pressure and surface tension or directly by producing toxic components, antibiotics and other antimicrobial agents. This antagonistic interaction among the microbiota can be used as biological attribution in food and pharmaceutical industries.The carotenoids showed potential activity against seven bacterial strains and *Pseudomonas aeruginosa* showed maximum activity. Cucco*et al.,* (2007) suggest that beta carotene could lead to accumulation of lysozyme, an immune lytic enzyme that digest bacterial cell walls, therefore generate the antibacterial activity.From the above results summarized in this present study, the microbial pigments have remarkable biological effects with therapeutic applications in clinical and biomedical research on further level. These qualities make the microbial pigment to use asecofriendly therapeutic molecules in human health concern.New ways of formulating and improving the existing pigments can be done by genetic engineering using functional genomics approaches to enhance the quality, quantity and stability during processing and storing. Thus the present study will help to understand further therapeutic application of the microbial pigments for food additives or pharmaceutical agents by animal model studies.

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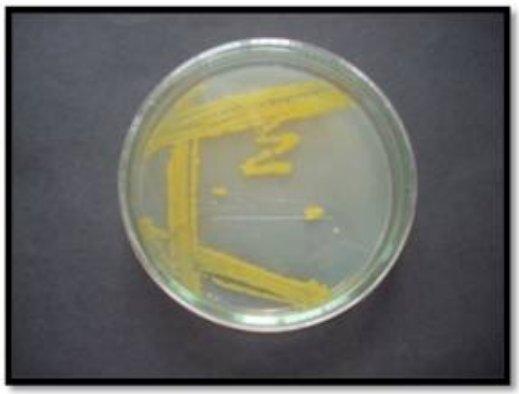

Figure 1. Yellow Colour pigmented colonies of Micrococcus luteus on nutrient agar medium

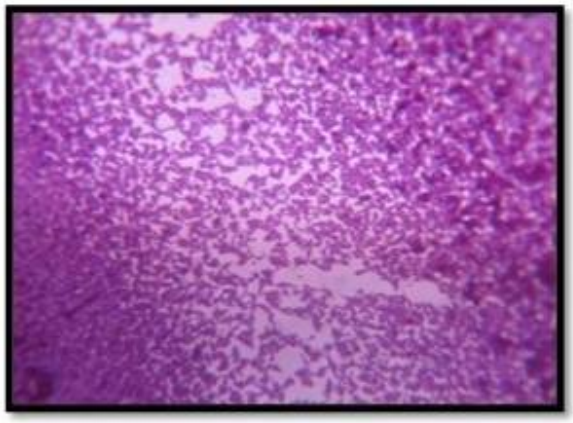

Figure 2 Microscopic view of Micrococcus luteus (Gram Positive-Cocci)

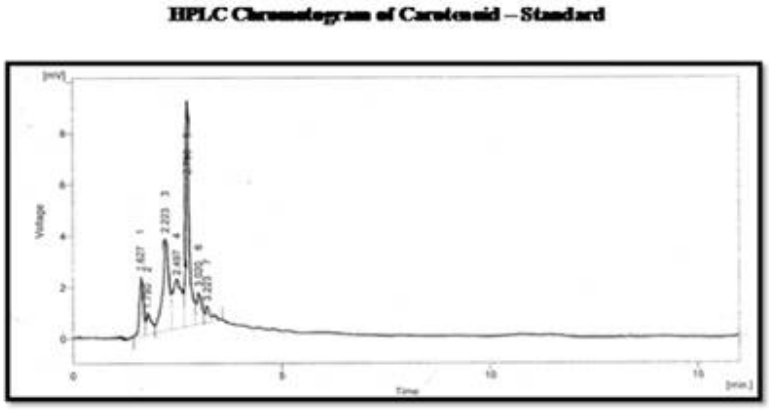

Figure 3a

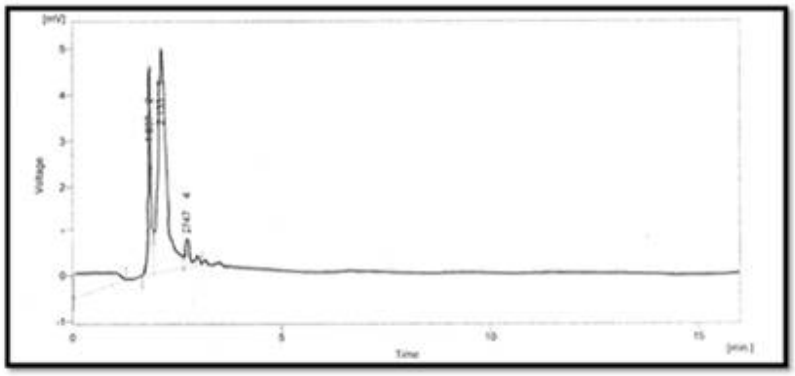

Figure 3b

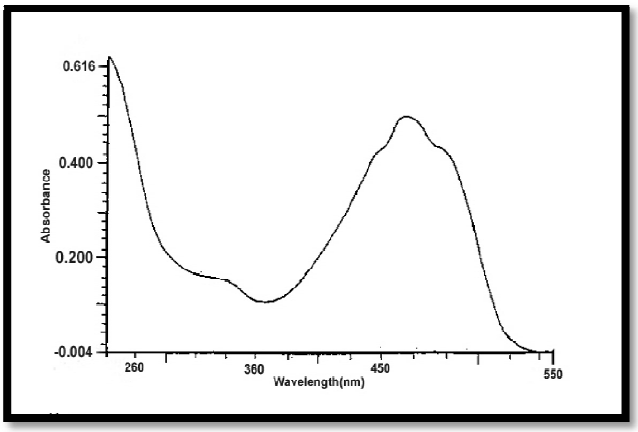

Figure 4: UV Spectra of Bacterial Pigment Carotenoid

Table 1: Functional groups of yellow pigment from *Micrococcus luteus*

Wavenumber (cm^{-1})	Functional groups
881	Aromatic CH bend
1380.94	C-H bending in CH_3 groups
1415.65	Alkene, Monosubstituted group
1452.3	C-H bending in CH_2 Groups
1647.1	C-C multiple bond conjugated stretching groups
2893.02 2927.74	C-H stretching- alkane groups
2974.03 3367.48	Symmetric & asymmetric stretching modes of C-H groups(polymeric associations)

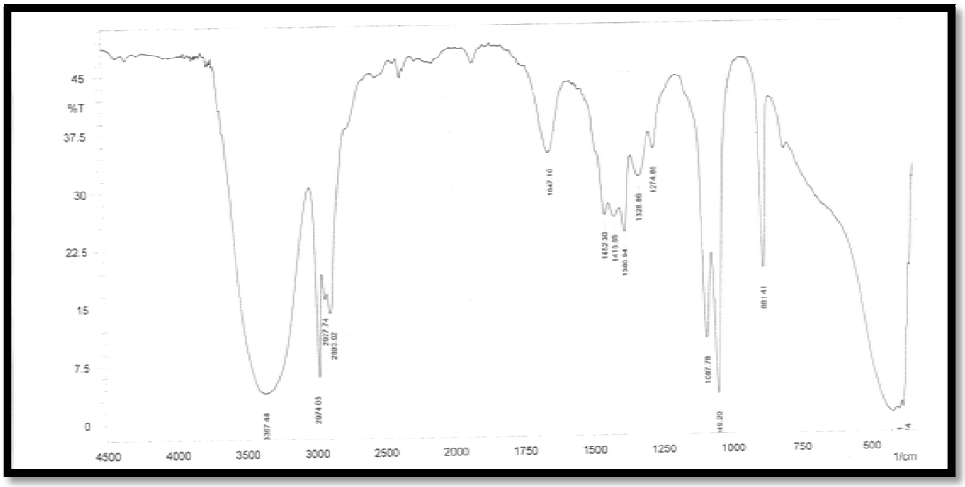

Figure 5: IR Spectra of Bacterial Pigment Carotenoid

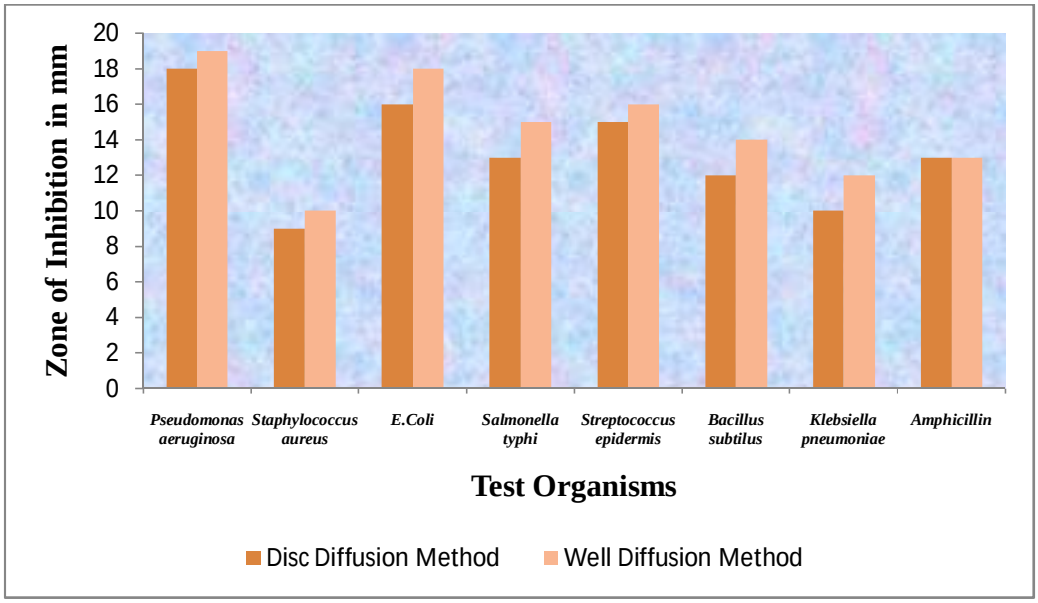

Figure 6: Antibacterial Activity of Bacterial Biopigment from *Micrococcus Luteus*

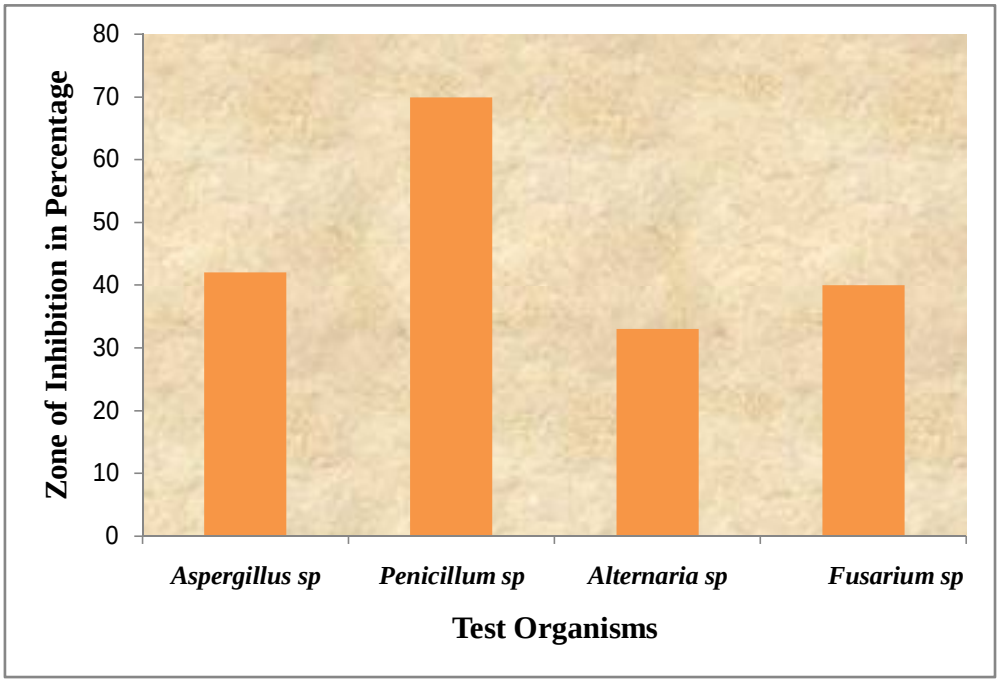

Figure 7: Determination of Antifungal Activity of Biopigment from *Micrococcus Luteus*

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RESEARCH PAPER

Efficacy of Organic Amendments aganist *Sclerotinia sclerotiorum* (Lib.) De Bary

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ABSTRACT

An attempt was made to find out the efficacy of different oil cakes against Sclerotinia *sclerotiorum* (Lib.) de bary causing Sclerotinia rot of Indian mustard. Extracts of six oil cakes viz., neem cake, mustard cake, castor cake, groundnut cake and sesame cake were evaluated aganist aforesaid pathogen. The result, indicate that all the organic cakes except groundnut, significantly reduced mycelia growth of *Sclerotinia sclerotiorum*.

Keywords: organic cakes, *Sclerotinia sclerotiorum*, Indian mustard.

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the major oilseed crops in Rajasthan. The largest cultivation of *Brassica* crops is done for edible vegetable oil production. They also play a pivotal role in world's agricultural economy and are recognized for their long history of cultivation and varied uses. The oilseed *Brassica* crops are generally grouped as rape and mustard. Commonly cultivated species are *B. compestris* var. Sarson. Yellow and brown sarson; *B. campestris* var. toria; *B. juncea*; rai and *Eruca sativa*: taramira. *Sclerotinia sclerotiorum* (Lib) De Bary, the causal fungus of *Sclerotinia* rot or white blight or stem rot disease is a necrotrophic pathogen with world wide distribution known to infect over 400 species of plants (Boland and Halls, 1994). The pathogen affects many crops in india, particularly rapeseed-mustard and has become a wide spread and destructive in mustard growing parts (Ghasolia *et. al., 2004)* and take a heavy toll of yield (Chauhan *et. al.*, 1992). In mustard growing areas, this disease led to complete crop failure, as the disease incidence has been recorded up to 80 per cent in some parts of Punjab and Haryana states (Kang and Chahal, 2000 and Sharma *et al.* 2001). Maximum incidence was recorded at Dausa (29.2%) district in Rajasthan followed by Rohtak (24.8%) district in Haryana and minimum 7.0 and 7.4 per cent

at Jaipur and Bharatpur districts, respectively (Yadav *et al*., 2013). Once the pathogen is established, it is extremely difficult to control. This ascomycete can cause systemic and aerial infection by myceliogenic and carpogenic germination of sclerotia of *Sclerotinia scletiorum* surviving in soil. Being ubiquitous necrotroph, it severely affects cultivated oilseed *Brassica* grown in different geographical regions of the world. The present study was under taken to asses the potential of different organic oil cakes for management of *Sclerotinia* rot of Indian mustard.

MATERIALS AND METHODS

The pathogen (*Sclerotinia sclerotiorum*) was isolated from diseased mustard plants collected from the fields of Jaipur district of Rajasthan. Five organic amendments *viz.*, neem cake, mustard cake, castor cake, groundnut cake and sesame cake were screened *in vitro* to evaluate their inhibitory effect on radial growth of the pathogen. Hundred g oil cake was taken in 1000 ml water and preserved in earthen pot for extraction. Pots were wrapped by polythene bags to preserve moisture. Oil cake extract was filtered with cheese cloth, mixed @ 3% in PDA in conical flask and autoclaved. Twenty ml PDA was poured in each sterilized Petridish and allowed for solidify. The each plate was inoculated with 5 mm diameter bit of 7 days old culture of pathogen. Inoculated plates were incubated at 25 $\pm$ 1^{0}C for 7 days. The fungitoxicity of organic cake in each treatment and average of three replications was calculated. Per cent inhibition over control was calculated by the following formula (Bliss, 1934).

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

C = growth of fungus in control
T = growth of fungus in treatment

RESULTS AND DISCUSSIONS

Efficacy of five organic oil cakes was tested *in vitro* against *Sclerotinia sclerotirum.* The castor cake (Table 1) was found significantly superior over all the tested oil cakes with maximum (44.44%) inhibition of mycelial growth of *Sclerotinia sclerotiorum* over control followed by neem cake (40.00%). Groundnut cake was found least effective (5.55%) in inhibiting mycelial growth of *S. Sclerotiorum.* The variation of inhibition effect of different organic oil cakes may be attributed to the presence of antifungal compounds in the cakes. Eariler workers have also reported soil amendments as a source for inhibition of the fungal growth. A number of plant extracts have been found to inhibit the mycelial growth of the pathogen (Chand and Rai, 2008 and Tripathi *et. al.,* 2011)

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Table: 1 Effect of different oil cakes against *Sclerotinia sclerotiorum in vitro.*

Organic amendments	Concentration (%)	Radial mycelial growth (mm)*	Per cent growth inhibition
Mustard cake	3.00	83.00	7.77 (16.17)
Neem cake	3.00	54.00	40.00 (39.23)
Castor cake	3.00	50.00	44.44 (41.81)
Groundnut cake	3.00	85.00	5.55 (13.63)
Sesame cake	3.00	70.00	22.22 (28.12)
Control	-	90.00	0.00 (0.00)
SEm±	-	-	0.28
CD (p=0.05)	-	-	0.88

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RESEARCH ARTICLE

Association Analysis among Oleic Acid Content and Oil Yield Component Traits in Sunflower (*Helianthus annus* L.)

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ABSTRACT

A population of BC_2F_1 was developed with a low oleic inbred as recurrent parent and a high oleic inbred as donor parent. Oil yield per plant possessed significant positive correlation with plant height, head diameter, seed yield per plant and oil content. Oil content exhibited positive and significant association with plant height. Seed yield per plant showed positive and significant correlation with plant height and head diameter. Oleic acid content has no correlation with any of the yield and other component traits in sunflower. Hence high oleic genotypes can be developed without sacrificing oil yield and other component traits.

Keywords: Sunflower, correlation, oleic acid content, oil yield.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the world and the nutritional quality of its edible oil ranks second among the best vegetable oils in cultivation. Sunflower seed contains 38 - 42 per cent oil. Sunflower oil is considered as premium oil due to its light color, mild flavor, low level of saturated fatty acids and ability to withstand high cooking temperatures (Robert *et al.,* 1993). Quality of sunflower oil is judged on the basis of the oleic and linoleic acid content. It has been determined that their contents are genetically controlled and that these contents are heavily influenced by environmental factors. Breeders focus their entire attention in developing sunflower genotypes with high oil yield. It is obvious that the important aspiration of plant breeders is to know the extent of relationship between seed yield and oil traits which will ultimately enhance their selection efficiency for above traits. Thus, it is necessary to measure the mutual relationship between various plant characters so as to determine the component traits on which selection can be based for genetic improvement in yield and other

important traits. The association analysis is more important whenever quality parameters included in the breeding programme as most of the quality and yield may not associate positively. Thus, the correlation analysis helps to form selection indices to balance two contrary but desirable characters affecting the primary character.

It has been generally accepted that correlation between different characters represents a coordination of physiological processes which is often achieved through gene linkages (Mather and Jinks, 1971). Knowledge of the strength and type of association is an important pre requisite for the formulation of breeding procedure. Thus idea about the nature of association will be useful to identify the key characters for which selection can be fruitfully made. Hence, the present investigation focused the attention on some important yield components through which changes in oil yield could be predicted in sunflower.

MATERIAL AND METHODS

Experimental design: The material of the present study comprised of backcross progenies of BC_2F_1 generation from a backcross in sunflower. In the backcross, the female parent (recurrent parent) COSF 1B was low oleic genotype and the male parent (donor parent) HO-5-13 was high oleic genotype. The field experiment was carried out at Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during *Rabi,* 2014-15. Observations were recorded on single plants *viz.,* plant height, head diameter, 100-seed weight, volume weight, seed yield per plant, oil content, oil yield per plant and oleic acid content. A total of 66 plants were observed.

Estimation of oil and oleic acid content: In the test population oil and oleic acid contents were measured on a BRUKER Matrix-I NIR spectrometer in transmittance mode. The samples were scanned in a wave number range of 12000 - 4000 cm^{-1} with a resolution of 2 cm^{-1}. The sample scan time was 32 scans. To develop and optimize the calibration models, partial least squares (PLS) method was used with cross validation.

Statistical analysis*:* Statistical analysis by resorting to simple correlation coefficients was performed as proposed by Goulden (1952) to assess the association between oil quality and other yield component traits.

RESULTS AND DISCUSSION

Correlation coefficients are useful to determine the component character on which selection can be based in order to improve oil quality. The perusal of Table 1 reveals that oleic acid content has no correlation with any of the yield and other attributing traits in sunflower. Similar findings were reported by Premnath *et al. (*2014). Oil yield per plant possessed significant positive correlation with plant height, head diameter, oil content and single plant yield. These results were earlier reported by Nehru and Manjunath (2003), Binodh, *et al. (*2008), Anandhan, *et al.* (2010), Sivamurugan (2011), Vanitha (2012) and Saranya (2013). Oil content exhibited positive and significant association with plant height. Seed yield per plant showed positive and significant correlation with plant height and head diameter. Plant height and head diameter documented positive and significant correlation. Strong and positive association of head diameter with yield and oil related traits suggested that increased head diameter will lead to higher seed yields and greater oil yield. This type of association might be due to the presence of higher source and sink capacities. Hence head diameter can serve as a good selection criterion to increase the above traits. These findings were in accordance with that of Premnath *et al.* (2014). The trait volume weight has no correlation with any of the traits studied in the present investigation.

Simple correlation coefficients showed that all the investigated traits did not have any influence on oil quality in terms of oleic acid content in sunflower. Hence sunflower breeding programmes to improve oleic acid content can be formulated without any adverse effect on oil yield and component traits. The comparison of correlation coefficients

of seven different traits against oil yield per plant showed that plant height, head diameter, oil content and single plant yield had significant positive correlations with oil yield per plant. It thus became apparent that these traits are the main characters and seem to be good selection criteria for improving oil yield per plant.

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Table 1. Simple correlation coefficients in BC2F1 population of cross COSF 1B X HO 5-13

Characters	Plant height (cm)		Head Diameter (cm)		Volume weight (g/100ml)	Oil content(%)		Oleic acid content(%)	Seed yield (g/plant)		Oil yield (g/plant)
Plant height(cm)	1.00										
Head diameter (cm)	0.42	**	1.00								
Volume weight (g/100 ml)	-0.23		-0.24		1.00						
Oil content (%)	0.27	*	0.01		-0.16	1.00					
Oleic acid content (%)	-0.08		-0.10		-0.01	0.02		1.00			
Seed yield (g/plant)	0.41	**	0.52	**	-0.01	0.20		0.09	1.00		
Oil yield (g/100 ml)	0.43	**	0.50	**	-0.03	0.33	**	0.11	0.99	**	1.00

* , ** Significant at 5% and 1% level respectively

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RESEARCH PAPER

Management of *Sclerotinia* Rot of Indian mustard through Plant Extracts

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ABSTRACT

An attempt was made to find out the efficacy of different plant extracts against *Sclerotinia sclerotiorum* (Lib.) de bary causing stem rot of Indian Mustard (*Brassica juncea* (L.) Czern & Coss.). Extracts of six plants viz., *Allium sativum*, *Azadirachta indica* , *Ocimum sanctum*, *Alstonia scholaris*, *Curcuma longa* and *Zingiber officinalis* were evaluated against aforesaid pathogen. Maximum inhibition of mycelia growth was observed with *Allium sativum* (68.63%) followed by *Azadirachta indica* (66.01%), *Ocimum sanctum* (56.08%) and *Curcuma longa* (51.83%).

Key words: Plant extract, Indian mustard, *Sclerotinia sclerotiorum*

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the major oilseed crops in Rajasthan. The largest cultivation of *Brassica* crops is done for edible vegetable oil production. They also play a pivotal role in world's agricultural economy and are recognized for their long history of cultivation and varied uses. The oilseed *Brassica* crops are generally grouped as rape and mustard. Commonly cultivated species are *B. compestris* var. sarson. Yellow and brown sarson; *B. campestris* var. toria; *B. Juncea,* rai and *Eruca sativa*. taramira. *Sclerotinia sclerotiorum* (Lib) De Bary , the causal fungus of Sclerotinia rot or white blight or stem disease is a necrotrophic pathogen with world wide distribution known to infect over 400 species of plants (Boland and Halls, 1994). The pathogen affects many crops in india , particularly rapeseed-mustard and has become a wide spread and destructive in mustard growing parts (Ghasolia *et. al., 2004)* and take a heavy toll of yield (Chauhan *et. al.*, 1992). In mustard growing areas, this disease led to complete crop failure, as the disease incidence has been recorded up to 80 per cent in some parts of Punjab and Haryana states (Kang and Chahal, 2000 and Sharma *et. al.* 2001). Maximum incidence was recorded at Dausa (29.2%) district in Rajasthan followed by Rohtak (24.8%) districts in Haryana and minimum 7.0 and 7.4 per cent at Jaipur and

Bharatpur districts, respectively (Yadav *et al.*, 2013). Once the pathogen is established, it is extremely difficult to control. This ascomycete can cause systemic and aerial infection by myceliogenic and carpogenic germination of Sclerotia surviving in soil. Being ubiquitous necrotroph, it severely affects cultivated oilseed *Brassica* grown in different geographical regions of the world. In recent years, an increasing consciousness about environmental pollution due to chemicals has forced plant pathologists to search for eco-friendly methods of disease management. In this context, the plant extracts can be a good alternative for disease management therefore ,the present study was undertaken.

MATERIALS AND METHODS

To find out the fungitoxicity of six plant extracts viz., *Allium sativum* Linn. Clove extract, leaf extract of *Azadirachta indica*, *Ocimum sanctum*. *Alstonia scholaris*, *Curcuma longa* and *Zingiber officinalis*. against the pathogen were evaluated. Hundred gram from each plant was collected and washed 2-3 times with water and allowed to dry at room temperature (25±1^{0}C) for six hours. Before extraction 100g part of each plant were crushed separately with 100 ml sterilized distilled water. The extract was filtered through muslin cloth and centrifuged at 5000 rpm for 30 min. These extract were then sterilized by passing them through a Millipore filter using a swimming filter adapter.

The extracts of each plant species was diluted in order to achieve three concentrations viz., 5, 10 and 15 per cent. Petri plates containing PDA The extract of each plant species was diluted in order to achieve three supplemented with different phyto-extracts, each with three concentrations and replicated three times were inoculated with 5-day-old culture (5 mm dia disc). A suitable check (without plant extract) was also maintained. Fungal colony was measured after 7 days of incubation at 25 $\pm$ 1 ^{0}C. The efficacy of plant extracts in each treatment and average of three replications was calculated. Per cent inhibition over control was calculated by the following formula (Bliss, 1934).

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

C = growth of fungus in control
T=growth of fungus in treatment

RESULTS AND DISCUSSION

The efficacy of six plant leaf extracts (Table 1) was tested *in vitro* at three concentrations viz., 5, 10 and 15 per cent against *S. sclerotiorum* on PDA by poisoned food technique. Among six plant extracts, extract of garlic cloves was found most effective in inhibiting mycelial growth (52.22, 65.66 and 88.00 %) of *S. sclerotiorum* at 5, 10 and 15 per cent, respectivley followed by neem (50.74, 67.20 and 80.0%) over control. Extract of *Alstonia*, tulsi and turmeric were found least effective in inhibiting mycelial growth of *S. sclerotiorum* over control. All the cocentrations (5, 10 and 15%) of garlic extract were found significantly superior over each other while 10 and 15 per cent of *Alstonia,* tulsi and turmeric were found at par to each other. Similar results have also been observed by Tripathi and Tripathi (2009) while working with *S. sclerotiorum in vitro*. Extracts of ten plants have been evaluated *in vitro* against *S. sclerotiorum* by Yadav (2009) and reported effective in inhibiting mycelial growth. The study demonstrates the potential of the natural disease control tools for management of Sclerotinia rot of Indian mustard. A closer look at the data seems to incidence that some plant extracts treatments in future may provide a better management of the disease.

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Table:1 Effect of different plant extracts on mycelial growth of *Sclerotinia sclerotiorum*

Plant extract	Plant part used	Per cent growth inhibition at different concentration (%)*			
		5	10	15	Mean
Garlic	Cloves	52.22 (46.27)	65.66 (54.13)	88.00 (69.73)	68.63
Turmeric	Rhizomes	25.96 (30.36)	61.11 (51.42)	67.82 (55.42)	51.63
Neem	Leaves	50.74 (55.39)	67.20 (45.17)	80.00 (63.43)	66.01
Ginger	Rhizomes	41.00 (39.82)	56.20 (48.56)	71.04 (57.44)	56.08
Tulsi	Leaves	45.07 (42.17)	49.30 (44.60)	60.26 (50.92)	51.54
Alstonia	Leaves	35.82 (36.76)	50.93 (45.53)	62.96 (52.51)	49.90
Control	-	0.00	0.00	0.00	0.00

	SEm±	CD (p=0.05)
E	1.44	4.11
Con.	0.94	2.69
E x Con.	2.49	7.12

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RESEARCH ARTICLE

Hydrocarbon Exposure and the Effect on Body Thermoregulation during Aircraft Fuel Tank Inspections

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ABSTRACT

Those involved in aircraft fuel tank maintenance are at risk to these exposure where hydrocarbon based jet fuel are used.In this study, the immediate effects of hydrocarbon exposure on body thermoregulation of aircraft maintenance workers during fuel tank inspection were conducted to determine the risk of developing heat distress. Twelve individuals male (N=12) aged 21 to 35 who work as aircraft maintenance workers were studied in this project. Each of the subjects stayed 30 minutes inside the Aircraft Fuel Tank to perform the inspection procedures and maintenances. The subject body temperatures were measured using aAxillary Body Thermometer before and after they enter the Aircraft Fuel Tank. The difference in the body temperature was noted. A significant increase on body temperature after entering the Aircraft fuel Tank was noted for all subjects (r2= 0.855*, p=0.000) suggesting the risk of workers being subjected to an increase in chemical exposure as they perform their task.

Keywords: Aircraft fuel, alkyl benzene, chemical exposure, hydrocarbon exposure and thermoregulation.

INTRODUCTION

The clinical effects of hydrocarbon exposure have been reported since the dawn of the 20th century and human exposure to hydrocarbons are possible through one of the following routes: inhalation, ingestion with or without aspiration, or dermal exposure. Jet fuel is a complex mixture containing more than 200 aliphatic and aromatic

hydrocarbon compounds with nine to 17 carbon atoms, including thousands of isomeric forms that distil at 170-325/C, and three to six non hydrocarbon performance additives[1]. The hydrocarbon portion of jet fuels is made from low sulfur or desulfurized distillate kerosene streams, usually blended with cracked or hydro cracked heavier streams to produce a fuel that meets specific performance specifications. In addition to hydrocarbons, jet fuel contains small amounts of sulfur and nitrogen as heterocyclic substituent's generally in structures containing one or two rings. Among these, the alkylbenzenes (single-ring aromatic compounds with single or multiple aliphatic side chains) are constituents of jet fuel. Toluene (methylbenzene) and mixed xylenes (*o*-, *m*- and *p*-) are present in jet fuel and have been identified as potential neurotoxic chemicals after sufficiently high intentional, accidental or occupational exposures [2, 3]. The major determinants of hydrocarbon toxicokinetics following systemic uptake are disposition-related physiologic measures, such as alveolar ventilation, cardiac output and blood flow to organs, partition coefficients, and organ volume. Hydrocarbons with high blood air partition coefficients will be absorbed to a greater extent than chemicals with poor blood solubility [4].

In this study, the immediate effect of jet fuel exposure to the body thermoregulation system ofaircraft maintenance workers was conducted using Axillary Body Thermometer base on Sundlevandermethods [5]. It has been suggested that the physiological response to environmental toxicants and drugs is modulated by the thermoregulatory system and normally involve an increase in body temperature[6].

MATERIALS AND METHODS

Twelve individual male (N=12) age 21 to 35 who work as aircraft maintenance workers were invited to participate in this project. Prior to the test, the subjects consent were obtained through Consent Forms. Thereafter, the subject were required to undergo basic physiological screening to ensure their basicphysiological health status and their basic psychological conditions evaluated by inviting them to fill the Depression Anxiety Stress Scale-21 (DASS 21) questionnaires.For evaluating the effect of the exposure to the hydrocarbons on body temperature while conducting the fuel tank maintenance work, body temperature of the subjects was taken using Axillary Body Thermometer right before they enter the fuel tank. To enter the fuel tank, the subjects wore 100% cotton attire which include cotton cap (without visor), socks, and coverall (with no buttons or zippers shown and no pockets) with cotton shorts and cotton tee shirts without pockets under the coveralls. Rubber gloves were worn by each of the subjects.The subjects spent 30 minutes each inside the fuel tank for the maintenance procedures. During these inspection procedures, the subjects were tied to a safety line which is tied to their waist and one end held by another subjects acting to pull on the line when 30 minutes time is up. This is to ensure that subjects do not spend longer than necessary on the fuel tank.Another measurement of their body temperature was then taken when the subjects are out of the fuel tank. Statistical analysis of the differences in temperature was done and performed using a commercial Statistical Package for Social Science (SPSS version 17.0).

RESULTS AND DISCUSSION

DASS 21 is a set of questionnaire to measure the three states of negative emotions which relates to a person's depression, anxiety and stress status. The participants were found to be on a healthy scale where the test is concern. The results showed a significant increase on the body temperature of all the subjects after they entered the aircraft fuel tank (r^2= 0.855*, p=0.000) as shown in Figure 1.

Description of Figure 1

In mammals, increase in body temperature causes an increase blood flow to the skin to allow the release of sweat as a way to cool the body which also leads to moistening of the skin. The moistening of the skin has a positive effect on the skins permeability which can exacerbate chemical toxicity as it increases the permeability of the skin to the

chemicals [7]. This could be true in the case of exposure to hydrocarbons vapours such as those found in jet fuel.Our study found a significant increase on the body temperature of all the subjects after they entered the aircraft fuel tank (r^2= 0.855*, p=0.000). Data showing correlation coefficient between temperature before (TB) and temperature after(TA) were shown in Table 1.

Description of Table 1

This suggests the risk of workers being subjected to an increase in toxic exposure when they perform the inspection and maintenance task. Perhaps, a procedure to immediately cool the body of workers involve in such work can be put in place as a reduction in body temperature has been observed in mice exposed to toxicants and was suggested to be a natural response to reduce toxicity [8].Simulation experiments already verified that xylene vapour can be introduced inside the room with ventilation system[9].Contrast, xylene induced changes in workers under unventilated rooms were also noted [10]. An immediate increase in body temperature is possible for aircraft maintenance workers when they work inside an Aircraft Fuel Tank. Further study could be done to determine the effect on other physiological parameters to ensure the safety of workers involved.

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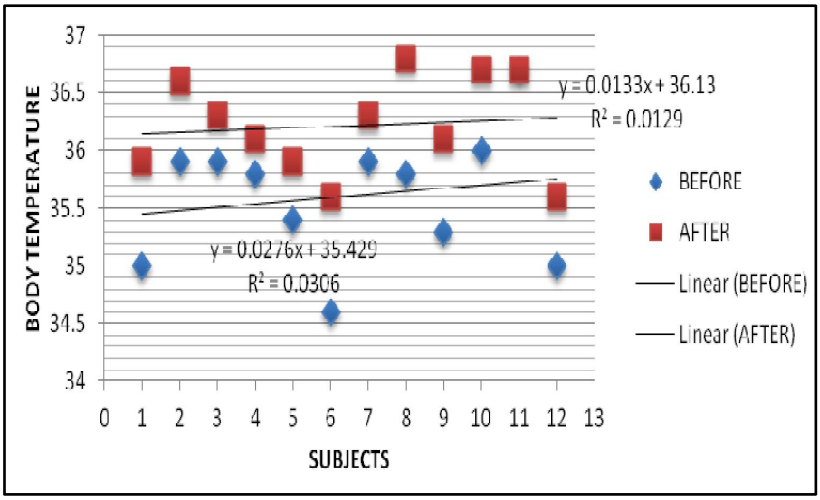

Figure 1: Graph showing changes in the body temperature of aircraft maintenance workers before and after fuel tank inspection

Table 1: Correlation coefficient between Temperature Before and Temperature After.

Correlations		Temperature Before	Temperature After
Temperature Before	Pearson Correlation	1	0.855*
	Sig. (2-tailed)		0.000
	N	12	12
Temperature After	Pearson Correlation	0.855*	1
	Sig. (2-tailed)	0.000	
	N	12	12

In table, Data is presented as mean ±SD. *Correlation is significant at the 0.01 level.

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RESEARCH ARTICLE

Effect of Dietary Incorporation of Nutraceutical Residue (Alfalfa Pellet) on Growth Performance and Haematological Parameters in Cross Bred Calves

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ABSTRACT

An experiment was conducted in crossbred calves for a period of three months to assess the effect of dietary incorporation of nutraceutical residue (alfalfa pellet) on their growth performance and blood biochemical profile. Twelve healthy crossbred calves of fifteen days of age were selected and divided into two groups of six each, as uniformly as possible with regard to age, sex and body weight and allotted randomly to two experimental rations T1(control) and T2 (calf starter containing 20 per cent nutraceutical residue). All the experimental animals were fed with concentrate mixture containing 24 per cent crude protein and 70 per cent TDN and were fed as per ICAR (2013). Data on haematological parameters such as haemoglobin, plasma protein, plasma glucose, BUN, serum calcium, serum phosphorus, serum cholesterol and triglyceride were the criteria employed for evaluation and they did not show any significant difference among treatment groups ($p > 0.05$). The results of present study indicated that nutraceutical residue can be included in the calf ration up to 20% level without any adverse effect on their growth performance and blood biochemical profile.

Key words: Nutraceutical residue, crossbred calves, blood, growth

INTRODUCTION

Efficient management and economic rearing of calves provide the foundation for profitable dairying. Feed account for 60-65 % of total cost in dairy farming. In traditional calf rearing methods whole milk is fed up to three months along with calf starter. Calf starter is a concentrate mixture containing all the essential nutrients in proper proportion which act as crucial link in rumen development and successful weaning. For obtaining optimum growth rate in calves both quality and quantity of protein in calf starter is very important. Moreover protein is the major constituent which determines the cost of the feed. An animal protein source like fishmeal is commonly incorporated as source of protein in calf starter. In India, the fish protein which available for livestock feed is unsalted dried fish and is usually the leftover of human market. Moreover, unsalted dried fish is prone to autolytic, lipolytic, microbial spoilage and may lodge harmful pathogens which are dangerous for the calves. Apart from that, proteins from plant sources are poorer in quality due to adulteration.

In such scenario, there is a need for alternate source of protein in the diet of calves. There are plenty of protein rich residues available from nutraceutical industries and alfalfa pellet is one among them. This nutraceutical residue (alfalfa pellet) contains 48 to 53 per cent crude protein and it can be used as a good alternative source of protein in calf starter. Hence, this research work is planned to determine the effect of feeding nutraceutical residues (alfalfa pellets) on growth performance and nutrient utilization in crossbred calves.

MATERIALS AND METHODS

Twelve healthy cross bred calves of fifteen days of age, selected from University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy, formed the experimental subjects for the study. Calves were housed individually in well ventilated, clean and dry pen with facilities for feeding and watering. The calves were divided into two groups of six animals each as uniformly as possible with regard to age, sex and body weight and were allotted randomly to two treatmentsT_1 (calf starter) and T_2 (calf starter containing 20 per cent nutraceutical residue). All the rations were made isonitrogenous and isocaloric (24 per cent CP and 70 per cent TDN). Proximate composition of nutraceutical residue is presented in Table 1. The ingredient and chemical composition of experimental rations are presented in Table 2.

Weighed quantity of calf starter was given in the forenoon and fresh green grass was fed in the afternoon to the calves throughout the experimental period. Individual data on quantities of calf starter and green grass offered daily were recorded. The left over portion of the calf starter and green grass were weighed daily and their moisture content was analyzed to calculate the dry matter intake. Body weights of all the calves were recorded at fortnightly intervals. Based on the body weight, feed and fodder allowances were reviewed fortnightly. Calves were fed as per ICAR standard (2013) and maintained on their respective feeding regime for a period of three months.Blood samples were collected from all animals at the end of the experiment. These samples were used to determine haemoglobin (cyanmethaemoglobin method using standard kits), plasma total protein (Jong and Vegeter, 1950), plasma glucose (GOP-PAP methodology using standard kits), serum calcium, serum phosphorus (Bernhart and Wreath, 1955), plasma cholesterol(Lie *et al.,* 1976) and triglycerides (McGowan *et al.,*1983). Blood haemoglobin was estimated by cyanomethaemoglobin method using reagents from Agappe diagnostics Ltd, Ernakulam, India. Plasma protein, calcium, phosphorus, cholesterol and triglycerides were determined using the blood analyzer (Mispa plus, SEAC radim group) and kits supplied by Agappe diagnostics, Ernakulam, India.

Calf starter and fodder samples were analyzed for proximate principles (AOAC, 2012). Data gathered on various parameters were analyzed statistically using Analysis of Variance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The per cent chemical compositions of nutraceutical residue and concentrate mixture were presented in Table 1 and 2 respectively. Fortnightly average body weight of calves was presented in the Table 3.Average initial body weight of calves belonging to two dietary treatments T1 and T2 were 35.60 and 36.96 kg respectively. The average body weight was 55.51 and 60.40 kg respectively for T1and T2 at the end of experiment.The average daily gain was 237 and 279 g respectively for T1and T2. There was no significant difference (P>0.05) in the body weight between two dietary treatments. These results are in agreement with findings of Rani *et al.* (2006), Pankaj *et al.* (2008), Sarker *et al.* (2010) and Ramniwas *et al.* (2013).Data on haematological studies were documented in Table 5 and 6. The values of various haematological parameters, (haemoglobin, MCV, MCHC, RBC count, WBC count, granulocytes, lympmocytes, and monocytes) were similar in both groups indicating that dietary incorporation of nutraceutical residue did not affect these parameters to any significant effect. The average plasma protein concentration at the end of the experiment for group T1and T2 was 6.73 and 6.96g/ dl, respectively. The average serum calcium values in experimental animals were 10.23 and10.36 mg/dl for group T1 and T2 respectively. The average serum phosphorus concentrations in the experimental calves were 6.03 and 6.07 mg/dl for group T1 and T2respectively. The average serum cholesterol and triglyceride values recorded at the end of the experiment were 94.33 and 89.10, 35.89 and 30.74 mg/ dl, respectively for group T1and T2.The average glucose and BUN values recorded at the end of experiment were 64.42 and 66.79,10.15and 10.53 mg/dl, respectively for group T1 and T2. There was no significant difference (P >0.05) in any of the haematological parameters between the two groups.

Value recorded in the present study falls in the normal range reported for the species (Rani *et al.* 2011, Vinu 2013 and Jini 2014). Present results are in agreement with Roshma *et al.* (2014) who reported incorporation of Ksheerabala residue at 0,10 and 20% level in the diet of Malabari kids showed no significant difference between treatments in haematological parameters such as haemoglobin, plasma protein, serum calcium, phosphorus, cholesterol and triglyceride.From critical evaluation of the results obtained in the present study it could be inferred that calves fed with ration containing 20% nutraceutical residue had similar growth rate as that of calves fed with control ration. Inclusion of nutraceutical residue in calf starter had no adverse effect on haematological parameters and values were within normal range of species. On summarizing the overall results of the study, it could be inferred that nutraceutical residue (alfalfa pellet) can be included in the calf ration up to 20 per cent level without any adverse effect on growth performance and blood biochemical profile.

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Table1. Proximate composition of Nutraceutical residue (on % basis)

Parameter	Percentage %
Dry matter	92.55
Crude protein	48.2
Ether extract	3.46
Crude fibre	12.78
Total Ash	5.42
Nitrogen Free Extract	30.14
Acid insoluble ash	1.23
Neutral detergent fibre	28.45
Acid detergent fibre	16.21

Table 2.Ingredient and chemical composition of calf starters, (on % basis)

Ingredients composition	T1	T2
Maize	35	34
Wheat bran	25	30
Soya bean meal	28	10
Dried fish	9	3
Nutraceutical residue	0	20
Salt	1	1
Mineral mixture	2	2
Total	100.00	100.0
Chemical composition(%)	**T1**	**T2**
Dry matter	91.93	91.90
Crude protein	24.34	24.92
Ether extract	4.23	4.54

Crude fibre	5.36	5.43
Total ash	6.67	7.21
Nitrogen free extract	59.4	57.9
Acid insoluble ash	2.34	2.65

Table3. Fortnightly average body weight of experimental calves (kg)

Fort night	T1	T2
0	35.6 ± 2.82	36.96 ± 4.00
1	38.96 ± 5.41	40.7 8± 3.95
2	42.66 ± 5.57	44.36 ± 4.42
3	45.48 ± 6.09	49.84 ± 4.66
4	47.90 ± 6.42	53.70 ± 5.61
5	49.64 ± 6.81	55.48 ± 5.17
6	55.51 ± 7.9	60.40 ± 7.07

Table4. Summarized data on body weight, total weight gain and average daily gain of experimental calves

Parameters	T1	T2
Initial body weight (kg)	35.6 ± 2.82	36.96 ± 4.00
Final body weight (kg)	55.51± 7.9	60.40 ± 7.07
Total weight gain (kg)	21.1 ± 3.59	23.44 ± 1.01
Average daily gain (g)	237.02 ± 0.05	279.05± 0.04

Table 5.Blood biochemical parameters of experimental calves

Parameters	T1	T2
Albumin,g/dl	3.31 ± 0.13	3.62 ± 0.14
plasma protein, g/dl	6.73 ± 0.09	6.96 ± 0.11
Calcium, mg/dl	10.23 ± 0.11	10.36 ± 0.15
Phosphorus, mg/dl	6.03 ± 0.10	6.07 ± 0.13
Cholesterol, mg/dl	94.33± 2.41	89.10 ±1.51
Triglyceride, mg/dl	35.89 ± 5.89	30.74 ± 2.16
BUN, mg/dl	10.15 ± 0.02	10.53 ±0 .14
Glucose,mg/dl	64.42 ± 0.49	66.79 ± 0.99

Table 6.Haematological parameters of experimental calves

Parameters	T1	T2
Haemoglobin (mg/dl)	11.78 ± 2.60	11.82 ± 0.22
MCV (fL)	38.04 ± 0.58	38.24 ± 1.20
MCH (pg)	12.20 ± 0.43	12.65 ± 0.19
MCHC (g/dl)	30.15 ± 0.39	30.28 ± 0.23
RBC count (x 10^6 /µl)	7.84 ± 0.51	8.12 ± 0.31
WBC count (x10^3/µl)	10.38 ± 0.14	10.18 ± 0.12
Granulocyte (%)	36.78 ± 0.78	36.62 ± 0.66
Lymphocyte(%)	59.42 ± 1.56	59.75 ± 0.82
Monocyte(%)	3.82 ± 0.23	3.83 ± 0.28

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RESEARCH ARTICLE

A Descriptive Study on Lifestyle Factors Influencing Gastritis among University Students of UniKL RCMP in Malaysia

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ABSTRACT

Gastritis is the inflammation of protective lining of the stomach. The present study aimed to identify the lifestyle factors that, favours gastritis among UniKL RCMP pre-clinical students and the results were closely analysed. A descriptive survey approach was used to assess the factors influencing gastritis. Extraneous variables were age, gender, health risk, behaviour, dietary pattern, family background in terms of education and economy, use of counter medication and source of information regarding gastritis. Simple random method was used for this research. Data was collected and recorded from 120 respondents who are mostly of age 17 to 26 years old and the survey was regarding gastritis related to lifestyle factors like stress, eating habits and smoking. The frequency value for the consumption of spicy food was the highest above all other factors with regards to eating habits in relation to this study followed by citrus fruits consumption, skipped meals and seasonal stress. This study demonstrate that incidence of gastritis among the respondents was seasonal especially during examination. Besides, the probabilities of developing gastritis among our respondents were also closely related to their tendency of skipping more than 1 meal on a particular day. Eventually, gastritis among them was also evidently due to consumption of spicy food.

Keywords: Adolescence, food habits, gastritis, stress and smoking.

INTRODUCTION

Gastritis is more common among the adolescents, even though it can affect anyone at any age. A very mild to severe stomach symptoms may indicate gastritis. Gastrointestinal system in our human body is responsible for intake of diet, absorption, metabolism and elimination(1). The stomach is an intra-peritoneal organ located between oesophagus and small intestine, lined by special cell for protection against producing acids. Gastritis can develop when stomach's lining become thinner or damaged(2). When hydrochloric acid comes in to contact with irritated mucosal lining of stomach, abdomen and epigastric discomfort or pain occurs that leads to gastritis. Acute gastritis involves sudden, severe inflammation, while chronic gastritis involves long-term inflammation that can last for years if left untreated (3)

Factors that increases the risk of gastritis include bacterial infection such as *Helicobacter pylori* (*H.pylori*), a gram-negative rod, and lives in human stomach exclusively and survives in the highly acidic environment by producing urease enzyme to neutralize the acid and raising the local pH(4). The risk factors like smoking, spicy and citrus foods, NSAIDS and stress can lead to excessive gastric secretion and ruptured the stomach mucosal lining. Infection of H.pylori can easily occur once the lining of the stomach is damaged and results in injury. Therefore, changes in lifestyle patterns can be significant in the development of gastritis. Ageing also can increase the risk of gastritis as the stomach lining tends to get thin. A proper stress management is significant as it is one of the major causes of gastritis among university students. The factors like pressure and excess freedom give a gate to achieve lifestyle changes through alcoholism, smoking, fast and spicy food which influence the occurrence of gastritis(5). The studies among adults conducted in various parts of the world have shown high prevalence of *H.pylori* infection. About 2.7 million people around the world are affected with gastritis in year 2013. Adolescence is prone to face a dilemma and stress in transition of their life. They tend to modify their lifestyle to overcome their problem. This give a gate for them to smoke cigarette and eating fast food. As a part of global health programme, it is necessary to explore alternative approaches to provide better health services to people(6). Hence, the present study aimed to identify which are those lifestyle factors that, favours gastritis among UniKL RCMP pre-clinical students as such there may be chances of more stress towards the balancing of the education, food timings and food patterns may occur during the course of study.

MATERIALS AND METHODS

A descriptive survey approach was used to assess the factors influencing gastritis among pre-clinical students of UniKL RCMP. Convenient sample comprises of 100 students out of 230 students in the age group of 17-26 years were selected due to limitation. Simple random method was used for data collection.Questionnaires were distributed, their knowledge, attitude and practice were assessed using a structured self-administered questionnaire. The consent forms were also provided along the questionnaires.

RESULTS AND DISCUSSION

Data was collected from the survey regarding gastritis related to lifestyle factors like stress, eating habits and smoking. From the figure 1, the descriptive frequency analysis showed 39 respondents (32.5%) have experienced gastritis. Out of 120 respondents, 24 of them were females and another 15 were males meanwhile the remaining 81 respondents (67.5%) have not experienced gastritis.A less common form of the condition, erosive gastritis, typically doesn't cause much inflammation but can lead to bleeding and ulcers in the lining of the stomach. Stress is closely related with the occurrence and incidence of gastritis(7). Accordingly, there was a strong relationship between stress and gastritis in the present study. From the figure 2, it was shown that over 120 respondents, 74 respondents (61.7%) considered that their daily routines and schedules were hectic whereas 97 respondents (80.8%) answered that they were mainly stressed out due to examination which was seasonal. Meanwhile, 70 respondents (58.3%) were stressed

out due to personal matters. In addition, 68 respondents (56.7%) were stressed out with their studies and 52 respondents (43.3%) were stressed out due to their peers. From the survey, it was also found that out of 120 respondents, 52 respondents (43.3%) were stressed by their results or academic performance.Previous studies also shown that stress is associated with gastrointestinal disorders (8)

As for eating habits with regards to this study, figure 3 showed that 74 respondents (61.7%) do take snacks in between meals. The survey also reported that out of 120 respondents, 79 respondents (65.8%) consume citrus fruits and 87 respondents (72.5%) like spicy food. More than half of percentage of respondents (65.8%)per total respondents have the optimal meal frequency of 3 times daily. Even taking foods that are spicy and irritant like hot pepper and coffee seems to damage the gastrointestinal system causing gastritis and colitis (9).In case of medication intake or administration, the survey analysis reported that out of 120 respondents, only 8 respondents (6.6%) have taken medications like NSAIDs and antacids. In the survey, the question about smoking was answered positive only by two respondents (1.7%) out of 120 respondents. Even tough, smoking is one of the important factor influencing the gastritis (10), the study excluded this factor due to negligible percentage.

CONCLUSION

The frequency value for the consumption of spicy food was the highest above all other factors with regards to eating habits in relation to this study followed by the frequency value for the consumption of citrus fruits. The incidence of Gastritis among pre-clinical students of UniKL RCMP was seasonal especially during examination. Eventually, gastritis among them was also evidently due to consumption of spicy food. Besides, the probabilities of developing gastritis among the respondents were also closely related to their tendency of skipping more than 1 meal on a particular day. In the study, out of 120 respondents 97 respondents were mainly stressed out due to examination which was seasonal. There was very least of them who smoke and only few who have taken medications like NSAIDs or Antacids. Based on this, the study cannot include those factors such as smoking and administration of medications like NSAIDs or Antacids for conclusion.

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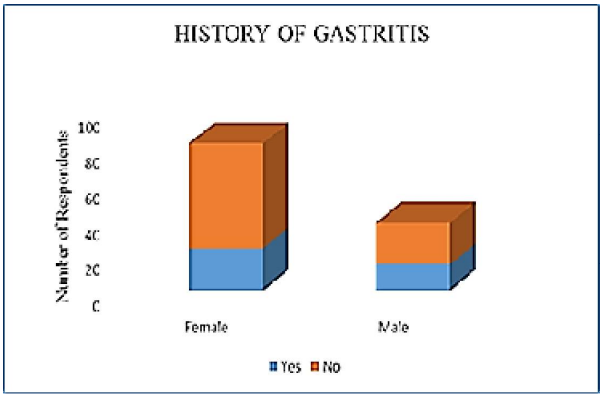

Figure 1: Respondents (male and female) showing history of gastritis as mentioned in the survey

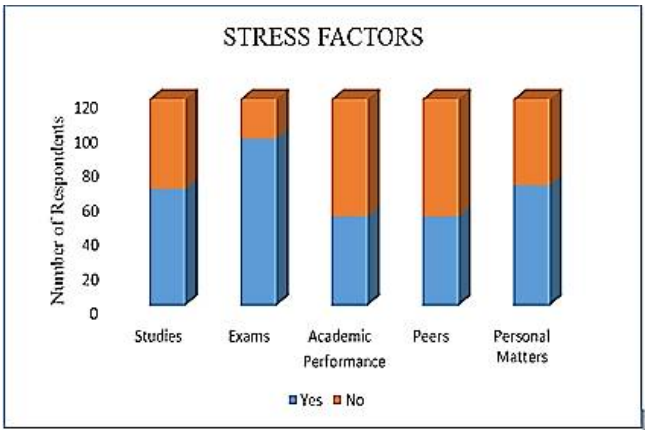

Figure 2: Several stress factors influencing gastritis among respondents.

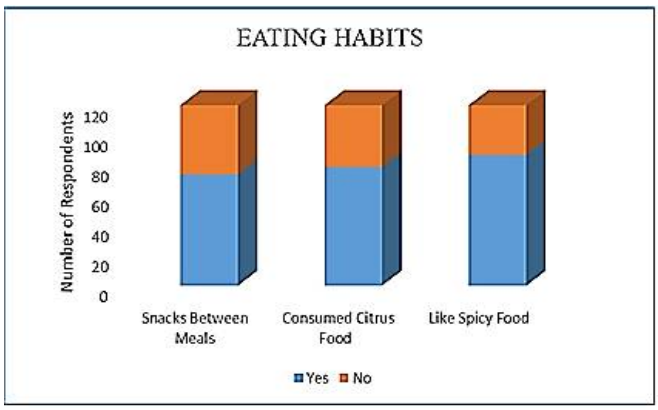

Figure 3: Dietary patterns (eating habits) influencing gastritis among respondents.

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RESEARCH ARTICLE

Prevalence of *Salmonella*, *Shigella* and *E.coli* in Vegetables of Various Markets in Kalaburagi (India)

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ABSTRACT

The present study reports the prevalence of enteric pathogenic bacteria on different vegetable samples in the markets of Kalaburagi, Karnataka (India). A total of 550 different fresh vegetable samples were analysed for the enteric pathogens on EMB, XLD and Mac Conkey Agars and found that 194 (35.27%) were culture positive. The food pathogens were identified by the conventional methods. Incidence of *E .coli* was maximum (38.14%) followed by *Salmonella* sp (36.59%) whereas the *Shigella* showed less incidence of (25.25%). Among all the collected vegetables, tomato was the highest contaminated.

Key words: Food borne pathogens; *E.coli, Salmonella*, *Shigella*, vegetables.

INTRODUCTION

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and low in fat and calories are thus vital for health and well being (Fasakin, K. 2004 , Anonymous, 2009) They give the body the necessary vitamins, fats, minerals and oil in the right proportion for human growth and development (Akinmusire *et al*., 2011). Vegetables contain a great variety of other phytochemicals, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties (Gruda *et al*., 2005). The consumption rates of vegetables and vegetable salads have greatly increased based on their proven medical and nutritional benefits (Abdullahi and Abdulkareem, 2010; Adeshina *et al*., 2012). India is the second largest producer of vegetables in the world and accounts for about 15% of the world's total production. Several outbreaks of gastroenteritis caused by eating fresh vegetables are increasing in recent years (Ankita *et al*., 2014). Contamination of fresh produce during washing and rinsing, unhygienic human handling improper storage, processing and packaging

leads to food poisoning Coliforms were observed in fresh vegetables (Titarmare *et al*, 2009). Viswanathan and Kaur (2001) examined different salad vegetables such as carrots, radishes, tomatoes, lettuce, cabbage, cucumbers, coriander and reported the presence of *P. aeruginosa*.

In India, traditional practice of growing vegetables is mainly organic based. Such organic farming has been described as representing an increased risk to public health (Tango *et al,* 2014). Vegetables and sprouts can become easily contaminated with food borne pathogens at several steps such as - growing in fields, harvesting, post harvest handling, processing and distribution (Beuchat *et al,* 1998). Depending upon the surface properties of vegetables, bacterial (including pathogens) adherence and colonization varies. To remove dust and soil, these vegetables or sprouts generally processed by simple water wash, which is insufficient to remove all the bacteria (Haute *et al,* 2015). Also, the water used to wash these vegetables itself could be the source of contamination (Rai *et al,* 2007). Therefore, the occurrence of pathogens on vegetables and sprouts can't be denied. These food items are then directly consumed or used in salads or similar preparations, hence, hazardous to public health. Some of the earlier studies from India reported the occurrence of *E. coli* in fruits, vegetables and sprouts sold locally (Rasheed *et al.,* 2006). The occurrence of bacterial populations on vegetables is recognized as a source of potential health hazard to man and animals. This is due to production of toxins and other compounds which are capable of inducing several critical clinical symptoms in man following ingestion or inhalation (Effiuvwevwere *et al,* 2000).

Recently, association between fresh vegetables and outbreaks of food borne infections has led to a greater concern about contamination of vegetables with fecal pathogenic bacteria like *E. coli, Vibrio, Salmonella,* norovirus, *Shigella, Listeria* etc (Mahima *et al*, 2013) and safety of using animal manures as fertilizer in vegetable production (Pell, 1997; Tauxe *et al*, 1997). The animal faeces and manure contain a number of zoonotic bacteria, such as *Escherichia coli, Campylobacter* spp. *Salmonella* spp. and *Mycobacterium* spp. (McGarvey *et al*, 2004; Dhama *et al*, 2013; Malik *et al*, 2013; Singh *et al*, 2013). The majority of diseases associated with fresh vegetables are primarily those transmitted by the fecal-oral route, and therefore, are a result of contamination at some point in the process (De Roever, 1998). Gastroenteritis, Giardiasis, *Hepatitis A*, *Hepatitis E, Shigellosis* (bacillary dysentery), Typhoid fever, Vibrio *parahaemolyticus* infections and *Cholera* are very evident examples of fecal-oral route transmitted diseases. Cultivation of trees/plants of fruits and vegetables in the areas with a presence of potentially harmful microbes like sewage, sludge, animal faeces, and toxic weeds can lead to the contamination during growth, harvesting and storage; also the places where operations with livestock or birds are made can cause the contamination. In India, the presence of coliforms and Staphylococci in kinnow and mandarin juices in Patiala city were reported (Ganguli *et al*., 2004). The present study therefore is aimed at investigating the prevalence of different bacterial species on vegetables sold in Kalaburagi super markets,

MATERIAL AND METHODS

Sample collection

Fresh vegetable samples were collected from, different areas of Kalaburagi such as Supermarket, Kanni market, markets at Timmapuri Chowk and RTO cross. The samples were collected in sterile polythene bags and transported to the laboratory within 2 hours of collection under aseptic conditions. A total of five hundred and fifty (550) samples of various vegetables were analysed.

Sample processing

Half gram of each sample was homogenized in 5 ml of buffered peptone water and was filtered through sterile Whitman No. 1 filter paper to remove the solid particles. The filtrate was incubated for enrichment at of 37^0C.

Enrichment and screening

After six hour enrichment a loop full of the sample was taken and streaked on the specific differential Media such as EMB, XLD and MacConkey Agar and incubated for 18-24hr at 37^{0}C.

Isolation and identification

Well isolated colonies formed on various media plates were picked up, purified by re-streaking on the same medium and stored as slant culture. The isolates were further characterised and identified by cultural, morphological and biochemical tests (Holt *et al,* 1994; Sherman 2005).

RESULTS AND DISCUSSION

Among the 550 vegetable samples analysed from different markets of Kalaburagi (Karnataka, India), 194(35.27%) samples showed cultural positivity (Table 1). The cultural characteristics of the isolates and important biochemical tests are depicted in (Fig.1 and Fig.2) *E.coli* constituted the majority of the isolates (38.14%) closely followed by *Salmonella* (36.59%) and significantly *Shigella sp*, the causative agent of bacillary dysentery was also present in 49 (25.25%) samples (Fig.3). Most highly contaminated vegetables are chilli 87.50%, dill 75.00%, capsicum 70.00%, spinach 66.67%, parsley 66.67%, peas 60.00%, cauliflower 50.00%, Potato 43.33%, fenugreek leaves 42.86%, carrot 42.86% and gongura 41.67%. cucumber 34.15%, tomato 30.26%, onion 24.44%, ivy gourd 38.46%, coriander leaves 26.67%, bitter gourd 30.00%, drumstick 21.42%, ladies finger 20.00%, French beans 22.73%, pumpkin 21.73% are moderately contaminated while zucchini 14.71%, snake gourd 12.5%, brinjal 7.69%, sponge gourd 6.67% are the least contaminated vegetables. Fortunately mint leaves, lemon and bitter gourd have not shown the presence of pathogens. It is alarming to note that, the most extensively and daily used vegetables like chillies, tomato, potatoes, spinach and the vegetables consumed raw like carrots, cucumbers are among the most extensively contaminated vegetables.

Consumption of fresh vegetable produce in the form of salads has increased in recent years and a large number of minimally processed fresh-cut vegetables are available in supermarkets, food service facilities and are also prepared household. Unfortunately, the increase in the consumption of raw vegetables has resulted in the increase in frequency of outbreaks of illness (Brackett, 1999; Thunberg et al., 2002; Bhagwat, 2004). Salmonella spp have been isolated from several raw vegetables from many countries (Wachtel and Charkowski, 2002; Zhao et al., 2001). Pathogenic Escherichia coli and *Salmonella* sp. enteric bacteria are involved in large food borne outbreaks worldwide, causing symptoms of gastroenteritis and chronic infections (De Oliveira *et al*., 2011)According to the study conducted by Joy *et al* (2004), 66.6% of the vegetable samples analysed were contaminated with food pathogens. The prevalence of *E.coli* was 27.7, *Shigella* 16.6 and *Salmonella* showed 33.8% prevalence. Wells *et el* (1997) studied the incidence of food pathogens from soft rot samples in his study 19 of 55 samples were confirmed positive and 50 samples were found to be positive for the occurrence of *Salmonella*. Among isolated organisms; *E.coli* (40%) was dominant followed by *Salmonella spp* (16%), *Enterobacter* spp (1%).

CONCLUSION

The study reveals the potential hazards of vegetables and indicates the importance of adoption of hygienic practices by food processors and vendors. The vegetables get contaminated with pathogenic microorganisms while growing in fields or orchards or during harvesting, post harvesting handling, processing and distribution. Pre-harvest and post-harvest practices play significant roles as sources of contamination. Adaptation and application of Hazard Analysis and Critical Control Point (HACCP) can reduce the chance of contamination and eliminate the pathogenic microorganisms.

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Table 1: Culture positivity of the vegetable samples.

Sl.No	Samples	Samples collected	Bacterial isolates (%)	Number of culture positive samples		
				E.coli **(%)**	***Salmonella*** **(%)**	***Shigella*** **(%)**
1	Vegetables	550	194 (35.27)	74 (38.14)	71 (36.59)	49 (25.25)

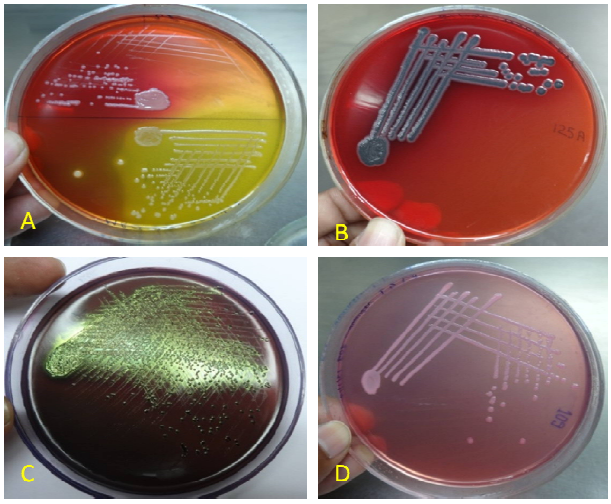

Fig.1. The cultural characteristics of the enteric pathogens on XLD agar (A) and (B), EMB agar(C), MacConkey agar (D).

Fig.2. Triple Sugar Iron (TSI) test.

Table-2: Sample wise distribution of enteric pathogens in vegetables

Sl no	Samples type	Number of samples collected	Total number of bacterial isolates (%)	*E.coli*	*Salmonella*	*Shigella*
1	Tomato	76	23 (30.26%)	12(15.79%)	5 (2.63%)	6(7.90%)
2	Carrot	35	15(42.86%)	7(20.00%)	7(20.00%)	1(2.85%)
3	Cucumber	41	14(34.15%)	1(2.43%)	7(17.07%)	6(14.63%)
4	Onion	45	11(24.44%)	7(15.55%)	3(6.67%)	1(2.22%)
5	Potato	30	13(43.33%)	9(30.30%)	1(3.33%)	3(10.00%)
6	Lady's finger	10	2(20.00%)	1(10.00%)	1(10.00%)	ND
7	Snake gourd	16	2(12.5%)	2(12.5%)	ND	ND
8	Brinjal	26	2(7.69%)	2(7.69%)	ND	ND
9	Sponge gourd	15	1(6.67%)	ND	1(6.67%)	ND
10	Chilli	24	21(87.50%)	8(33.33%)	7(29.16%)	6(25.00%)
11	Ivy gourd	13	5(38.46%)	1(7.70%)	4(30.77%)	ND
12	Beat root	4	ND	ND	ND	ND
13	Capsicum	10	7(70.00%)	3(30.00%)	3(30.00%)	1(10.00%)
14	French Beans	22	5(22.73%)	ND	3(13.63%)	2(9.09%)
15	Drumstick	14	3(21.42%)	ND	3(21.42%)	ND
16	Lemon	8	ND	ND	ND	ND
17	Pea	5	3(60.00%)	1(20.00%)	ND	2(40.00%)
18	Pumpkin	23	5(21.73%)	2(8.70%)	2(8.70%)	1(4.34%)
19	Zucchini	34	5(14.70%)	2(5.89%)	2(5.89%)	1(2.94%)
20	Bitter Gourd	10	3(30.00%)	1(10.00%)	ND	2(20.00%)
21	Cauliflower	34	17(50.00%)	5(14.70%)	5(14.70%)	7(20.58%)
22	Spinach	9	6(66.67%)	1(11.11%)	3(33.33%)	2(22.22%)
23	Coriander leaves	15	4(26.67%)	3(20.00%)	1(6.67%)	ND
24	Dill	4	3(75.00%)	ND	3(75.00%)	ND
25	Parsley	3	2(66.67%)	ND	1(33.33%)	1(33.33%)
26	Fenugreek Leaves	14	6(42.86%)	2(14.28%)	3(21.42%)	1(7.14%)
27	Gongura	12	5(41.67%)	4(33.33%)	1(8.33%)	ND
28	Mint Leaves	12	ND	ND	ND	ND

ND- Not detected.

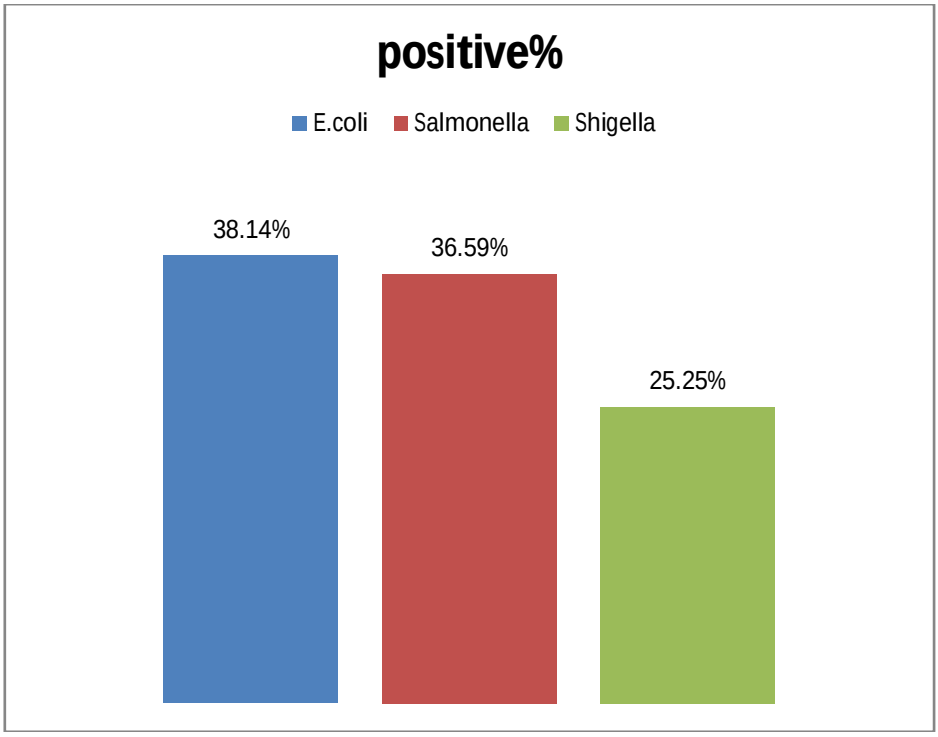

Fig 3: Positive percentage of food pathogens

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RESEARCH ARTICLE

Antimicrobial Activity of *Bignonia magnifica* and its Silver Nanoparticles against Urinary Tract Infection Pathogens

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ABSTRACT

Medicinal plants are extensively used to cure various infectious diseases in human beings, hence (*B. magnifica*) leaves was investigated for its activity against *Staphylococcus aureus and Escherichia coli* bacteria. Extract of the leaves was prepared using various solvents such as methanol, ethylacetae and hexane. Preliminary phytochemical analysis of the extracts showed the presence of various active principles. Methanolic and ethylacetate extract of the leaves showed maximum phytochemical principles; hence it was taken for further investigation. Methanolic and ehylacetate extract of the plant showed a dose dependent inhibition of the two bacterial species taken. The silver nanoparticles were synthesized using *Bignonia magnifica* and the nanoparticles were characterized using ultraviolet–visible (UV–Vis) absorption spectroscopy and FTIR studies. The synthesized silver nanoparticles were investigated to evaluate the antibacterial activity against urinary tract infections (UTIs) pathogens. The antibacterial activity of silver nanoparticles was evaluated by disc diffusion assay. Silver nanoparticles showed maximum sensitivity to *S. aureus* and *E.coli*. The results provide evidence that, the silver nanoparticles might indeed be the potential source to treat urinary tract infections caused by *S.aureus* and *E.coli.*

Keywords: Bignonia magnifica, Staphylococcus aureus, Escherichia coli, UTI

INTRODUCTION

Urinary tract infection (UTI) is a common infection that usually occurs when bacteria enter the opening of the urethra and multiply in the urinary tract. Urinary tract infections are the second most common type of infection in the world. It is estimated that about 35% of healthy women suffer symptoms of UTI. The incidence of UTI is greater in women as compared to men which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors. About 80 to 90 percent of UTIs are caused by *Escherichia coli* (*E.coli*) and remaining 5 – 15 % of UTI are caused because of *Staphylococcus* species. Generally UTI are treated with anti-bacterial antibiotic such as Clindamycin, Vancomycin, Bacitracin, Ampicillin, Chloramphenicol and Erythromycin. The increasing drug resistance among these UTI pathogenic bacteria has made the UTI therapy difficult and has led to greater use of expensive broad-spectrum drugs. This resistance problem may be encountered by searching effective antibacterial agents against pathogenic microorganisms which is resistant to current antibiotics (Sundaram Ravikumar *et al*., 2012). Usually chemical compounds produce lot of side effects, in order to minimize these side effects; naturally available plant metabolites are used to treat diseases. The substances that can either inhibit the growth of pathogens or kill them, and have no or least toxicity to host cells; are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens (Lee. 1998). Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth is considered candidates for developing new antimicrobial drugs (Bruneton, 1995). Nanobiotechnology describes an application of biological systems for the production of new functional material such as nanoparticles. The nanoparticles were synthesized by using aqueous extract of *B. magnifica* and metal ions (such as silver). Silver is of particular interest due to its distinctive physical and chemical properties (Geeta Watal *et al*., 2012). Nanoparticles of noble metals such as silver have antibacterial properties (Sukumaran Prabhu *et al*., 2005).The synthesized silver nanoparticles were investigated to evaluate the antibacterial activity against UTIs bacterial pathogens. In the present investigation silver nanoparticles were synthesized using *B. magnifica* leaves extract and its antimicrobial activity was evaluated against UTI pathogens.

MATERIAL AND METHODS

Preparation of Extracts

Fresh *Bignonia magnifica* leaves were washed thoroughly in tap water and with distilled water and air dried in the shade at room temperature for five days. Shaded dried leaves were powdered. 10g of each powder(*B.magnifica*) was mixed with 100 ml of methanol, ethyl acetate and hexane to form methanolic, ethyl acetate and hexane extract; heated below the boiling point and stirred for 2 ½ - 3 hr. The extract was filtered by muslin and then by filter paper (Whatman No. 1) and then stored in the refrigerator at 5 ºC for further use.

Phytochemical screening of Extract

The different qualitative tests were performed for establishing profile of the given extracts for its chemical composition by Harborne method (1995).

Tests for Antimicrobial Activity of the Extracts

Sterile nutrient agar plates were prepared; microorganisms were evenly spread on the surface of the agar plate and sterile cork borer of 6 mm diameters was used to make 5 wells on the plates. Varying concentrations of the extracts i.e. 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml were made and 0.5 ml of the extract was added in each well;

incubated at 37°C for 24 h. After incubation the zone of clearance around each well was measured using a metric ruler.

Evaluation and synthesis of silver nanoparticles

Biosynthesis of Silver nanoparticles

10 g of dried leaf powder of *Bignonia magnifica* were added to 100 ml of distilled water and allowed to boil; then filtered with the Whatmann filter paper.1Mm of fresh silver nitrate solution was prepared and 10 ml *Bignonia magnifica leaf* extracts were added into 90 ml of silver nitrate and incubated at room temperature for 2 hrs.

UV-Vis spectra analysis

The bioreduction of Ag^+ in aqueous solution at different temperature interval was monitored by UV-Vis spectroscopy. 1ml of sample taken in Eppendroff's centrifuge tube, centrifuge at 10,000 rpm for 10 min, discard the supernatant; wash the pellet twice at 7000 rpm for 2 min and then dilute the pellet with 1ml of distilled water. The diluted pellet was used for further analysis.

Fourier Transform Infra-Red (FTIR) Analysis

FTIR Spectra for extract powder was obtained in the range 4000–400 cm−1 with IR-Prestige-21 Shimaduz FTIR spectrophotometer using KBr pellet method.

Antimicrobial activity of silver nanoparticles by well diffusion method

The silver nanoparticles synthesized from *Bignonia magnifica* were tested for their antimicrobial activity by well diffusion method against gram positive and gram negative organisms such as *Staphylococcus aureus and Escherichia coli.* The pure cultures of organism were subcultured on Muller–Hinton broth at 35°C on rotary shaker at 200 rpm. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm have been made on Muller–Hinton agar plates using gel puncture. Using micropipette 25 µl, 50 µl, 75µl and 100 µl of the sample of nanoparticles solution were poured into wells on all plates. After incubation at 35°C for 24 h, the different levels of zone of inhibition were measured.

RESULTS AND DISCUSSION

In this study the phytochemical screening were performed with hexane, methanol and ethyl acetate extract of *B.magnifica* leaf power (Table 1). Among all the solvents, methanolic and ethyl acetate extract proved to be the most prominent solvent for the extraction of antimicrobial substances from the selected plant and showed the presence of more number of phytochemicals. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available regarding anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties of plants (Cushnie and Lamb, 2005). Hence methanolic (Table 2) and ethylacetate(Table 3) extracts of *B. magnifica* leaves was taken for further investigation. The results of the antibacterial activity showed that the methanolic and ethyl acetate extracts of *B.magnifica* exhibited remarkable activity against *Staphylococcus aureus* and *Escherichia coli* with zone of inhibition ranging from 3 to 8 mm. The highest concentration 20 µl/ml showed the maximum zone of inhibition for the two bacteria taken for the investigation. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant, antitumor and antimicrobial activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, antimicrobial and anti-inflammtory activity (Abubakar, 2009). The UV-Vis spectra show peak at 427 nm which

confirms the presence of silver nanoparticles (Figure 1). An absorption peak between 410-460 nm confirms the presence of silver nanoparticles (Rajan *et al*., 2013).The FTIR spectrum of silver nanoparticles (Figure 2) confirms that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. The band at 3403.37 cm-1 corresponds to N-H stretching of amines. The peak at 2977.79 cm-1 corresponds to C-H stretching of alkanes. The peak at 2117.62 cm-1 corresponds to C-H of phenyl ring. The peak at 1635.31cm-1 corresponds to C=C of aromatic rings. The peaks at 1382.71 and 1049.49 cm-1 corresponds to stretching of alcohols, ethers, carboxylic acids, esters and the band observed at 878.56 and 676.06 cm-1 corresponds to C-H stretching in alkynes. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Ravi *et al*., 2013**)**. The results of the antibacterial activities of silver nanoparticles exhibited remarkable activity against the test organisms (S. *aureus* and *E. coli*) with zone of inhibition ranging from 8 to 11 mm (Table 4). The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria (Sondi and Salopek-Sondi, 2004). The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Morones *et al.,* 2005).

CONCLUSION

The results provide evidence that the active principles of *B. magnifica* and silver nanoparticle synthesized using *B. magnifica* might indeed be the potential source to treat urinary tract infections caused by *E.coli* and *S. aureus.* Further study is needed to identify the mechanism of action of nanoparticles against UTI pathogens.

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Table 1: Preliminary phytochemical analysis of *Bignonia magnifica* extracts.

PHYTOCHEMICALS	Methanol	Hexane	Ethyl acetate
Alkaloids	+	-	-
Flavonoids	+	-	+
Glycosides	+	+	+
Saponins	-	-	-
Phenolic compounds	+	-	-
Tannins	-	-	-
Terpenoids	+	-	+

Table 2: Antimicrobial properties of methanolic leaf extracts of *Bignonia magnifica* against *Staphylococcus* aureus and *Escherichia coli.*

Pathogens	Methanolic leaf Extracts	Antimicrobial activity*			
		Conc. Of 5 µl/ml	Conc. of 10 µl/ml	Conc. of 15 µl/ml	Conc.of 20 µl/ml
Staphylococcus aureus	*Bignonia magnifica*	4mm	5mm	6mm	8mm
Escherichia coli	*Bignonia magnifica*	3mm	4mm	5mm	7mm
*measured by the diameter of zone of inhibition in mm Zones are mean diameter of five replicates.					

Table 3: Antimicrobial activities of ethyl acetate extracts of *Bignonia magnifica* leaves against UTI pathogens.

Pathogens	Ethyl acetate leaf Extracts	Antimicrobial activity*			
		Conc. Of 5 µl/ml	Conc. of 10 µl/ml	Conc. of 15 µl/ml	Conc.of 20 µl/ml
Staphylococcus aureus	*Bignonia magnifica*	3.3mm	4.2mm	5.1mm	6.3mm
Escherichia coli	*Bignonia magnifica*	3.5mm	4.3mm	5.5mm	6.4mm
*measured by the diameter of zone of inhibition in mm Zones are mean diameter of five replicates.					

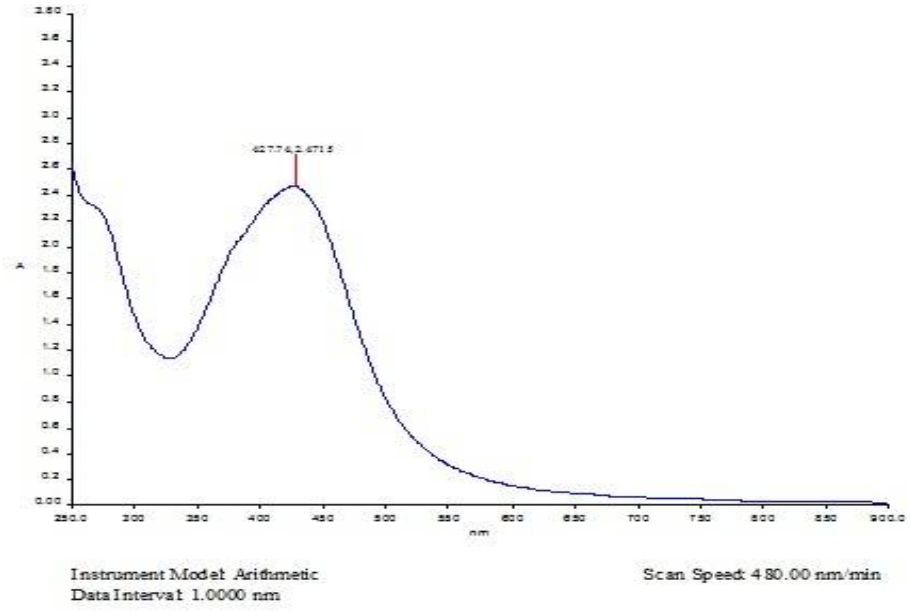

Fig: 1 UV-VIS spectroscopy of silver nanoparticles synthesized using *B.magnifica* leaves.

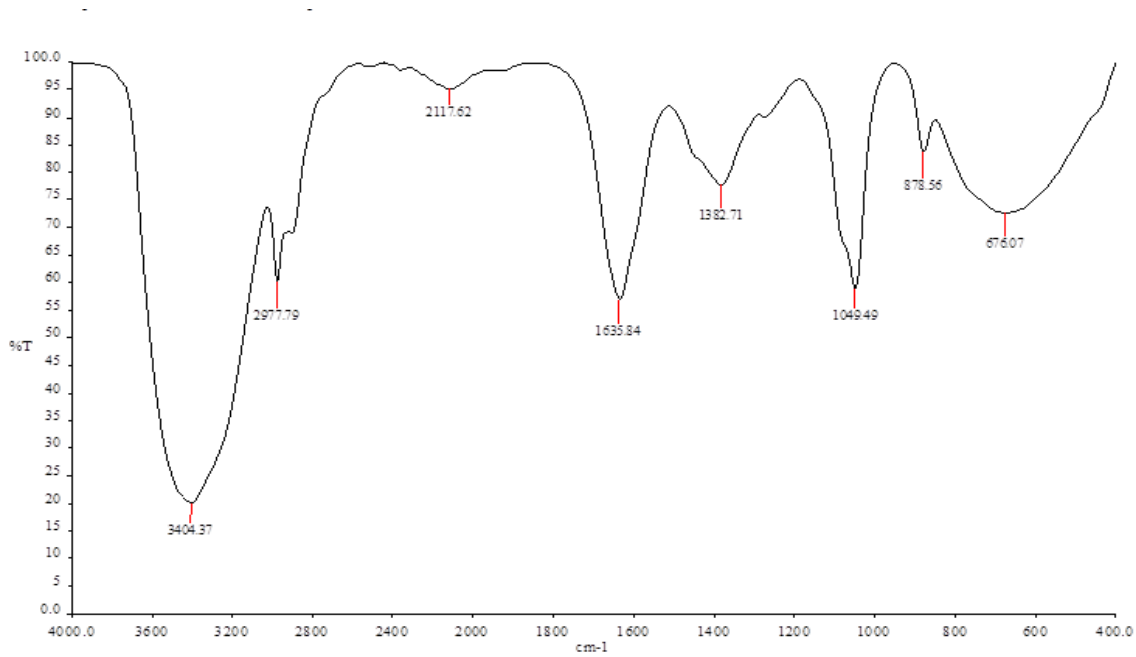

Figure 2: FTIR analysis of silver nanoparticles synthesized using *B.magnifica* leaves

Table 4: Antimicrobial properties of nanoparticles synthesized using *Bignonia magnifica* leaves against *Staphylococous aureus* and *Escherichia coli*.

Pathogens	**nanoparticles**	**Antimicrobial activity***			
		Conc. Of 5 µl/ml	Conc. of 10 µl/ml	Conc. of 15 µl/ml	Conc.of 20µl/ml
Staphylococcus aureus	*Bignonia magnifica*	8mm	9mm	10mm	11mm
Escherichia coli	*Bignonia magnifica*	8.mm	8.8mm	9.7mm	11mm
*measured by the diameter of zone of inhibition in mm Zones are mean diameter of five replicates					

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RESEARCH ARTICLE

Genetics of Yield Traits, Seed Cotton Yield and Fibre Quality Traits in Upland Cotton (*Gossypium hirsutum* L.)

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ABSTRACT

Present study was conducted to evaluate the genetics of yield and fibre quality traits in seven upland cotton (*Gossypium hirsutum* L.) cultivars i.e. Narasimha, TCH 1726, TCH 1705, KC2, MCU13, BW4-1 and MCU 3were crossed in a complete diallel mating design. Genetic analysis revealed that mean degree of dominance was found to be less than one for all characters studied except boll weight. H_1 value was significant and greater than D for all traits studied except boll weight. Narrow sense heritability found to be very high in plant height, 2.5% span length. $H_2/4H_1$ ratio found to less than 0.25 is recorded for all characters studied except for seed index, and boll weight.The character number of bolls per plant, single plant yield, seed index, 2.5 percent span length showed partial dominance nature in the (Vr), (Wr) covariance graph. All other characters exhibited over dominance. The parent TCH 1726 was the dominant parent for plant height and boll weight. The parent Narasimha was the dominant parent for days to first boll bursting in view of its position near the origin in the regression graph. The parent MCU 13 was dominant parent for, micronaire and 2.5% span length.The parent BW4-1 carried recessive genes for plant height.

Keywords: Cotton, diallel analysis,fibre quality traits, gene action,heritability

INTRODUCTION

Cotton (*Gossypium spp*.) is an important fibercrop and it plays a vital role as a cash crop in many countries. Cotton provides fibre for textile industry, cellulose from its lint, oil and protein rich meal from its seed (Ashokkumar andRavikesavan, 2011a & b).Cotton, also known as "King of fibres" plays aremarkable role in Indian economy. Development of cotton varieties with high yield and fibre quality is the primary objective of cotton breeders.The first step in a successful breeding program is the selection of appropriate parents. Diallel analysis provides asystematic approach for detection of appropriate parents and crosses in terms of investigated traits and it has beenwidely used by plant breeders in the selection of parents and crosses in the early generations (Kiani et al., 2007; Karademir and Gencer, 2010; Senthilkumar et al., 2013; Senthilkumar et al., 2015).Numerousstudies have been reported on yield and yield attributing traits, but little work has been reported on the genetics of fibre quality traits in cotton breeding. A few recent reports in the literature (Karademir et al., 2011; Bolek et al., 2011; Ashokkumar and Ravikesavan, 2008; Ashokkumar and Ravikesavan, 2010; Ashokkumar et al., 2010; Ashokkumarand Ravikesavan, 2011a; Ashokkumar et al., 2013; Ashokkumar et al., 2014) have determined that cotton genotypes differ in fibre quality traits.

The previous studies reports showed that inheritance of seed cotton yield and its components were controlled with additive and non-additive gene action. Earlier studies ofMukhtar et al. (2000), Iftikhar et al. (2001), and Subhan et al. (2002) revealed additive type of gene action with partial dominance and additive type of gene action with partial dominance for boll weight was observed Shakeel et al. (2001).Though, Ajmal et al. (1998) and Shakeel et al. (2001) reported the presence of genes showsoverdominance for yield and yield attributing traits. Additionally, Saravanan et al. (2003) and Soomro et al. (2009) studied diallel set of crosses of some parents of cotton for genetic effects ondifferent traits. The additive type of gene action was noticed for boll weight; however plant height, number of bolls per plant and seed cotton yield perplant revealed effects with over dominance type of gene action.Hence, present study was conducted to obtain the genetic information for yield and fibre quality traits of cotton plant by using diallel analysis method. Theinformation thus generated may help the breeders to launch a successful cottonbreeding programme.

MATERIALS AND METHODS

Genetic materials

The field experiment was conducted to evaluate the growth, yield and fibre quality traits performance of seven commercially cultivated varieties of cotton (*Gossypium hirsutum* L.) using seven parents, viz., Narasimha, TCH 1726, TCH 1705, KC2, MCU13, BW4-1 and MCU 3. All the seed materials were obtained from Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, India. The commercial cultivars were cultivated in southern states of India.

Experimental design, sampling, and traits measurements

The cotton cultivars were evaluated in randomized block design (RBD) with three replications at Cotton Breeding Station, Tamil Nadu Agricultural University, Coimbatore, and Tamil Nadu in India. The seed of each parental genotype was sown in 20 rows of 6m length in crossing block with a spacing of 90 x 45 cm. Crosses were made between parents in a 7 x 7 full diallel mating design. The crossed bolls were collected and ginned to obtain F_1 seeds. Seven parents and 42 hybrids were raised along with the standard check with three replications. For each genotype and its cross combinations, data were recorded on five randomly selected plants per replicationfor twelve characters namely, days to boll bursting, number of sympodia per plant, plant height (cm),number of bolls per plant, boll weight (g), lint index, seed index, ginning percent, single plant yield (g), 2.5% spanlength (mm), elongation percent and fiber fineness. The diallel analysis was performed as model 1 and method 1 suggested by Griffing (1956).

Genetic and graphical analysis

The graphic and genetic component analysis of the diallel cross was analysed following the methods suggested by Jinks and Hayman (1953) and Aksel and Johnson (1962).The diallel hypothetic assumptions were tested by "t^2" test and uniformity of W_r and V_r (Hayman, 1954).

$$t^2 = \frac{n-2}{4} \quad x \quad \frac{(Var.Vr - Var.Wr)^2}{(VarVr.Var.Wr) - (Cov^2(Vr\ Wr))}$$

where, n is the number of parents under study.

t^2 values were compared with statistical tabular values for 4, n-2 degrees of freedom, which is a 'F' test. The t^2value when non-significant denotes the uniformity of Vr - Wr and confirms the valuable application of Hayman Postulate (1954). Otherwise, significance of t^2 values indicates the failure of at least one of the assumptions postulated.

Deviation of Regression co-efficient (b) from zero and unity

The occurrence of non-allelic interactions was further tested and confirmed by calculating the regression co-efficient. The regression of covariance on variance and its standard error were calculated as follows:

$$b_{yx} = \frac{Cov(Wr.Vr)}{Var(Vr)}$$

$$\text{Standard Error } (b_{yx}) = \left[\frac{Var\ Wr - b\ Cov(Wr.Vr)}{Var\ Vr\,(n-2)}\right]^{1/2}$$

The significance of b_{yx} from zero and unity was tested as follows:

$$\frac{b-0}{SE(b)} \quad \text{and} \quad \frac{1-b}{SE(b)} \text{ both following 't' distribution}$$

The significance of 'b' from zero and unity was tested against the table value of 't' for n-2 degrees of freedom. A non-significant value justifies the existence of regression of Wr and Vr.

Estimation of genetic components

The theory and method of Hayman (1954) were extended to estimate the genetic components or parameters viz., D, F, H_1, h^2 and E. Hayman (1954) derived the expectations for the statistics calculated for F_1 diallel table and also the expected values of the parameters of the variation using least square technique as follows:

(D) = $V_{0L0} - E$

(F) = $2\,V_{0L0} - 4\,W_{0L01} - 2\,(n\text{-}2)\,E/n$

(H_1) = $V_{0L0} - 4\,W_{0L01} + 4\,V_{1L1} - (3n\text{-}2)\,E/n$

(H_2) = $4V_{1L1} - 4V_{0L1} - 2E$

(h^2) = $4\,(M_{L1} - M_{L0})^2 - 4\,(n\text{-}1)\,E/n^2$

(E) = $2\,(V_{0L0} - W_{0L01} + V_{1L1} - Wr - Vr) - 2\,(n\text{-}1)\,E/n$

Where,

D = Component of variation due to additive effects of the genes

F = The mean of Fr over the arrays. For being the covariance of additive and non-additive effects in the r^{th} array. Additive x dominance variance.

H_1 = Component of variation due to dominance effects of the genes

H_2 = H1 $(1-(u-v)^2)$ measures the dominance variance due to positive (u) and negative (v) effects of genes, where, u = proportion of positive alleles while, v = proportion of negative alleles since u + v = 1.

L_2 = Net dominance effects (as the algebraic total over all loci in heterozygous state in all cross combinations)

E = The expected environmental component of variation derived from error variance divided by number of replications.

$V_{0L1}$is the variance of mean arrays.

$M_{L1} – M_{L0}$ is the difference between the mean of the parents and the mean of their n^2 progenies. When F parameter is on positive direction, it explains higher proportion of dominant alleles, while a negative direction reveals more of recessive alleles.All these genetic parameters were tested by 't' test for significance by the use of the respective standard errors of parameters.

These standard errors were estimated from

i) the formula $S^2 = \frac{1}{2}$ Var (Wr - Vr)

ii) the main diagonal or covariance matrix (Hayman, 1954) as corresponding multipliers

$$\text{SE of D} = \left[\frac{\left(S^2 n^5 + n^4\right)}{n^5}\right]^{1/2}$$

$$\text{SE of F} = \left[\frac{4S^2\left(4n^5 + 20n^4 - 16n^3 + 16n^2\right)}{n^5}\right]^{1/2}$$

$$\text{SE of H1} = \left[\frac{16S^2\left(n^5 - 41n^4 - 12n^3 + 4n^2\right)}{n^5}\right]^{1/2}$$

$$\text{SE of H2} = \left[16\,S^2\,\frac{\left(36n^4\right)}{n^5}\right]^{1/2}$$

$$\text{SE of } h^2 = \left[16S^2\,\frac{\left(16n^4 + 16n^2 - 32n + 16\right)}{n^5}\right]^{1/2}$$

$$\text{SE of E} = \left[\frac{S^2 n^4}{n^5}\right]^{1/2}$$

Where, n = number of parents

Test for significance of components

The calculated't' values of every parameter and error was derived by dividing the component value with its standard error and the value was compared with the tabular value of't' for n-2 d.f.

Proportion of genetic components

The proportionate ratios of different components of variance were compared by the equations given by Crumpacker and Allard (1962), as follows :

1. Mean degree of dominance over all loci

$= (H_1 / D)^{1/2}$

If it is > 1, reveals over dominance

< 1, partial or incomplete dominance

= 0, no dominance
= 1, complete dominance

2. Proportion of dominant and recessive genes in parents

$$\frac{KD}{KR} = \frac{4(DH_1)^{1/2} + (F)}{4(DH_1)^{1/2} - (F)}$$

If the proportion is equal to 1, it defines the equality of dominant and recessive genes greater than 1, predominance of dominant genes and less than 1, indicates predominance of recessive genes.

3. Proportion of genes with positive and negative effects in the parents

$$= \frac{(H_2)}{4(H_1)}$$

when u and v are symmetrically distributed
(u = v = 0.5) the ratio $H_2 / 4 (H_1) = 0.25$

4. Number of groups of genes controlling the character and exhibiting dominance is derived by (h^2 / H^2)

5. Narrow sense heritability estimates

$$\frac{1/2(D) + 1/2(H_1) - 1/2(H_2) - 1/2(F)}{1/2(D) + 1/2(H_1) - 1/4(H_2) - 1/2(F) + E} \text{ (or)}$$

(Crumpacker and Allard, 1962)

$$\frac{1/4(D)}{1/2(D) + 1/2(H_1) - 1/4(F) + E}$$

6. The correlation between parental order of dominance (Wr + Vr) and parental measures (Yr) was computed. A significant positive correlation indicates that most of the dominant genes had negative effects for those characters, while significant negative value indicates that most of the dominant genes had positive effects (Hayman, 1954).

Graphical Analysis

Wr. Vr. Graph: The essential factors for the graph Vr and Wr were calculated from the diallel table and these values were used in constructing the Wr. Vr. Graph for each character.

Where,

Vr is the variance of offspring of the r^{th} parental array
Wr is the covariance of the offspring of the r^{th} array with respect to non-recurrent parent.

Data from F_1 and parents were subjected the analysis proposed by Jinks and Hayman (1953) as illustrated by Aksel and Johnson (1962). In this analysis the following statistical parameters were estimated from the data.

Vr - The variance of offspring of the r^{th} parental array
Wr - The covariance of offspring of the r^{th} array with respect to non-recurring parent.
V_{OLO} - The variance of all parental means
V_{OL1} - The variance of the means of the arrays
W_{OLO1} - The mean covariance between parents and the arrays
V_{1L1} - The mean variance of arrays

$(M_{1L1} - M_{LO})^2$ – The square of difference between the mean of the parents and the mean of their n^2 progeny.

By utilizing the regression values, the Wr, Vr graph was drawn for all the characters studied and the limiting parabola was constructed using the formula

$$W_r^2 = Vr \times V_{OLO}$$

i.e. by plotting Vr. $(Vr \times V_{OLO})^{1/2}$ points starting with $Vr = V_{1L1}$. A few interpolations and extrapolations make it possible to draw the parabola. The hypothesis was tested as indicated earlier.

RESULTS AND DISCUSSION

Genetic analysis

The proper understanding of genetic architecture of the characters of the parents involved in hybridization is essential to genetically improve the crop plants. The genetic analysis of different characters was estimated adopting the method suggested by Hayman (1954) and Griffing (1956). This analysis provides certain additional information such as a) Mean degree of dominance b) proportion of genes with positive effects in parents c) the proportion of dominant and recessive genes in parents d) heritability estimate in narrow sense. In genetic analysis, various genetic parameters D_1, H_1, H_2, F, h^2 and E was estimated and furnished (Table 1).Predominance of dominance effect was observed for plant height, ginning percentage, days to first boll bursting, single plant length, 2.5% staple length, micronaire value, number of sympodial branches, number of bolls per plant, lint index, seed index and bundle strength.The similar results are in confirm with the findings of Sandhu et al., (1992) for plant height, Jagtap, (1994) and Tomer and singh (1996) for number of bolls per plant, Singh et al. (1988) for lint index,Tomer and singh (1996) for seed index, Gururajrao et al. (1977) and Bhandari et al., (1981) for 2.5 per cent span length.

The mean degree of dominance was found to be less than one indicating the partial dominance for boll weight. The ratio $H_2/4H_1$ was less than 0.25 for all characters studied except for boll weight and seed index.F value was positive for all characters studied. The heritability in narrow sense was found high for plant height and 2.5 % span length. Heritability in narrow sense is high it indicated that the character is largely governed by additive genes and selection for improvement of such character would be rewarding, Deskmuchet al. (1999) reported similar results for plant height, number of bolls per plant and 2.5% span length.The moderate heritability in narrow sense was recorded in ginning per cent, lint index, days to first boll bursting and micronaire. Similar results are also reported by Gururajaraoet al. (1977) for lint index. The characters namely bundle strength, seed index, number of sympodial branches, boll weight, number of bolls per plant, and single plant yield recorded low estimates of heritability. Jagtap and Kolte (1986) also reported low estimates of heritability for seed cotton yield per plant. If narrow sense heritability is found to be moderate/low heritability indicated that preponderanceof non-additive gene action and heterosis breeding can be exploited for improvement of such characters.

Graphical Analysis

The graphical analysis was carried out as suggested by Hayman (1954). The validity of assumptions was tested by the t2 tests for all the traits. T2 static for VrWr regression was significant for all the characters studied. Validity of assumptions was also verified by the regression value of b for Vr/Wr graph.Graphical Analysis measures the genetic diversity of the parents involved in diallel cross in terms of the proportion of dominant and recessive genes along with net direction dominance either with favourable effects or unfavorable effects. The values of vrand wr for parents are given (Table 2).

The regression line of Vr-Wr graph passes exactly the origin indicating the presence of complete dominance for days to first boll bursting. The parents Narasimha, MCU 3 and TCH 1705 were located near to the origin showing the presence of dominant genes in these lines (Fig. 1A). However, In Vr-Wr graph, the regression lines passes below the origin indicating the presence of over dominance in Number of sympodial branches per palnt, plant height and Number of bolls per plant (Fig. 1B – 1D).In Vr-Wr graph the regression lines passes above the origin indicating the presence of partial dominance for boll weight. The parents TCH 1726, MCU 13, TCH 1705 and Narasimha are located near to the origin indicating the presence of more dominant genes (Fig. 1E). However, additive type of gene action was noticed for boll weight,Shakeel et al. (2001). For lint index trait, the regression line passes below the origin indicating the presence of over dominance. The parents KC 2 and MCU 3 are located very far from origin and indicated that it contains more recessive genes for this trait (Fig. 2A). However, the regression line of the VrWr graph passes above the origin indicating the presence of partial dominance for seed index. The parent Narasimha and MCU 3 were located very far from origin and it contains more recessive genes (Fig 2B). For ginning percentage trait, regression line of the VrWr graph passes below the origin indicating the presence of over dominance. The parent BW 4-1 and MCU 13 are located near to the origin indicating the presence of more dominant genes. The parent TCH 1726 was located far from origin indicating that it contained more of recessive alleles (Fig 2C).The regression line passes below the origin showing the presence of over dominance in single plant yield. The parent BW4-1 and TCH 1726 was located far from origin indicating that it contained more of recessive alleles (Fig. 2D). It was supported by previous studies of Ajmal et al. (1998) and Shakeel et al. (2001) reported that the presence of genes shows overdominance for seed cotton yield and yield attributing traits.

For 2.5% Span length trait, the regression line passes above the origin, showing the presence of partial dominance. The parent MCU 13 and TCH 1726 was located far from the origin indicating found to contain more of recessive alleles (Fig. 3A). However, regression line passes below the origin showing the presence of over dominance for bundle strength trait. The parent KC 2, TCH 1726, TCH 1705 and BW- 4 -1 were located far from origin indicating that it contained more of recessive alleles (Fig. 3B). Furthermore, for micronaire trait, regression line passes below the origin showing the presence of over dominance. The parent MCU 13 was located near to the origin indicating the presence of more dominant genes. The parent TCH 1726 was located far from origin indicating that it contained more of recessive alleles (Fig 3C). Similar results also reported by Shimna (2004) that T2 statistic for Vr-Wr regression was significant for all the characters studied except boll weight, single plant yield and bundle strength. Similar also confirm with the findings of saravananet al. (2003) reported that parent MCU 12 had most of dominant genes for plant height and number of bolls per plant. Parent Suvin had most dominant genes for boll weight, number of seeds per boll and 2.5 per cent span length.

CONCLUSION

The present study conclude that predominance of dominance effect was observed for plant height, ginning percentage, days to first boll bursting, single plant yield, number of sympodial branches, number of bolls per plant, lint index, seed index ,2.5% span length and bundle strength and micronaire. The mean degree of dominance was found to be less than one indicating partial dominance for boll weight. The ratio $H_2/4H_1$ was less than 0.25 for all characters studied except for boll weight and seed index. The heritability in narrow sense was found high for plant height and 2.5 % span length. For single plant yield and bundle strength the parent Bw4-1 had located far from origin indicating that it contained more of recessive alleles as against ginning percentage where dominant gene was noticed by that parent. Parent Narasimha had recorded both dominant and recessive alleles for boll weight and number of bolls, respectively. The distribution of array points along the regression line for different plant traits indicated the existence of genetic variation in plant material. Thus variation could be exploited through appropriate breeding programme.

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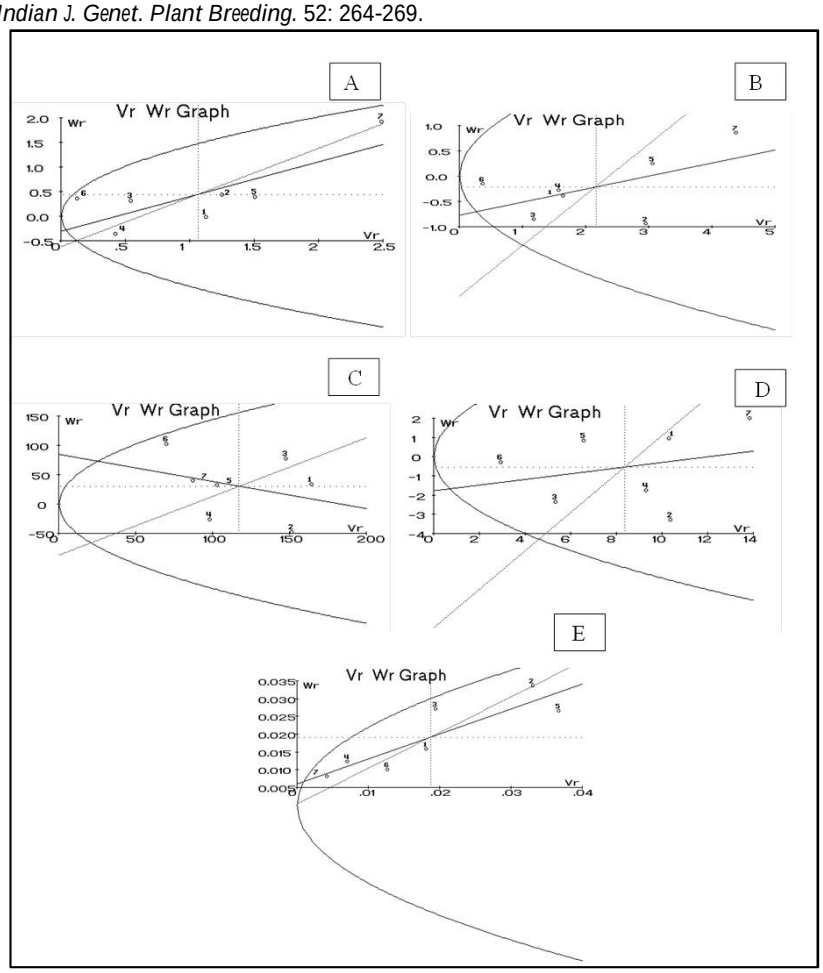

Fig. 1.Vr-Wr graph analysis for metric traits. A), Days to first boll bursting; B), Number of symbodial branch per plant; C), Plant height, D), Number of bolls per plant;and E), Boll weight.

Note: (1).Narasimha, (2). KC 2, (3). MCU 3, (4). TCH 1705, (5). BW4-1, (6). MCU 13 and (7). TCH 1726.

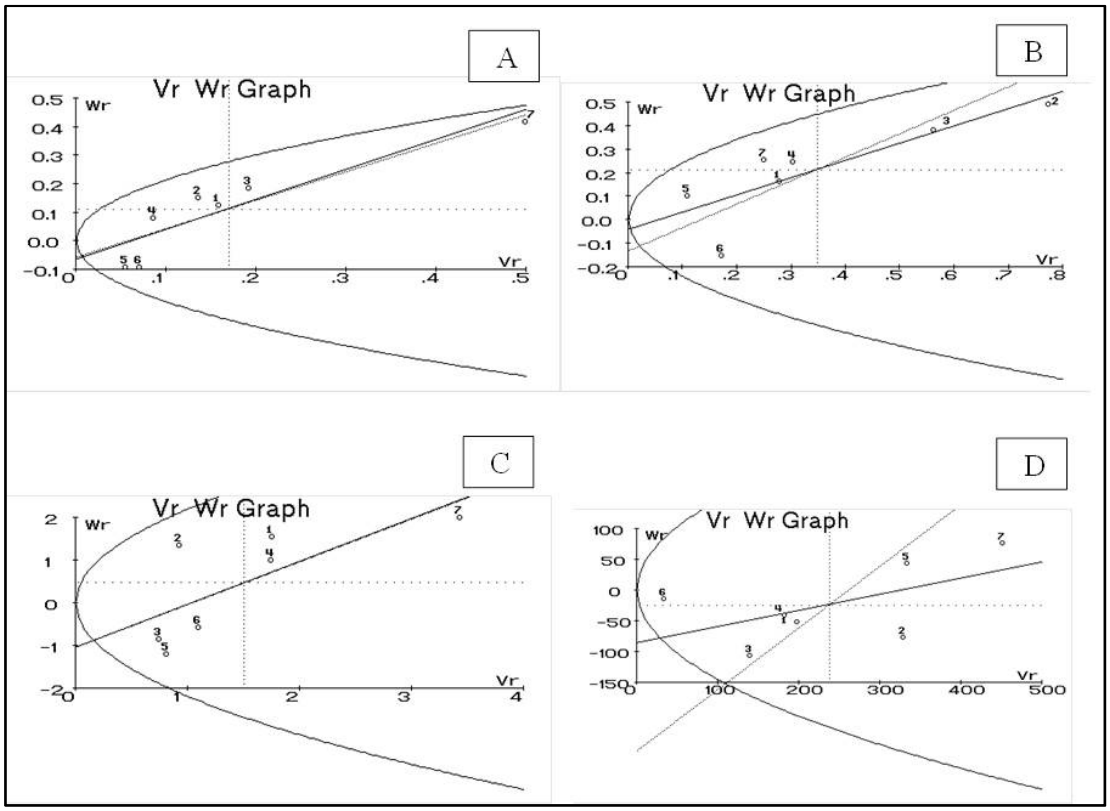

Fig. 2.Vr-Wr graph analysis for seed traits and single plant yield. A), Lint index; B), Seed index; C), Ginning percentage, and D), Single plant yield.

Note: (1).Narasimha, (2). KC 2, (3). MCU 3, (4). TCH 1705, (5). BW4-1, (6). MCU 13 and (7). TCH 1726.

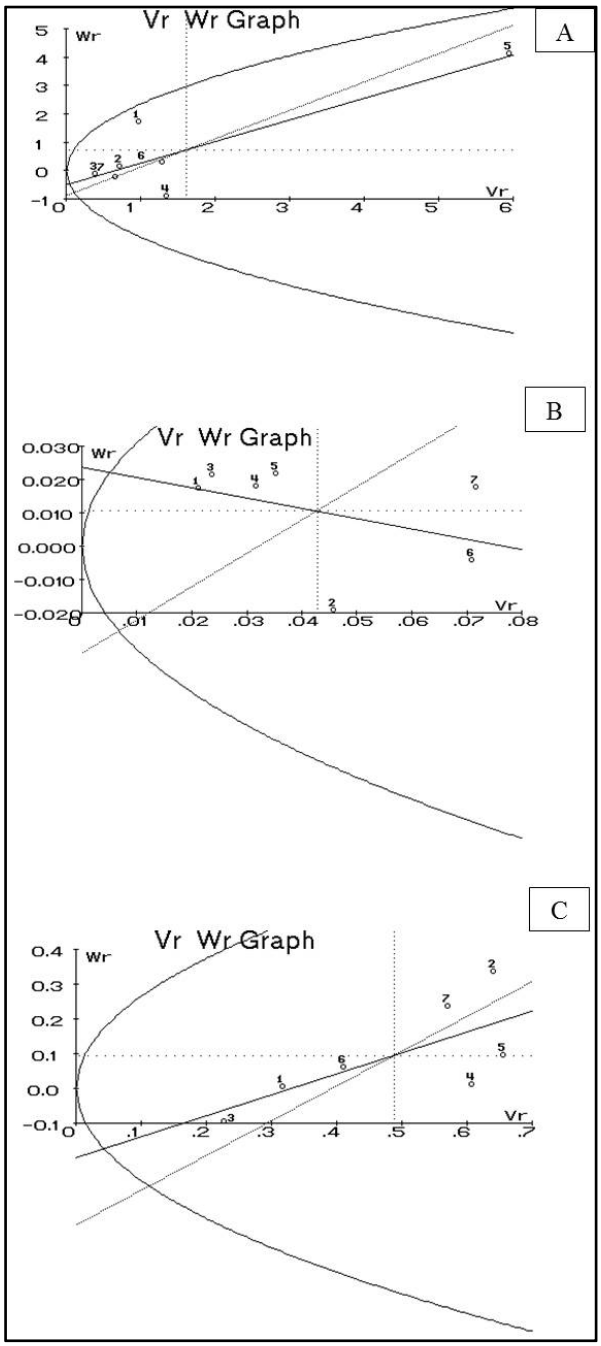

Fig. 3.Vr-Wr graph analysis forfibre quality triats. A), 2.5% span length; B), Bundle strength, and C), Micronaire value.

Note: (1).Narasimha, (2). KC 2, (3). MCU 3, (4). TCH 1705, (5). BW4-1, (6). MCU 13 and (7). TCH 1726.

Table 1. Expression of genetic parameters for yield and fibre quality characters.

Character	$(H_1/D)^{1/2}$	$H_2/4H_1$	$\frac{4DH_2)^{1/2}+F}{4DH_2)^{1/2}-F}$	h^2/H_2	Heritability in narrow sense (%)
Days to first boll bursting	1.46	0.203	2.17	0.522	58.8
Number of sympodial branches	2.74	0.172	3.18	0.202	33.4
Plant height	1.69	0.165	2.51	0.168	89.1
Number of bolls per plant	3.99	0.171	2.54	1.199	15.8
Boll weight	0.17	0.880	0.40	-2.434	30.9
Lint index	1.16	0.187	2.36	2.119	60.6
Seed index	1.25	0.254	1.04	4.699	29.7
Ginning percentage	1.34	0.14 1	4.05	0.204	75.3
Single plant yield	2.65	0.169	3.22	0.171	37.6
2.5% Span length	1.24	0.144	3.81	1.701	98.7
Bundle strength	2.02	0.130	2.18	1.521	31.7
Micronaire	1.51	0.148	3.17	-0.053	70.5

Table 2. Graphical analysis of genetic diversity of parents through expression of Vr and Wr

Character	Narasimha		KC 2		MCU 3		TCH 1705		BW 4-1		MCU 13	
	Vr	Wr	Vr	Vr	Wr	Vr	Wr	Vr	Wr	Wr	Vr	Wr
Days to first boll bursting	1.12	-0.008	1.24	0.42	-0.36	1.50	0.39	0.12	0.35	0.43	0.54	0.32
Number of sympodial branches	1.64	-0.39	2.95	1.57	1.64	3.06	2.95	0.36	0.14	-0.93	1.18	-0.84
Plant height	60.2	34.2	151.5	164.4	34.2	151.5	-48.0	147.5	77.2	-48.0	147.5	77.2
Number of bolls per plant	10.29	0.95	10.37	9.29	-1.75	6.55	0.82	2.93	-0.28	-3.27	5.32	-2.36
Boll weight	0.018	0.016	0.033	0.007	0.012	0.037	0.027	0.013	0.010	0.034	0.019	0.027
Lint index	0.15	0.12	0.13	0.086	0.081	0.055	-0.091	0.070	-0.091	0.15	0.19	0.18
Seed index	0.27	0.16	0.77	0.30	0.24	0.10	0.10	0.17	-0.15	0.49	0.56	0.38
Ginning percentage	-2.77	0.002	5.44	1.74	0.99	0.81	-1.20	1.09	-0.58	0.07	0.57	0.032
Single plant yield	198.0	-52.1	328.3	181.5	39.9	322.8	43.8	32.9	-14.2	-76.9	138.7	-106.8
2.5% span length	0.97	1.74	0.71	1.34	-0.89	5.94	4.15	1.28	0.32	0.15	0.38	-0.11
Tenacity	0.31	0.007	0.64	0.607	0.011	0.65	0.097	0.41	0.063	0.33	0.22	-0.093
Micronaire	0.021	0.017	0.04	0.032	0.018	0.035	0.022	0.071	-0.004	-0.019	0.024	0.021

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RESEARCH ARTICLE

Effect of Fungicide Cumine Phenol on Three Isolated Fungal Species in Leather Manufacturing

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ABSTRACT

Leather is a natural product which gets decomposed overtime, by the airborne bacterial or fungal spores present everywhere. These microorganisms quickly colonize and cause increased biodeterioration of leather. The aim of the present study is to isolate, standardize and investigate the antifungal efficacy of fungicide cumine phenol on various fungal species of vegetable tanned leather.Various application studies were also performed to study the effect of fungicide on infected leather. *Aspergillus niger*, *Aspergillus flavus* and are the three isolated fungal species which were used for the present study. Physical properties of the leather were also tested in all the three isolated fungal species.Antifungal susceptibility and the Minimum Inhibitory Concentration (MIC) of cumine phenol against *Aspergillus niger* and *Trichophyton rubrum* was found to be 40 µl and against *Aspergillus flavus* was found to be 80 µl. Application study conducted showed that 0.05% of cumine phenol is sufficient to control the growth of isolated fungal species on leather. The studies on physical properties showed moderate leather biodeterioration on fungal attack.The results obtained from the present study showed that cumine phenol can be use as an effective fungicide in protection against fungal attack of all three isolated species.

Keywords: *Aspergillus flavus, Aspergillus niger,* fungicide, leather manufacturing, *Trichophyton rubrum*.

INTRODUCTION

Leather industry, leads the world economy and it has occupied a unique place in terms of its distinct ability towards employment, massive growth as well as exports. The tremendous growth of this industry is primarily due to its planned development in utilizing the optimal availability of raw materials to maximize its returns from exports. India, a developing nation is fortunate in being a storehouse of good raw hide base but they are susceptible to aggressive attack of fungi which results in biodeterioration of leather during its manufacture, finishing and in storage[1, 2]. The microbial deterioration of leather during manufacturing depends on various factors such as pH values, temperature, duration taken for the process, and varying compositions of chemicals used [3, 4]. Among various types of leather, the vegetable tanned leather is found to be more susceptible to fungal attack when compared with that of chrome tanned leather [5].Various precautionary measures taken during tanning process has failed to prevent damage in leather. The acidic and basic properties of skin expose them to damage by bacteria or fungi. The bacteria degrades leather in the initial process of production such as pre-soaking and main soaking while the fungi undergoes degradation during the pickling, tanning and post-tanning processes [6] .Wet leathers are susceptible to fungal attack unless they are effectively protected. The most suited conditions which represent the growth of fungi are temperature range of 20-30, with humidity ranging from 65-80% and with slightly acidic pH i.e. around 5.5. Various type of fungi are present in deteriorated leather which includes the most frequently identified species such as *Aspergillus niger* and *Penicillium* while some other species includes *Curvularia, Fusarium, Cladosporium, Verticillium, Cephalosporium and Scopulariopsis species* [7, 8].

Damaging effects of fungi on leather leads to significant reduction in terms of costs such as during staining, lack of non-uniformity during processing, physical properties changes which results in decreased satisfaction of customers. [9]. In order to provide necessary protection to leather from the attack of fungi during the storage and export, the use of fungicides could to more beneficial. Fungicides are referred to as the chemical substance which is used in leather industry to inhibit the fungal growth that causes degradation of leather. Fungicides have become the primary means of fungal control in leather industry due to its cost effectiveness and ease of use [10].Fungicides which are used in leather industry are classified under two main categories i.e. phenolics and heterocylics. Phenolics includes 4-chloro-3-methylphenol (PCMC) and 2-phenylphenol (OPP), heterocyclics includes 2-(thiocyanomethylthio)-benzothiazole (TCMTB), 2-Octyl-3(2H)-isothiazolone (OIT) and 2-mercaptobenzothiazol (MBT) [11]. In the present study three different isolated fungal species from leather industry were used. The fungal species used includes *Aspergillus niger, Aspergillus flavus and Trichophyton rubrum.* Hence the aim of the current study is to investigate the antifungal efficacy of cumine phenol on the above three isolated fungal species and to perform various application studies of the fungicide on the infected leather.

MATERIALS AND METHODS

Leather-for sourcing microorganisms

The microorganisms (fungal species) were isolated from the Mildew attacked East India (EI) Cow calf leather obtained from the leather industry.

Haemocytometer

Haemocytometer was used for counting the spore suspension to know the number of spores.

EI leather-for application study

Vegetable tanned (EI) cow calf leather tanned using Wattle and Myrabolan were used as a substrate for the application study of the various fungicides (to study the extent, degree and pattern of the inhibition of the identified fungal species).

Fungicide

Cumine Phenol

Known concentration of cumine phenol was taken and dissolved in sterile distilled water. Various concentrations (10 µl, 20 µl, 40 µl, 80 µl, 100 µl, 200 µl) were prepared by serial dilution method.

Media used

Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar (SDA) medium consists of 10 g of Peptone, 40 g of dextrose, 15 g of agar in 1000 ml of distilled water. The ingredients are mixed well and the pH was adjusted to 5.6. The agar was dissolved by boiling followed sterilization at 121 for 10 minutes.

Aspergillus flavus / parasiticus agar

The ingredients present in the medium includes 20 g of yeast extract, 10 g of peptone, 0.5 g of ferric ammonium citrate, 15 g of agar and 0.02 mg dichloran dissolved in 100 ml of distilled water and sterilized at 121 for 15 minutes.

Urease medium

About 2.9 g of urea agar base was dissolved in 10 ml of distilled water, sterilized and filtered. To that add and dissolve 1.5 g of agar in 90 ml of distilled water. Sterilize the contents by autoclaving at 15 lb/in^2 for 15 minutes. Then the urea agar base was added to the cooling agar at about 50 and mixed well in aseptic condition. This was dispensed in the sterile tubes as slant.

Corn meal agar

2 g of corn meal was heated with 50 ml of distilled water and maintained at 60 for 30 minutes. The other ingredients such as 2 g of peptone, 2 g of dextrose, 1.5 g of agar were mixed and dissolved in 50 ml of distilled water separately and mixed with the cornmeal extract and made upto 100 ml. Then the medium was autoclaved at 121 for 15 minutes.

Reagents used

Lactophenol cotton blue stain

The ingredients of the stain consist of 20 ml of lactic acid, 20g of phenol crystals, 40 ml of glycerol, 0.05 g of cotton blue. Phenol was dissolved in lactic acid, glycerol and water gently by heating. Then the cotton blue was added and mixed well.

Materials for application study

Cow calf leathers were collected for studies which do not have any preservative, biocide or any other fungicides.

Methods

Isolation of fungal species from mildew attacked leather

Mildew attacked leather sample (1 g) was cut into small pieces and transferred into 100 ml of sterile 0.85% saline solution. [9] The samples and the saline solution were agitated for 4 hours at 25 in a lab line orbital environment shaker incubator in order to extract the fungi. 1 ml portion of the extract was inoculated on SDA in a petridish. This was held for 4-7 days at 25 for complete sporulation. Fungal isolates were purified and pure cultures were maintained of SDA slants. Then the fungi were identified. The sterilized SDA media was poured on the sterile petriplates in aseptic condition. Then the media was allowed to solidify. After solidification, the fungal species that had grown on the EI leather was scrapped with the help of inoculating needle and was directly inoculated on the surface of primary isolation agar (SDA). Then the inoculated plates were incubated at 25 to 30 for complete sporulation.

Slide culture technique-for morphology study

This technique will enhance the fungi to grow and to adhere to the glass slide and the cover slip so that the organism can be examined without disturbing, distorting and disrupting the various characteristic structures [12]. In a glass petridish a V shaped bent glass rod, a microscopic glass slide, and two cover slips are placed and wrapped and then sterilized in a hot air oven at 160 for 1 hour. From the plates of SDA, a block of agar was cut with sterilized scalpel and it was transferred to the centre of the sterile glass slide. With an inoculation needle, the mold culture was inoculated along the four sides of agar block. A sterile coverslip was placed over the agar block with mild pressure. About 10 ml of sterile distilled water was poured into the petridish, which was then covered and incubated at room temperature for about 4 to 7 days. When the sporulation was developed, the coverslip was carefully removed with forceps and placed on glass slide containing a drop of lactophenol cotton blue. With forceps, agar block was tapped from the original slide into a container with antifungal disinfectant. A drop of lactophenol cotton blue and coverslip was placed on the slide. Slide was observed under microscope.

Antifungal susceptibility test

Inoculum preparation

The isolates from the mother culture were sub cultured on SDA slants [13]. Isolates of all species were incubated at room temperature for 3 to 5 days. Inoculum suspensions were prepared from the fresh mature cultures grown on SDA slants. The colonies were covered with 5 ml of sterile 0.85% NaCl to each tube, and the surface was rubbed with a sterile applicator stick, and the suspension was transferred to a sterile tube. The spore suspension was vigorously shaken for 15 to 20 seconds in a vortex mixer. The prepared inoculum was filtered through one or more layers of muslin cloth depending on the size and morphology of conidia to remove the majority of hyphae. Then the filtrate was used as inoculum.

Inoculum standardization

The prepared spore suspension was quantitatively measured by direct microscopic count using counting chamber [14]. A drop of fungal spore suspension was put into the counting chamber. Number of cells was counted and the total cell number was determined. A drop of conidial suspension was placed on the engraved grid and the

preparation was allowed to stand for 1 to 2 minutes to settle the conidia at the bottom. The cover glass was placed over the grid carefully so that no air bubble enters between the slide and cover glass. The smaller size spores were counted in the middle square (E) which consists of 25 groups of 16 small squares, each group 0.2 mm square. The larger spores or fewer spores are counted in 4 corners of large squares (A, B, C, D) and in the middle one (E) to have a total count of 200 to 250 (i.e. 20 to 25 spores/square).

Calculation

- For small spores:

Spores/ml = number of spores counted on the middle square of the grid x 10000 v spores per cm^3 [14].

- For larger spores:

Spores/ml = (A+B+C+D+E) / (5) x 10^4 spores per cm^3

Aspergillus niger

Spores = (559 + 495 + 477 + 417 + 507)/5 x 10^4

1 ml = 4.91 x 106 spores per cm^3

Aspergillus flavus

Spores/ml = (572 + 561 + 516 + 532 + 488)/5 x 10^4

= 525 x 10^4

1 ml = 5.25 x 10^6 spores per cm^3

Trichophyton rubrum

Spores/ml = (288 + 320 + 296 + 262 + 275)/5 x 10^4

= 288 x 10^4

1 m = 2.8 x 10^6 spores per cm^3

Assessment of antifungal susceptibility

The fungicides were serially diluted in the sterile distilled water and prepared in various concentrations (10 µl, 20 µl, 40 µl, 80 µl, 100 µl, 200 µl) [15]. It was added to the respective tubes containing SDA (45 to 50) and then it was mixed well. After thorough mixing, the media was poured into sterile petriplates. It was allowed to solidify. After solidification the known volume of spore suspension (5 µl) was added to the medium. Using L rod the spore suspension was uniformly spreaded over the surface of the medium. The inoculated plates were incubated for 2 to 5 days and examined for every 24 hrs. The concentration of antifungal agent that exhibited no growth is the MIC.

Application of fungicides on leather

Four EI cow calf leathers were taken. No preservative or fungicide or biocide had been administered during the manufacturing of the EI leathers. Each leather is marked as 1, 2, 3, 4 and cut along the back cone. The four right halves are the control leathers, where no fungicide is administered. Four left halves were treated with varying amounts of the fungicides with 100% water in drum for 60 minutes. The leathers (both controlled and experimented) were dried and inoculated with fungal spores. They were incubated at room temperature for 30 days.

Physical testing of leather samples

Samples of standard dimension of various physical tests were obtained as per international union of leather technology chemists society (IULTCS) methods. The leather specimen was conditioned at 200c and 65 ± 2% relative humidity over a period of 48 hours. The physical properties such as tensile strength, tongue tear strength and stitch tear strength were then investigated with the help of instruments called Instran.

RESULTS AND DISCUSSION

Leathers are highly susceptible to airborne spores and the fungal growth can occur within few days when it is not protected in a safer way. Increased fungal growth results in short term allergenic response. Even some strains of fungi under conditions can produce mycotoxins which will further result in severe long term consequences [9]. The microorganism affects the raw skin resulting in damaging effects and it decreases the quality and yield of leather as well. The major signs of biodeteriorated leather are reduction in surface area, colour of the flesh changes, putrefactive odour occurs, reduction in hair root bonding, pigmentation occurs, reduction of mechanical strength and hairslip occurs following exfoliation [16].

Isolation and identification of fungal species from vegetable tanned leathers

Three different fungal species were isolated from different sources of vegetable tanned (EI) leathers obtained from the leather industries. The isolated organisms were inoculated in SDA medium, and designated as J_1, J_2, and J_3.

Aspergillus niger

Fig. 1 show the macroscopic pictures of *Aspergillus niger* which was designated as J_1 and later confirmed. Cultural and morphological characteristics were studied for the isolated organisms to ascertain the species. Table 1.a represents the culture and morphological characters of *Aspergillus niger*. The microscopic observation of *Aspergillus niger* under 40X is depicted in fig. 2.

Aspergillus flavus

The organism *Aspergillus flavus* species (J_2) was inoculated in *Aspergillus flavus*/Parasiticus Agar (AFPA) medium. The presence of growth in this organism was observed by the yellow orange pigmentation on the reverse of the media and it was confirmed by the absence of pigmentation in negative control. Culture and morphological characteristics of *Aspergillus flavus* was well depicted in table 1.b. Fig. 3 shows the microscopic observation of *Aspergillus flavus* under 40X. The confirmation of the species *Aspergillus flavus* was represented in fig. 4.

Trichophyton rubrum

Trichophyton rubrum species was inoculated in urease agar medium and designated as J_3. The observation of red colour pigmentation (Fig. 5) which was weakly positive confirmed the presence of *Trichophyton rubrum.* Table 1.c shows the cultural and morphological characteristics of *Trichophyton rubrum.*Increased fat content in leather is positively correlated with relative decrease in water content which makes the leather resistant to action of various microorganisms [17]. Absence of sufficient quantities of biotin and vitamin B2 results in drematitis and hair loss in skin which results in increased microbial penetration in leather. The microorganisms isolated from deteriorated leather may be bacteria like *Bacillus mesentericus* and presence of some fungal species such as *Aspergillus niger, Penicillium chrysogenum, Penicillium cyclopium* [18].

Standardization of isolated organisms

The inoculum of three isolated fungal species had been standardized using haemocytometer (Neubaur chamber) following the procedure which was described earlier. The results of standardization of isolated fungal species were depicted in table 2. The standardized and adjusted inoculum for *Aspergillus niger* was found to be 4.91 x 10^6 spores/cm^3, 5.25 x 10^6 spores/cm^3 for *Aspergillus flavus* and 2.8 x 10^6 spores/cm^3 for *Trichophyton rubrum*. From the above, about 5 µl of standardized inoculum was taken for antifungal tests of selected fungicides.

Inhibition study

Inhibition study was performed on commercially obtained cumine phenol to test antifungal susceptibility. The result of antifungal susceptibility is presented in table 3. The MIC of cumine phenol was depicted in fig. 6a, b, c. The MIC of cumine phenol against *Aspergillus niger* was found to be 40 µl among the concentrations of 10, 20, 40, 80 and 100 µl. The MIC of cumine phenol against *Aspergillus flavus* was found to be 80 µl among the concentrations of 10, 20, 40, 80 and 100 µl. The MIC of cumine phenol against *Trichophyton rubrum* was found to be 20 µl among the concentrations of 10, 20, 40, 80 and 100 µl.The antifungal tests were done and in accordance with the results obtained we conducted application studies. The samples are taken from each fungicide and four experiments had been conducted. Varying concentrations of fungicides had been offered to the EI cow calf leathers. The concentrations used for the present study were 0.05%, 0.1%, 0.15% and 0.2% based on the weight of the EI leathers which were used in the experiments. After treatment of leather with varying concentrations of three fungicides, they were also inoculated with isolated fungal species independently. The leathers were inoculated and incubated for a period of 30 days. The growth of the organisms was observed. Fig. 7 shows the leathers treated with selected fungicides. The results of the application studies conducted were represented in table 4. It is evident from all these experimental findings that 0.05% cumine phenol is sufficient to control the growth of the isolated representative fungal species on leather. Phenolic compounds are found to inhibit the bacterial growth and activity of protease resulting in damaging effects of cell wall and its cytoplasm [19]. This damaging effect of phenolics results in destruction of the vegetative structure of bacteria and it slows down the growth of the spores [20].

The decrease in leather tissue density is closely associated with decrease in collagen density and denser packing of structural elements. The decrease in leather tissue density has resulted from increased duration of microbiological impact on leather tissue of raw hide and tanned leather [21].The leather which were inoculated and treated was then tested to understand the physical characteristics of the leathers. The physical properties of the experimental leathers and their corresponding control leathers were compared. The result shows the pattern and damage of the leather that is caused by isolated fungal species if they are not treated with suitable fungicide. Table 5 represents the physical characteristics of the fungal damaged leathers exhibited a moderated deterioration which was not very significant. If the damage persists, then the deterioration caused by isolated fungal species would be very significant. The increased growth of mould leads to loss of lipids and biodeterioration of leather resulting in reduced physical strength of leather[22].Finished leather products which are good sources of foreign exchange are prone to high risk of fungal attack even during the storage conditions. From the studies carried out the fungicide cumine phenol was found to be better in inhibiting the fungal growth in finished leather. The physical properties picturized a *moderate* deterioration of leather but if it persists can lead to serious damage in leather. Although present study provides supportive evidence of using cumine phenol as an effective fungicide, conducting further research will help in standardizing the application process.

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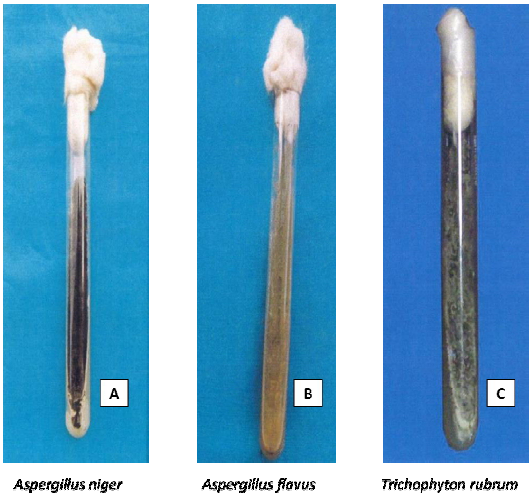


Figure 1. Macroscopic appearance of isolated fungi.

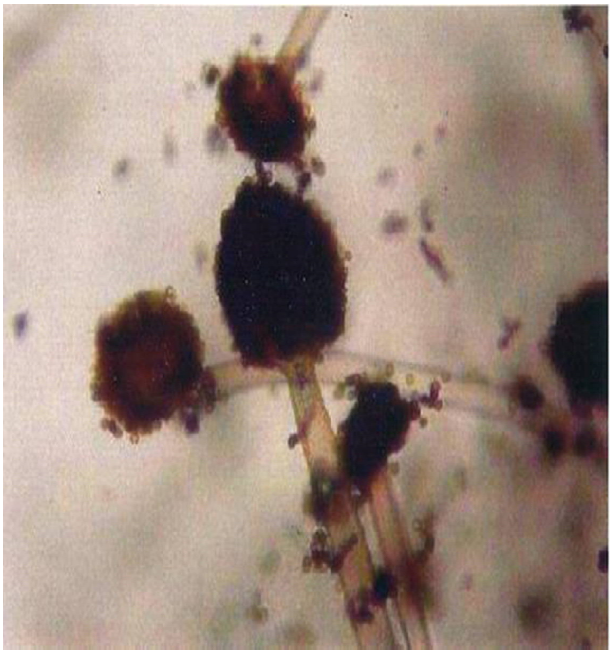
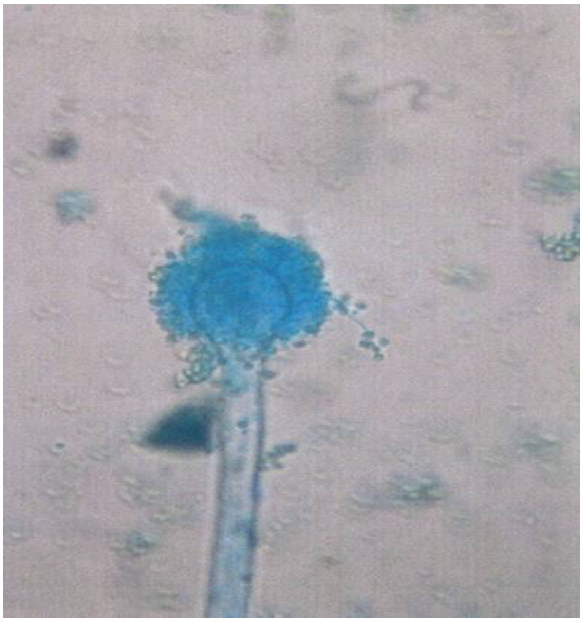


Figure 2. Microscopic observation of *Aspergillus niger* under 40X magnification.



Microscopic observation under 40X

Figure 3. Microscopic observation of *Aspergillus flavus* under 40X magnification.

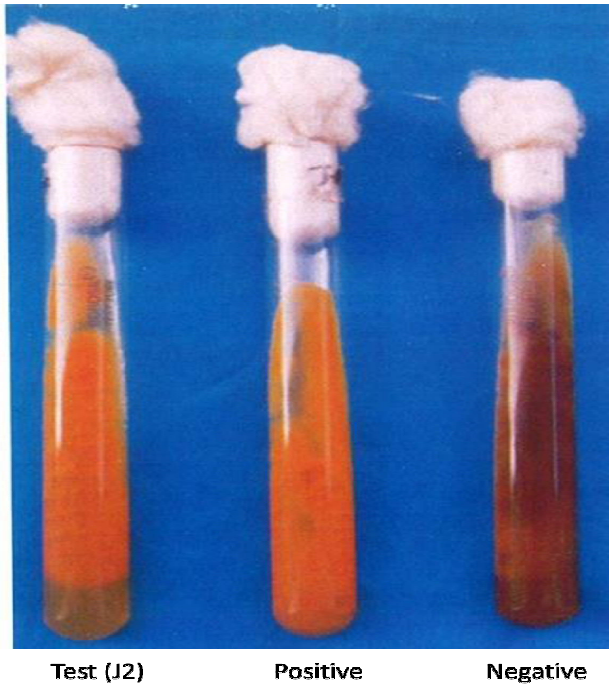


Figure 4. Confirmatory test for *Aspergillus flavus* in AFPA medium.

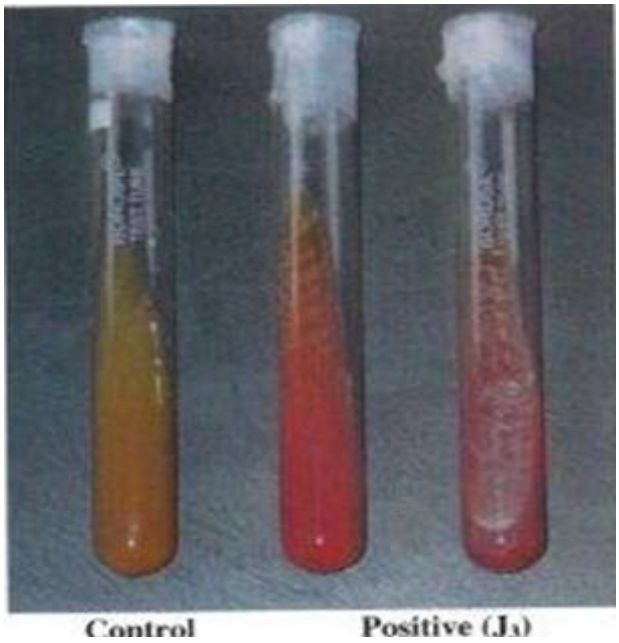


Figure 5. Confirmatory test for *Trichophyton rubrum* in Urease agar medium

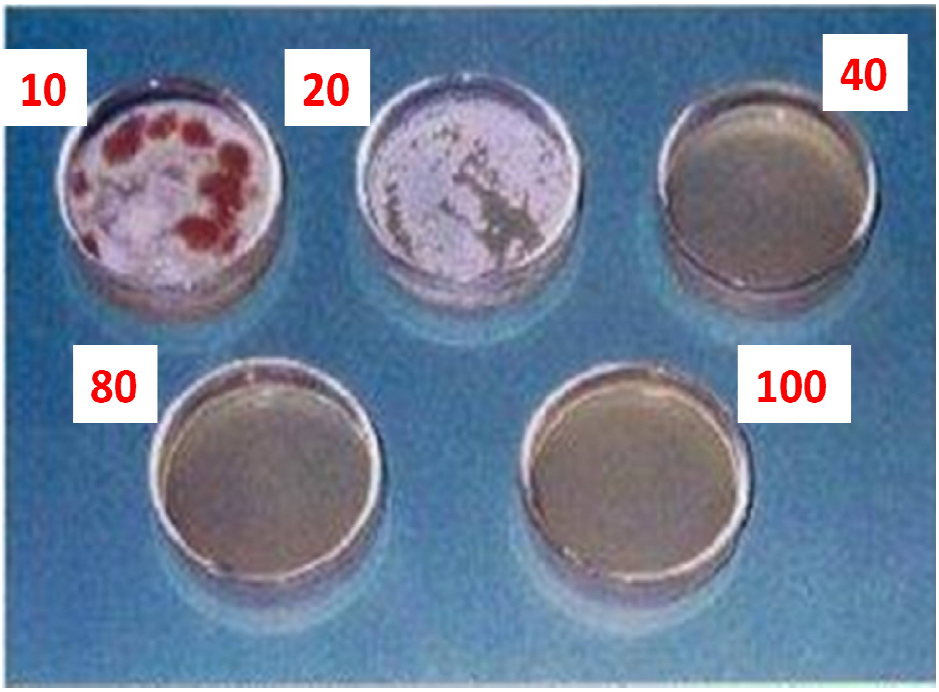


Fig 6a

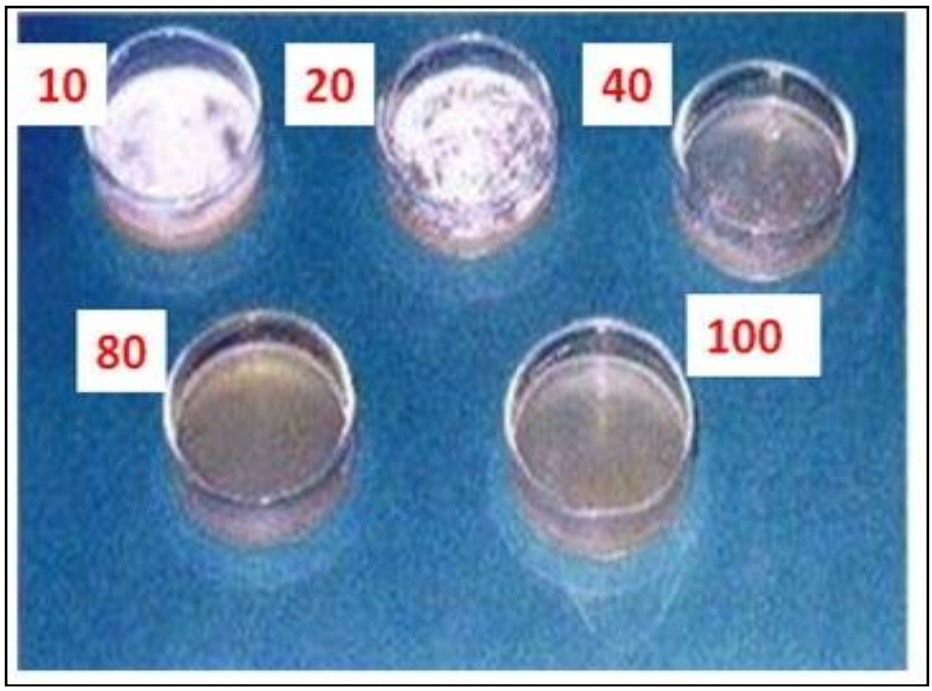


Fig.6b

Fig.6c

Figure 6.(a,b,c) MIC of cumine phenol against three isolated fungal species.

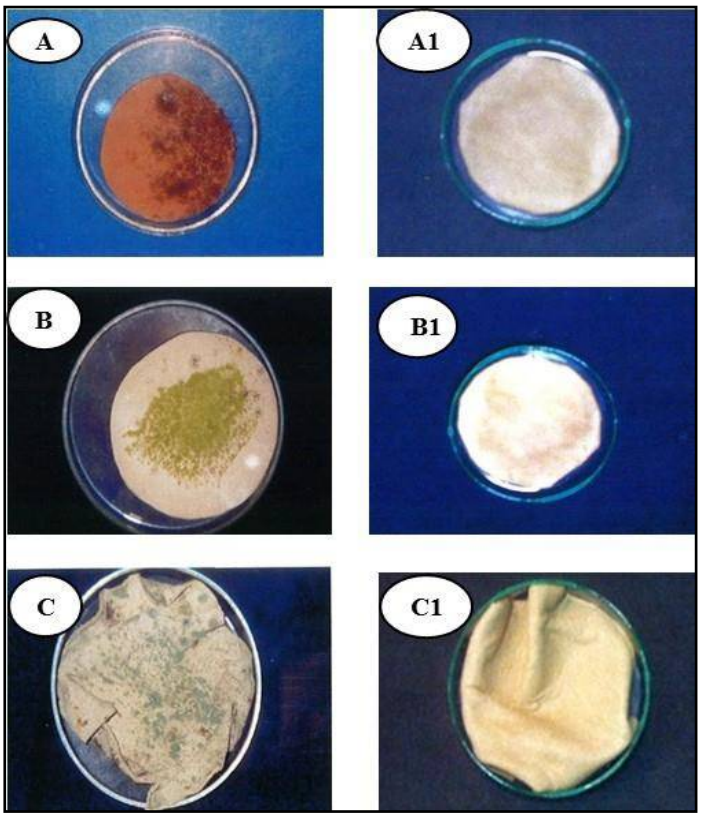


Figure 7. The leather treated with fungicide cumine phenol.

Table 1.A.Identification of Fungal Species *Aspergillus niger*

Species isolated	Characteristics		Species Inferred
	Macroscopic	**Microscopic**	
J1	• Media was covered with white fluffy mycelia when immature • On maturation colonies exhibit "salt and pepper" effect • Covered with black spores • Reverse of the colony was buff colour	• Conidial head were large black to brownish black • Conidial head appear globose • Conidiophores was brownish near the vesicle • Vesicle had a concave under the surface • Brownish sterigmata was produced in two series • Primary sterigmata were long • Secondary sterigmata were short • Conidia were globose and echinulate	*Aspergillus niger*

Table 1.B. Identification of Fungal Species *Aspergillus flavus*

Species isolated	Characteristics		Species Inferred
	Macroscopic	Microscopic	
J2	• Colonies were yellowish green in colour	• Conidial head were radiate and loosely columnar • Conidiophore was hyaline and thick walled • Vesicle were elliptical • Sterigmata were covered entire surface of the vesicle • Monoseriate sterigmata were seen • Primary and secondary sterigmata were nearly equal in size • Conidia were elliptical and echinulate • Conidia appeared yellow green	*Aspergillus flavus*

Table 1.C. Identification of Fungal Species *Trichophyton rubrum*

Species isolated	Characteristics		Species Inferred
	Macroscopic	Microscopic	
J3	• Surface is granular or fluffy, white to buff • Reverse is deep red or purplish; occasionally it is brown, yellow orange, or even colourless • The pigment production is best seen on cornmeal dextrose agar • Reverse of the colony was buff colour	• Clavate tear drop microconidia were seen along the sides of the hyphae • Long, septate hyphae were seen • Pencil shaped macroconidia were seen • Conidia were elliptical and ectinulate	*Trichophyton rubrum*

Table 2 Standardization of Isolated Fungal Species

S. No.	Fungal species isolated	Spores/ml (spores/cm^3)
1	*Aspergillus niger*	4.91 x 10^6
2	*Aspergillus flavus*	5.25 x 10^6
3	*Trichophyton rubrum*	2.8 x 10^6

Table 3 Antifungal Susceptibility of Cumine Phenol

Fungicide	Concentration (9 ml of distilled water + 1 ml of fungicide) in µl	*Aspergillus niger*	*Aspergillus flavus*	*Trichophyton rubrum*
Cumine Phenol	10	+	+	+
	20	+	+	-
	40	-	+	-
	80	-	-	-
	100	-	-	-

Table 4: Results of Applications Studies

Sample	Fungal growth			
	0.05%	0.1%	0.15%	0.2%
Cumine Phenol control	+	+	+	+
Cumine Phenol experiment	-	-	-	-

Table 5: Physical Characteristics of Leather

Sample	Physical characteristics		
	Tensile strength (kg/cm2)	Tongue tear strength (kg/cm)	Stitch tear strength (kg/cm)
Sample treated with Cumine phenol	240	54	90
Sample not treated with Cumine phenol	220	52	87
% change	-8.3	-3.7	-3.4

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RESEARCH ARTICLE

Phytochemical Screening of Bark Extracts of *Gmelina arborea* Roxb.

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ABSTRACT

The prime step in detection of bioactive principles is screening of phytochemicals present in the plants which may lead to novel drug discovery. In the present study the phytoconstituents of the bark of "*Gmelina arborea* Roxb." belonging to family Verbenaceae is investigated. Screening of the plants was performed using standard methods. Phytochemical analysis confirmed the presence of alkaloids, tannin, glycosides, saponins, phenolic, flavonoids, flavon glycosides, cardiac glycosides in leaves. The presence of these phytochemicals can be correlated with medicinal potential of these plants. Further studies can be carried out with these barks to assess the pharmacological potentials for their medicinal values.

Keywords: Phytochemical Screening, *Gmelina Arborea*, Bark Extract, Verbenaceae

INTRODUCTION

Plants have been used as medicine by the mankind for the treatment of different types of ailments from time immemorial and also in several developed and developing country [1-3]. Plants and plant derived products are the main source of medicine in the past and also present centuries [4]. Even today, considerable attention has been paid to utilize plant based products for the prevention and treatment of different human diseases [5]. The general communities are familiar with their medicinal value as a source of new and complimentary medicines owing to their adaptable applications [6]. During the last decades, there is an increasing interest to unlock the secrets of ancient herbal remedies. The drugs obtained from the medicinal plant parts viz leaves, stem, bark and roots are cheap, secure, and efficient and without side effects as compare to synthetic drugs. The importance of medicinal plants in the management of human ailments cannot be overemphasized [7-8].*Gmelina arborea* Roxb. belongs to the family Verbenaceae is a deciduous tree grows to a height of 12 to 30 m and grows preferably in moist fertile area. The root

decoction is used in the ancient medicine to treat abdominal tumors. They are also used in folk remedy for anthrax, bites, blood disorders, cholera, colic, convulsions, diarrhea, dyspepsia, fever, gout, smallpox, snakebite, swelling and urticarial [9].Local healers recommend *Gmelina arborea* for treatment of various diseases such as alopecia, anemia, leprosy, thirst and vaginal discharges and leprosy. The root of the plant is used as laxative, stomachic, colic and in urinary discharges. The extract form of different parts of *G. arborea* has been proven scientifically for its Analgesic [10], Antidiabetic [11], Antimicrobial [12], Antioxidant [13] and wound healing activities [14].

Collection and Identification of Plant Material

The barks of GA were collected from south India, Kanyakumari district during the month of January and February. The plant was identified by S. Balasubramanium, ABS Botanical Garden, Salem.

Extract Preparation and Phytochemical Screening

The freshly collected barks were dried in shade, then coarsely powdered. For extraction of crude phytochemical, 25 g of powdered bark material was kept in closed conical flask with 20 mL various solvents like petroleum ether, benzene, chloroform, ethanol, acetone, ethyl acetate and distilled water in a shaker at room temperature for 24 h. After incubation, the extracts were filtered and the extracts were collected and stored in the refrigerator at 4^{o}C for further studies. All the extracts were subjected to preliminary phytochemical screening as per the methods given by Harborne [15].

Preliminary Phytochemical Screening

Qualitative phytochemical Screening of bark Extract of *Gmelina arborea*

The extracts obtained as above were then subjected to qualitative tests for the identification of various plant constituents.

Detection of Carbohydrates

A minimum amount of extracts were suspended in 5ml of distilled water. The suspension was subjected to General test, Starch test, Barfoed's test, Molisch's test, Fehling's test, Benedict's test, Iodine test as seen below.

a) General Test

The extracts were treated with a few ml of distilled water and sulphuric acid. Formation of dull violet precipitate indicates the presence of reducing sugar.

b) Starch Test

Aqueous extracts were treated with 5ml of 5% potassium hydroxide. Canary colored solution shows the presence of starch.

c) Barfoed's test

Aqueous extracts were treated with 1ml of Barfoed's reagent. The solutions were heated in a beaker of boiling water bath gives a red precipitate indicates the presence of reducing sugar.

d) Molisch's Test

The extracts were treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of purple ring between two layers shows the presence of carbohydrates.

e) Fehling's Test

The extracts were treated with Fehling's A and B solution and heated for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

f) Benedict's Test

The extracts were treated with Benedict's reagent and heated for few minutes. Formation of red precipitate shows the presence of reducing sugar.

g) Iodine Test

Add a few drops of iodine solution to 1ml of the extract. Formation of deep blue color indicates the presence of starch.

Detection of Glycosides

Minimum quantities of the extracts were hydrolyzed with hydrochloric acid for few minutes on a water bath and the hydrolyzate was subjected to Legal's test, Bontrager's test, Ferric Chloride's test as seen below.

a) Legal's Test

To the hydrolyzate 1ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide. The pink color changes in to red show the presence of glycosides.

b) Borntrager's Test

Hydrozylate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The pink color changes in to red show the presence of glycosides.

c) Ferric chloride Test

2ml of extracts were treated with 1ml of glacial acetic acid and 1ml of ferric chloride. Also add few drops of concentrated sulphuric acid. Formation of blue color showed the presence of glycosides.

Detection of Proteins and Amino Acids

A small quantity of extract was dissolved in few ml of water and they were subjected to Million's test, Ninhydrin test, Biuret test as given below.

a) Million's Test

The extracts were treated with Millon's reagent. The precipitate was formed with the extract, which shows the presence of proteins.

b) Ninhydrin test

The extracts were treated with Ninhydrin reagent. The purple color was formed with extract, which shows the presence of proteins.

c) Biuret Test

To the extracts equal volume of 5% sodium hydroxide solution and 1% copper solutions were added. A violet color formation indicates the presence of amino acids.

Detection of Fixed Oils and Fats

A small quantity of extract was subjected to Spot test, Saponification test as follows.

a) Spot Test

Small quantities of extracts were placed between two filter papers. The production of stains with alcoholic extract shows the presence of fats and fixed oils in the extract.

b) Saponification Test

Few drops of 0.5N alcoholic potassium hydroxide was added to the extracts with few drops on phenolphthalein solution. Later the mixture was heated on a water bath for 1-2 hours. The soap formation indicates the presence of fat and fixed oils in the alcoholic extracts.

Detection of Alkaloids

A small quantity of the extracts were treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid reagent such as Mayer's reagent, Dragondroff's reagent, Hager's reagent, Wagner's reagent and subjected to potassium dichromate test as given below.
The filtrate was tested with alkaloid reagent such as:

1) Mayer's reagent (Cream precipitate)
2) Dragondroff's reagent (Reddish brown precipitate)
3) Hager's reagent (Yellow precipitate)
4) Wagner's reagent (Reddish brown precipitate)
5) Potassium dichromate Test

The extracts were treated with concentrated sulphuric acid and add small amount of potassium dichromate. No color change indicates the presence of indole alkaloid.

Detection of Flavanoids

i) Ferric chloride Test

Aqueous extracts were treated with few drops of 10% ferric chloride. Formation of green precipitate indicates the presence of Flavanoids.

ii) Lead acetate Test

Aqueous extracts were treated with few ml of 10% lead acetate. Buff colored solution formation indicates the presence of Flavanoids

.

iii) Test for Anthraquinone

a) 0.5gm of extract was treated with 10ml of sulphuric acid. This solution was boiled and filtered while hot. From the filtrate add 5ml of chloroform. Pipetted out the formed chloroform layer add 1ml of dilute ammonia. No color change indicates the presence of Anthraquinone.

b) 0.5gm of Anthroquinone added a drop of benzene and ammonia. Formation of pink color indicates the presence of Anthraquinone.

iv) Test for Catechins

A drop of Erlich's reagent was added to the 0.5gm of extracts. Formation of pink color indicates the presence of catechins.

v) Test for Anthocyanin

2ml of plant extracts were treated with 1ml of 2M NaoH and heated for 5 min. Formation of yellow color indicates the presence of Anthocyanin.

Detection of Phytosterols

Small quantities of extracts were suspended in 5ml of chloroform separately. The above obtained chloroform solution was subjected to Libermann Burchard test, Salkowski test as given below.

a) Libermann Burchard Test

The above prepared chloroform solutions were treated with few drops of concentrated sulphuric acid. A bluish green solution indicates the presence of phytosterols.

b) Salkowski Test

To the above prepared chloroform solutions, a few drops of concentrated sulphuric acid were added. Formation of brown ring with chloroform extract indicates the presence of phytosterols.

Detection of Tannins- Phenolic Compounds

All the extracts were dissolved or suspended separately in minimum amount of water and filtered. The filtrate was subjected to General test, Ferric Chloride test, Lead acetate test and Phlonatannins as given below.

a) General Test

Plant extracts were treated with a few drops of sulphuric acid and 1 drop of 5% HCl. Formation of green color indicates the presence of tannins.

b) Ferric chloride Test

To the filtrates few drops of ferric chloride was added. Violet color precipitate indicates the presence of tannins.

c) Test for Phlonatannins

The ethanol extract of plant material was treated with 5ml of 1% hydrochloric acid. Formation of red precipitate indicates the presence of phlonatannins.

Detection of Saponins

The extracts were subjected to Foam test, Haemolysis test, as seen below.

a) Foam Test

The extract was diluted with 20ml of distilled water and then agitated in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of Saponins.

b) Haemolysis Test

About 2ml of blood was taken two test tubes separately. To one of the test tubes, equal quantity of water was added. To the other test tube, an equal quantity of ethanolic extract dissolved in water wad added. A clear red liquid was formed in the first test tube, which indicates the red blood corpuscles were haemolysed. The extract in the second tube also haemolysed. It indicates the presence of Saponins.

Detection of Steroids, Vitamins and Terpenoids

The extracts were subjected to various tests, as follows:

Detection of Steroids

To the ethanolic extract add few drops of acetic anhydride and a drop of concentrated sulphuric acid. Appearance of green or brown color was the end point.

Detection of Vitamins

One or two drops of plant extracts were treated with 1ml of chloroform and a drop of concentrated sulphuric acid. A color changes from violet to brown color indicates the presence of vitamins.

Detection of Terpenoids

Aqueous extract of plant materials were treated with 2ml of chloroform and few drops of concentrated sulphuric acid. Formation of reddish brown color at interphase indicates the presence of terpenoids.

RESULTS AND DISCUSSION

The phytochemical tests revealed the presence of many phytochemical components viz., flavonoids, saponins, alkaloids and glycosides in all the extracts but among the seven solvent ethanol extract was found to be more effective. The ethanol extract of the plant showed significant presence of almost all phytochemicals. The presence of

various phytochemicals in different solvent extracts is shown in the Table 1.Medicinal plants are the greatest sources of chemical ingredients for treatment of different ailment. In the present investigation, quantitative phytochemical screening test were analysed in bark extract of *GA*. The result was shown in Table 1, which indicated the presence of compounds of *GA* bark extract.Results showed that, flavonoids and carbohydrates was present in high intensity followed by compounds like alkaloids, phytosterols etc.,These compounds also can be correlated with the medicinal potential of the plant.

Most natural compounds are derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites. Secondary metabolites are considered products of primary metabolism but not involved in metabolic activity (alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, *etc.*) [16].The phenolic compounds are present in most widely distributed in the plant kingdom [17]. Mainly, the phenolic and flavonoids compounds extracted from the leaves samples antibiotics activity of the plant leaf extract [18].

CONCLUSION

The present study conclude that the ethanol extract of bark of *Gmelina arborea* possess a good source of phytocomponents like, carbohydrates, proteins, flavonoids etc., These components are mainly considered important for many pharmacological properties. Development of these pharmacological properties will engage the researchers for further investigation and studies. Hence the bark of the tree under study showed their medicinal potential and can be used for further pharmacological studies.

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Table 1. Phytochemical Analysis of Bark Extracts *Gmelina arborea*

S. No.	Name of the test	*Gmelina arborea* bark extracts						
		Petroleum ether	Benzene	Chloroform	Ethanol	Acetone	Ethyl acetate	Distilled water
1	CARBOHYDRATES							
	a) General test	+	+	+	+++	+	+	+
	b) Starch test	+	+	+	+++	+	+	+
	c) Barfoed's test	+	+	+	+++	+	+	+
	d) Molisch's test	+	+	+	+++	+	+	+
	e) Fehling's test	+	+	+	+++	+	+	+
	f) Benedict's test	+	+	+	+++	+	+	+
	g) Iodine test	+	+	+	+++	+	+	+
2	DETECTION OF GLYCOSIDES							
	a) Legal' test	+	+	+	++	+	+	+
	b) Borntrager's test	+	+	+	++	+	+	+
	c) Ferric chloride test	+	+	+	++	+	+	+
3	PROTEINS AND AMINO ACIDS							
	a) Million's test	-	+	+	++	+	-	-
	b) Ninhydrin test	-	+	+	++	+	-	-
	c) Biuret test	-	+	+	++	+	-	-
4	FIXED OILS AND FATS							
	a) Spot test	+	+	-	++	+	+	+
	b) Saponification test	+	+	-	++	+	+	+
5	DETECTION OF ALKALOIDS							
	a) Mayer's test	-	+	-	+++	+	+	-
	b) Dragondroff's test	-	+	-	+++	+	+	-
	c) Hager's test	-	+	-	+++	+	+	-
	d) Wagner's test	-	+	-	+++	+	+	-
	e) Potassium dichromate test	-	+	-	+++	+	+	-

6	DETECTION OF FLAVANOIDS							
	a) Ferric chloride test	+	+	+	++	+	+	+
	b) Lead acetate test	+	+	+	++	+	+	+
	c) Test for Anthraquinone	+	+	+	++	+	+	+
	d) Test for Catechins	+	+	+	++	+	+	+
	e) Test for Anthocyanin	+	+	+	++	+	+	+
7	DETECTION OF PHYTOSTEROLS							
	a) Libermann Burchard test	+	-	-	+	+	+	-
	b) Salkowski test	+	-	-	+	+	+	-
8	TANNINS AND PHENOLIC COMPOUNDS							
	a) General test	-	+	+	+	+	+	+
	b) Ferric chloride test	-	+	+	+	+	+	+
	c) Test for Phlonatannins	-	+	+	+	+	+	+
9	DETECTION OF SAPONINS							
	a) Foam test	-	-	-	+	+	-	-
	b) Haemolysis test	-	-	-	+	+	-	-
10	Detection Of Steroids	-	-	-	+	+	-	-
11	Detection Of Vitamins	-	-	-	+	+	-	-
12	Detection Of Terpenoids	-	-	-	+	+	-	-

+++ High ++ Moderate + Mild - Absent

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RESEARCH ARTICLE

The Effect of Nano Silver in D.C Conductivity of Biodegradable Polylactic Acid

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ABSTRACT

The present studies are focused on modification of the properties of biodegradable polylactic acid with additive nano silver by prepared of composites systems with percentage (2,4, and 6%) .The experimental results show that the D.C conductivity at (293-416 K) for additives nano silver is increased with increased nano silver additive and temperature and find the electrical conductivity of nano silver at room temperature is lie between ($2.3*10^{-11}$ -$0.9*10^{-11}$) $(\Omega.cm)^{-1}$. The activation energy is decreased when increased nano silver additive.

Keywords: D.C conductivity, nano silver, polylactic acid.

INTRODUCTION

Biodegradable polymers were first introduced in 1980s. Polymers from renewable resources have attracted an increasing amount of attention over the last two decades, predominantly due to two major reasons: firstly environmental concerns, and secondly the realization that our petroleum resources are finite. There are many sources of biodegradable plastics, from synthetic to natural polymers. Natural polymers are available in large quantities from renewable sources, while synthetic polymers are produced from non-renewable petroleum resources. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion. A vast number of biodegradable polymers have been synthesized recently and some microorganisms and enzymes capable of degrading them have been identified [1,2].Classification of biodegradable polymers Biopolymers can be classified in two ways: according to their renewability content (fully or partially bio-based or oil-based) and to their biodegradability level (fully or partially or not biodegradable).Classify biodegradable polymers into two main groups has been developed, these two groups being (i) the agropolymers obtained by

biomass fragmentation processes (polysaccharides, proteins…), (ii) and the biopolyesters obtained either by synthesis from bio-derived monomers (polylactic acid – PLA) or by extraction from micro-organisms (polyhydroxyalkanoate – PHA) or by synthesis from synthetic monomers (polycaprolactone – PCL, aromatic and aliphatic copolyesters – PBAT, PBSA…)appear in Fig(1) [3,4]. Polylactic acid (PLA) is prominent among the polymers that are biodegradable and biocompatible due to versatility of its applications and relatively low cost of production at industrial scale. PLA, is linear aliphatic thermoplastic polyester, produced from renewable resources, has several attractive properties such as biocompatibility, high strength, and thermo plasticity. It has been used in medical applications, such as surgical sutures, implants, tissue culture, and controlled drug delivery. Though PLA is biodegradable and has been useful in various biomedical applications, the high stiffness and brittleness at ambient temperatures associated with PLA must be improved to allow for more applications [5,6]

The basic building block of PLA is the lactic acid (LA). It is a simple chiral molecule which exists as two enantiomers, L- and D-lactic acid, optically active appear in Fig(2). It is can be produced by fermentative or chemical synthesis. The melting temperature of PLLA can be increased by 40–50 °C and its heat deflection temperature can be increased from approximately 60 °C to up to 190 °C by physically blending the polymer with PDLA (poly-D-lactide). PDLA and PLLA form a highly regular stereocomplex with increased crystallinity. The temperature stability is maximised when a 1:1 blend is used, but even at lower concentrations of 3–10% of PDLA, there is still a substantial improvement. In the latter case, PDLA acts as a nucleating agent, thereby increasing the crystallization rate. Biodegradation of PDLA is slower than for PLA due to the higher crystallinity of PDLA [7,8,9]. Many studies have been attempted to improve the properties of PLA , particularly the electric conductivity properties , by composites with other materials, such as montmorillonite, nano silver , CNT and ect.. within insulating polymer matrices generates a new species of smart materials termed “conductive polymer composites” (CPCs). The high electrical conductivity together with their large aspect ratio make nano silver particularly outstanding candidates as multifunctional fillers for CPC[10,11]

PLA is considered to have sufficient electrical properties that can be applied to electrical application. Table (1) shows a comparison of the general properties of PLA and other polymers, the volume resistivity ϱ is sufficiently high and safety to make application such as cables because that PLA decompose easily by two steps: the first step is hydrolysis of PLA and the second step is biodegradation by bacteria. Therefore, it is thought that PLA cables do not disintegrate easily by limiting the environment of the cable[12].Electrical resistivity (also known as resistivity, specific electrical resistance, or volume resistivity) is an intrinsic property that quantifies how strongly a given material opposes the flow of electric current. A low resistivity indicates a material that readily allows the flow of electric current. Resistivity is commonly represented by the Greek letter ϱ (rho). The SI unit of electrical resistivity is the ohm· metre (Ω·m), although other units like ohm·centimetre (Ω·cm) are also in use[13]. The relationship between the resistance and the electrical resistivity can be summed up by the following formula

$$R = \varrho L / A \quad \text{……..1}$$

Where

R is the resistance of the object in question in (ohms),ϱ is the electrical resistivity of the material the object in (ohms, Ω) , L is the length of the material in (m) , and A is the cross-sectional area the current flows through in (m^2). If current flows through a cylindrical object, then the cross-sectional area of the cylinder is a circle; that is $A = \pi r^2$

Conductivity σ is defined as the inverse of resistivity:

$$\sigma = 1/\varrho \quad \text{…….2}$$

Where:

σ: Conductivity has units Siemens per meter (S/m)

The change of electrical conductivity with temperature for most cases of materials are given by[28]:

$$\sigma = \sigma_0 \exp(-Ea/kBT) \quad \text{……(3)}$$

Where: Ea: is the thermal activation energy

T: is the absolute temperature

KB: is the Boltzmann constant

σ_0: is the minimum electrical conductivity at 0K

Composites consisting of polymers with embedded nanostructured metallic particles have unique properties and are of particular interest for optical, electrical, and opto-electronic applications. These composite materials exhibit unique optical characteristics originating from the strong interaction between incident light and metallic nanoparticles. This interaction results in collective oscillations of electron clouds, so called surface plasmons, at the interface of the metallic nanoparticles and the dielectric matrix. The resonance frequency of this interaction is strongly dependent on the metal, the surrounding dielectric medium, as well as the size and shape distribution of the nanoparticles[14,15].One of the best materials for electrical conduction (low resistivity) is silver appear that of electrical conductivity in Fig (3), but its use is restricted due to the high cost. Most widely used conductor is copper: inexpensive, abundant, high σ, but rather soft – cannot be used in applications where mechanical strength is important [16]

The incorporation of metal nanoparticles could effectively improve the electrical, optical and dielectric properties of the polymers composites. These properties are extremely sensitive to small changes in the metal content and in the size and shape of the nanoparticles. That the nanoparticles themselves could act as conductive junctions between the Polymer chains that resulted in an increase of the electrical conductivity of the composites. The electrical conductivity of such composites might also depend upon the molecular structure of the conductive polymer matrix (i.e., crystallinity). Since silver exhibits the highest electrical and thermal conductivities among all the metals, the combination of Polymers with silver could yield functional materials having enhanced electrical properties [17,18].

MATERIALS AND METHODOLOGY

Experimental

Lactic acid (99.9%), Tin chloride dehydrated ($SnCl_2.2H_2O$) P-toluene sulfonic acid (TSA)were purchased from Fluka . Methylene Chloride was purchased from Sigma-Aldrich. Nano silver was purchased Nano powder filler of silver was supplied by Cristal Globa Phama Company with particle size (13.69 nm) is shown in Fig (4) measured of nano Ag.

Preparation of pure PLA and PLA/Ag nanocomposites

The reaction was conducted in 250ml , three necked flask reactor equipped with a magnetic stirrer and a reflux condenser .100 gm of aqueous solution of lactic acid acid was mixed with methylene chloride for 5hrs at reflux temperature without any catalyst .After the removal of water of the condenser , the reaction vessel was cooled at 50°C, the required amount of catalyst SnCl2.2H2O (0.5wt%), TSA(0.4wt%) were add and this was followed by slow heating of the reaction mixture to the refluxing temperature of the solvent under mild stirring with the help of magnetic stirring bar . The temperature gradually increased to 120°C in 3 hrs, and the reaction mixture was stirred continuously. Polymerizations are done at 120-160°C for 5hrs .At the end of the reaction, the flask was cooled, and the product was dissolved in chloroform and subsequently precipitated in methanol .The resulting solid was filtered and dried Under vacuum at 60°C under vacuum for 24hrs .powder PLA weighted grade (2wt%) by using electronic balance of four digits type (Sartorius H51) and then dissolved in chloroform to obtain 20 wt% solution of PLA grade by slowly in 60°C for 2 hours warming until the solution become viscous using magnetic stirrer hot plate, then cast into petri dish at room temperature for 24 hour to ensure complete solvent removal.

PLA/ nano silver composite films were prepared by many steps a weight percent of nanoparticles were used with different weight percentages (2, 4 , 6%) with electronic balance of four digits type (Sartorius H51). Initially nano silver was swollen in chloroform by mixing for 2 hours while polylactic acid dissolved in chloroform then nano solution were mixed by shearing mixer at 800 rpm for one hour to have good distribution and less agglomeration. After two

solutions were mixed for an hour and sonication process was again introduced in order to improve dispersion of nano filler in the polymer matrix. Then cast on the glass plates and then kept at 60°C in the vacuum oven for 24 hour to ensure complete solvent removal. The thickness of PLA/ nano silver composite thickness of 150 µm.

Characterization

Fourier Transform Infrared (FTIR) Analysis

The infrared spectra were recorded using spectrophotometer type SHIMADZU FTIR -7600 in range 400 to 4000 cm^{-1}. FTIR spectra gave information about the chemical structures of all films.

D.C. Conductivity

The electrical resistance has been measured as a function of Temperature for pure PLA and PLA/Ag nanocomposites films in the range (298-413) °K by using the resistivity (ϱ) of the films is calculated by using the equation(1) and the conductivity of the films was determined equation(2).The activation energies could be calculated from the plot of Ln σversus l000/T.

RESULTS AND DISCUSSION

FTIR of pure PLA

FTIR spectra of pure PLA appear in Fig (5) Polylactic acid spectrum contains characteristic absorption bands at 2800–3000, 1300–1500 and 756 cm^{-1} which can be assigned to methylene groups (stretching and deformation vibrations, respectively). Strong, sharp peaks at 1757 cm^{-1} due to carbonyls and 1186, 1094 cm–1 attributed to C–O–C groups .

FTIR of PLA/Ag nanocomposites

FTIR spectroscopy was used to characterize the interaction of composites PLA/Ag 6% relative to PLA. In Fig (6) peak at 2878 cm^{-1} of O–H stretching of the –CH (CH_3)–OH end group of PLA. The splitting of the C=O carbonyl stretching at 1757 cm^{-1} might be due to the presence of –CH– in CO–O– group , peak at 1051 cm^{-1} assigned to the C–O.

DC-Electrical Conductivity for pure PLA

Polylactic acid is generally insulators and the electrical resistivity of the pure PLA is of about $4.6*10^{17}$ Ω·cm, that similar in the typical range generally reported for PLA [19]. Fig (7) shows the temperature dependence of DC-conductivity in the temperature range 293- 413°K for pure PLA that appear straight line and show one activation energy is 1.55ev.

DC-Electrical Conductivity for PLA/Ag nanocomposites

To illustrate the effect of Ag nanoparticles on the DC conductivity of polylactic acid material, a comparison of pure PLA and PLA/Ag nanocomposite was made. Fig (8) shows the temperature dependence of DC-conductivity in the temperature range 293-413 °K for PLA/Ag, it is evident that the DC-electrical conductivity of the composite is higher than that of pure polylactic acid , the homogeneous dispersion of nano silver in a polymer matrix is one of the most important requirements in achieving conductive nanocomposites and that a small amount of highly conductive nanoparticles, when well dispersed, could form a conducting network in the polymer matrix finally reduce in resistivity due to an increased Ag network density after partial removal of the amorphous phase of the polymer

matrix [20].It is clear from the figure that in all the samples, the plots of ln σDC vs 1000/T are not straight lines, indicating that the conduction in these samples through an activated process having single activation energy in the temperature. The activation behavior of the samples is studied by using Arrhenius Equation (3), at low temperature the thermal activation of the conductivity was almost negligible , activation energy of thermal degrees (293-328)K except for the transition of positive energy levels localized in the energy gap and as a results of a proposed high density of localized energy levels in the energy gap , after increased the temperature above Tg the conductivity increased strongly .So the calculated for this region because that the second region (333-373) K the results of activation energy at the granular ion by thermal emission thermal stimulation cross-border movement , and listed in Table(2) .The activation energy values decreased with increased of wt% nano silver .The low activation energy for PLA/nano silver composites should be to the electronic conditions mechanism which was resulted for a new kinetic path formation in polymer matrix. It has been reported that for these types of composites ionic, electronic and even mixed conductive process are possible [21].The values of activation energy calculated for pure PLA and PLA/Ag nanocomposites from Fig (7,8) are given in Table (2) . It was found that the activation energy for pure PLA is higher and decrease in PLA/Ag nanocomposite is found to be associated with a shift of Fermi level in doped samples [22].

CONCLUSION

In this work, electrical properties of PLA/Ag nanocomposites were studied. The nanocomposites were prepared using casting method. Electrical results showed that PLA/Ag nanocomposites increased in electrical conductivity when increased nano silver and decreases in activation energy when increasing temperature. The electrical results indicate that the prepared nanocomposites are suitable to be used in cables, because that polylactic acid (PLA), which is a plant-derived polymer, is an environment-friendly polymer from the point of view of not only having biodegradable property but also saving fossil resources and reducing carbon dioxide emission. Although the PLA has already been applied to many products, it has not yet been applied to cable.

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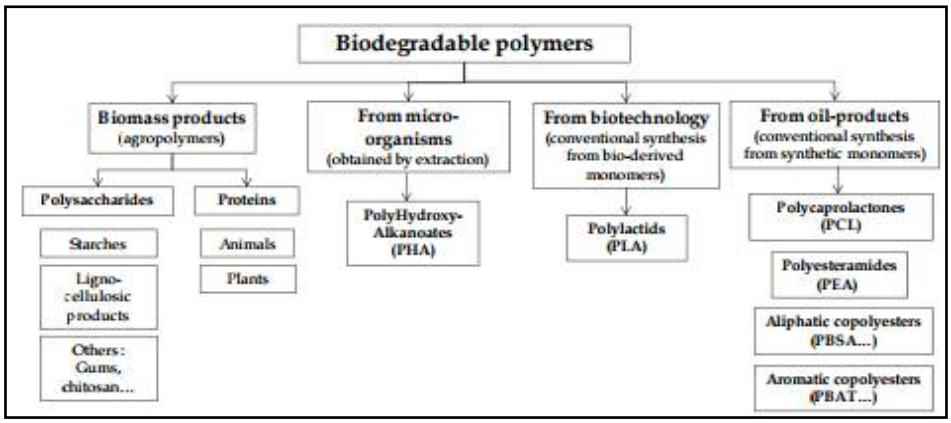


Fig. 1: Classification of biodegradable polymers

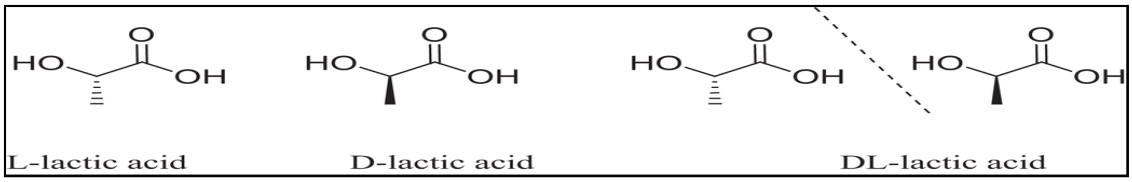


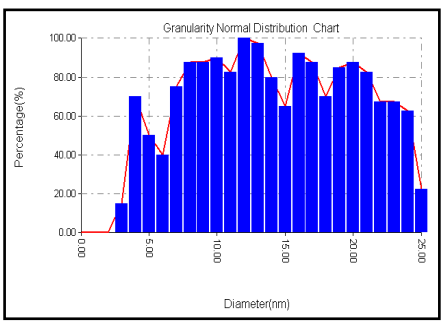
Fig. 2: Structure of L-, D-, DL Lactic acid

Table. 1: Properties of PLA and other polymers [12]

Polymer	Thermal properties			Mechanical properties		Electrical properties		
	Density g/cm^3	Glass transition temperature Tg °C	Melting point °C	Tensile elongation EL %	Tensile strength TS MPa	Dielectric dissipation factor tanδ %	Permittivity ε	Resistivity ρ Ω cm
PLA	1.3	60	170	4	59	0.01	3.1	4.3×10^{17}
LDPE	0.92	−120	110	600–700	15–20	0.01	2.3	$>10^{16}$
HDPE	0.95	−120	130	650	12	0.01	2.3	$>10^{16}$
PP	0.91	5	165	800	38	0.05	2.2	$>10^{16}$
PVC	1.4	70–100*	180*	300*	20*	0.10*	4–5*	10^{11}–10^{14}*

Resistivities at 20°C	
Material	Resistivity (Ω•m)
Aluminum	2.82×10^{-8}
Copper	1.72×10^{-8}
Gold	2.44×10^{-8}
Nichrome	$150. \times 10^{-8}$
Silver	1.59×10^{-8}
Tungsten	5.60×10^{-8}

Fig. 3: Resistivity of Materials



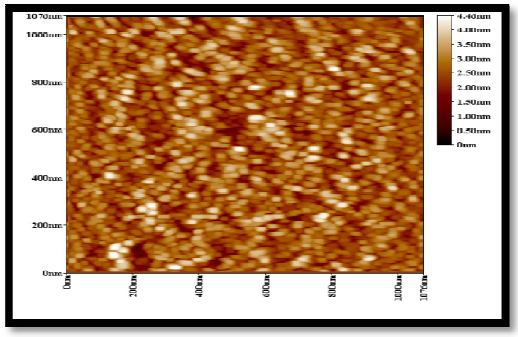


Fig. 4: Granuality normal distribution chart for nano Ag particles

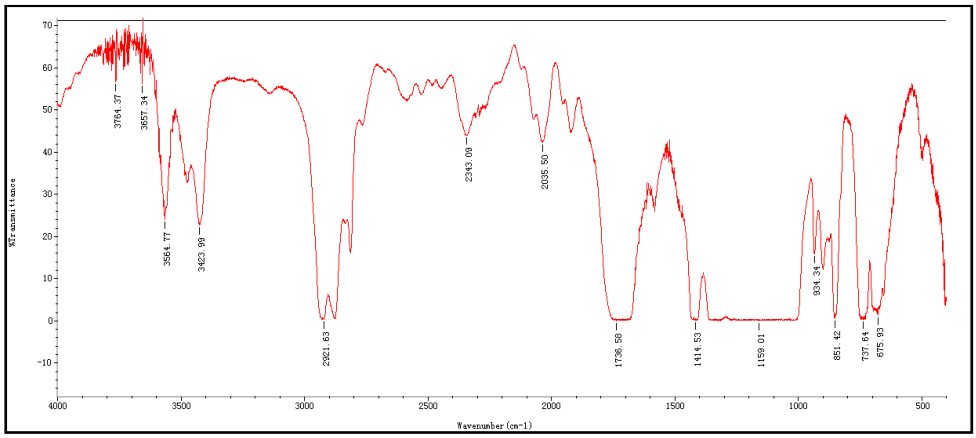


Fig. 5: FTIR of pure PLA

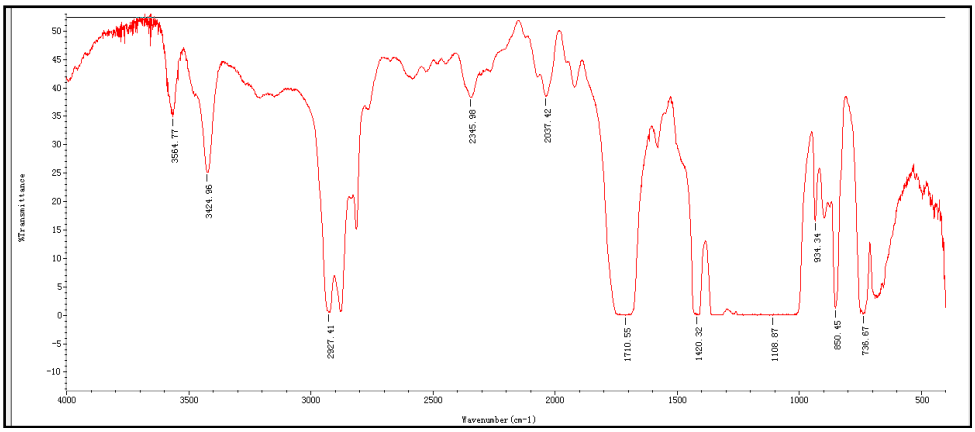


Fig. 6: FTIR of PLA/Ag nanocomposites

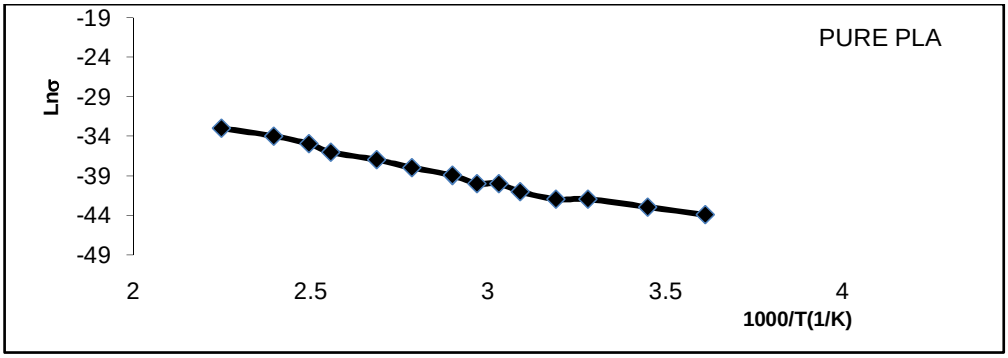


Fig. 7: ln σDC vs 1000/T

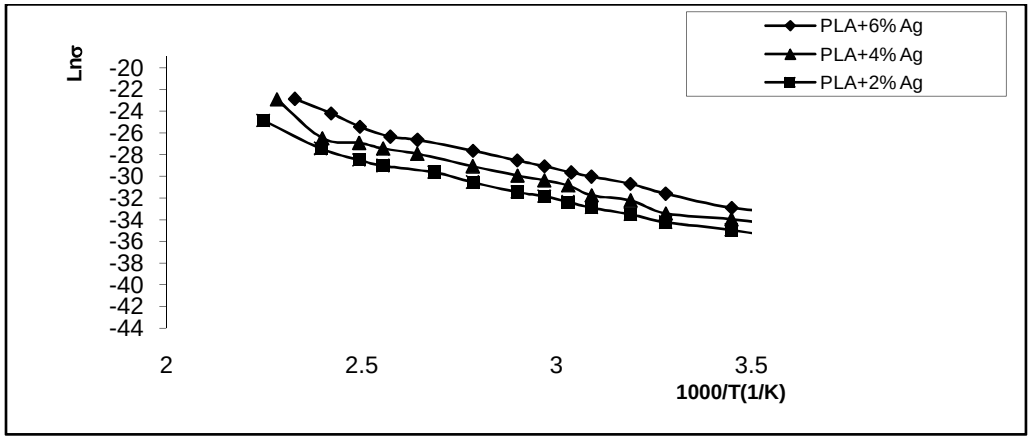


Fig. 8: ln σDC vs 1000/T

Table.2: Values of σ and activation energy of PLA/Ag nanocomposites

Samples	$\sigma(\Omega.cm)^{-1}$	E_{ev1}	E_{ev2}
Pure PLA	$2.1*10^{-17}$	1.55	-
PLA/Ag 2%	$2.3*10^{-11}$	0.44	0.32
PLA/Ag 4%	$1.4*10^{-11}$	0.19	0.159
PLA/Ag 6%	$0.9*10^{-11}$	0.06	0.11

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RESEARCH ARTICLE

Glandular Hairs and Essential Oils of Stem and Leave from the Promosing Medicinal Legume *Cytisus triflorus* L' Hérit. Using Ultrasounds: Comparison with Conventional Hydrodistillation

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ABSTRACT

Micromorphology of glandular andnon-glandular trichomes on the vegetative organs of the medicinal legume *Cytisustriflorus*L'Hérit.were investigated using light microscopy. Essential oil composition of dry stems and leaves isolated by hydrodistillation(HD) with and without ultrasonic pre-treatment prior hydrodistillation, were analysed by GC-MS. Anatomical study revealed the presence of two-celled non-glandular trichomes and monocellularcapitate glandular hairs, which were most abundant in leave.The ultrasonic pre-treatment positively affected the leave oil yield extraction and was achieved in a reducer extraction time, whereas the relative content of target volatiles increased in stem oil with application of ultrasound prior hydrodistillation (US-HD). A total of 43 and 50 compounds were identified in the leave and stem oils of *C. triflorus*, respectively, in the different hydrodistillation procedures.Acids (52,48%) dominated the stem HD oil while alcohols (34,12%) majored in US-HD oil. Contrary to stem oil, both HD and HD-US leave oilswere majored by alcohols (72,93%-61,32%, respectively). The relative content increase of volatiles differed with extraction method and depended on their functional class and target compounds. This is the first report on anatomical characterization of glandular hairs from species of the genus *Cytisus*, and identification of oils from vegetative parts of the genus *Cytisus*.

Key words: Essential oil, *Cytisustriflorus*, glandular hairs, Hydrodistillation, Ultrasounds, GC-MS, linalool, BHT, fatty acids

INTRODUCTION

Plant essential oils (EOs) are odorous and highly complex mixture of volatile secondary phytochemical metabolites derived from a great range of different chemical classes including mainly terpenoids such as monoterpenes, diterpenes and sesquiterpenes and a variety of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclicesters or lactones [1] In addition to their widespread use as flavoring material[2],essential oils represent a "green" alternative in the nutritional, pharmaceutical, and agricultural fields due to their biological activities[3-5]. Volatile oil compounds production and emissions have also ecological roles in modulating plant-pest interactions and the prevalence of host specificity and phytophagous insects [6]. Additionally, they are used in taxonomic and evolutionary studies[6,7].

Various processes are used to extract essential oils from various matrixes. Among themethods commonly used, the conventional steamdistillationand hydrodistillation which are the mostly very simple processes but suffer of many disadvantages: distillation takes several hours, thermal degradation, hydrolysis and solubilisation in water of some compounds, oxidation and losses of some volatile compounds, low extraction efficiency [2,7,8]. Lately, innovation methods such as supercritical fluid extraction (SFE)[8], microwave-assisted extraction (MAE) [**7**] and ultrasound-assisted extraction (UAE) [9]have been applied to alleviate these problems aswell as to increase the oil yield, reduce the processing time and moderate energy consumption. Moreover its use in various food technology processing [9], the ultrasound is nowwell known to have great potential effect inextraction of several natural products such as pigments, aromas, antioxidants and other organic and mineral compounds.Ultrasonic extraction has also been combined with other extraction processes such as distillation and recentlysoxhlet process. This novel procedures allowed enhancing oil extraction and target compounds extraction as limonene and carvone from caraway seeds [10,11].

The family *Fabaceae* is one of the largest families among Angiosperms which is extensively studied for anatomical, biological and phytochemical characters [12]. In the genus *Cytisus* belonging to that family, two types of metabolites were intensively investigated: flavonoids and alkaloids. While the phytochemistry and pharmacological activities of these natural compounds in the *Cytisus* L. species have been studied in depth, studies of volatile compounds from them are almost absent. Only three papers have previously been reported on the essential oil extracted from flowers of *C. sessifolius* L. [13]and *C. scoparius* Link.[14,15]. Additionally, the leaf and stem anatomics of some shurby species of the genus *Cytisus* have been carried out [16,17], however only non-glandular trichomes have been identified.Among the sixty species from the genus *Cytisus* spread in the spontaneous flora of many mediterranean countries,the broom*Cytisustriflorus*L'Hérit. isthe most one growing in the nord-est of Algeria. Commonly known as "Ilougui", its leavesare usedin traditional medicine fortreating abdominal pain, wound healing, dermatophytic and as haemostatic, traditionally recognized by healers and consumers. However, few reports have been traced concerning the phytochemistry and pharmacological potential of this plant [18-20]. Moreover, the taxonomic status of *C. triflorus* is critical in view of various taxonomic markers [21]. The aim of the study was to evaluate the influence of ultrasoundspre-treatment of stems and leavesof the medicinal plant *Cytisustriflorus*on their essential oil yields and chemical composition. Comparisons have been made with conventional hydrodistillation. Moreover, anatomical characteristics of hairs in both organs are described. To the best of our knowledge, such study has never been performed previously on *Cytisus* species.

MATERIALS AND METHODS

Plant Material

The aerial part of *Cytisus triflorus* was collected at flowering in May 2010, in the middle of a forest located in Azazga (Tiziouzou, Algeria). The plant was identified and authenticated by Dr. M. Zaoui from the Department of Biology,

Superior Normal School (Algeria).Voucher specimenwas deposited at the herbarium of the research unity VARENBIOMOL of the university of Constantine 1 (CTA 125/03/11) .

Anatomical exam

Cross-sectionfrom fresh leaves and stems were made manually. The presence of fats was revealed by red straining of lipophilic strain Sudan III (1 % solution in 96 % ethanol and glycerol (1: 2). The surface of the epidermis of the leaves and the stems were examined with a Moticbinocular loupe, whereas the anatomical samples were observed with aNovexbinocular light microscope.

Isolation of essential oils by hydrodistillation (HD)

Dried powdered materials (leaves 40 g, shoot 60 g) were submitted separately to hydrodistillation for 3 hours, using a modified Clevenger apparatus. The extracted oils were dried over anhydrous sodium sulfate and stored at 20°C until analysed by GC-MS.

Ultrasound extraction combined with hydrodistillation (US-HD)

Ultrasounds were applied on the plant materiasl as a pre-treatment beforehydrodistillation. Dried powdered leaves (40 g) and shoots (30 g) were separately mixed with 600 ml and deionized water, respectively, in a 1L erlenmeyer flask. The mixtures were submittedto ultrasound for one hour, using a Bandelin ultrasonic bath (35 kHz, 150 W). Subsequently, the mixture was hydrodistillated for 90 mn. The extracted oils was collected such as in the HD method and stored in same conditions until GC-MS analysis.

Gas Chromatography-Mass spectrometry Analysis (GC-MS)

The gas chromatography coupled with the mass spectrometry (GC-MS) was performed with a Hewlett-Packard 6890 gas chromatograph combined with an Agilent 5973 mass spectrometer and equipped with an HP-5 MS (5% phenyl-polymethylsiloxane) capillary column (30m x 0.25mm x 0.25µm). The column temperature was programmed from 40°C (10 min) to 220°C at a ramp of 3°C per minute with holding time 30 min. The injector temperature with splitting ratio or splitless was kept at 250°C. Helium was used as carried gas at a flow rate of 1ml/min. The mass spectrometer was operated in EI mode with ion source and quadruple temperature 230°C and 150°C, respectively. Mass spectra survey wasperformed using MS-libraries (Nist, 1998 and Nist, 2000).

RESULTS AND DISCUSSION

Anatomic observations

Through an adequate observation binocular loupe, it can be checked that both plant material surfaces were very hairy, especially the leave one (figure 1).Trichome density isone of the main factors correlating with resistance to herbivory [22].Microscopic observations of leave and stemtransverse sections indicated the presence of anunistratified epidermis with a thick cuticular layer which was more important on epidermal tissue of the stem than on that of the leaf (Fig. 2).Moreover, two types of hairs were distinguished using binocular loupe and light microscope:nonglandular and glandular hairs which showed a higher density on leave than on stem (fig.1). In both transverse sections, the non-glandular hairs were adpressed, simple, uniseriate and two-celledtrichomes, with a short base cell and an elongated terminal cell in the leaf, whereas in the stem, the terminal cell were generally found elongated hooked cell. The cell wall of the both leaf and stem trichomes showed warty ornamentations and a grooved cuticle surface. These results agree with those previously have been reported for *C. villosus, C. supinus* and *Chamaecytis ussessifolius*[16]. Similar description of the trichomes has also been noted for *C. supinus* and *C. hirsutus*

[17].However,*Cytisophyllumsessifolius, C. reverchonii* and *C. scoparius* lacked trichomes [16].The glandular hairs, reported here for the first time in the *Cytisus* group, showed a quite simple morphology, consisting in both stem and leaf sections of a simple epidermal basal cell with a single round cell head (fig. 3).Structural lipids were present on the whole cell walls, as shown by the positive reaction with Sudan red. This endodermal feature is commom in oil-secreting trichomes, which prevent backward-flow of the secreted material [23].

Both glandular and non-glandular trichomes may have many other functions as well, including attraction of pollinators, protection against UV due the presence of flavonoids and other UV-absorbing compounds in trichomes, temperature regulation and reduction of water loss [24].Glandular trichomes function particularly as important chemical barriers for plant parasites. The main classes of secondary chemicals that have been found to be produced in trichomes include terpenoids, phenylpropenes, flavonoids, methyl ketones, acyl sugars, and defensive proteins [22]. Thus, the identification of capitateglandular hairs in *C. triflorus* could be useful for identification of this species, as well as clarification of its ecological function and its taxonomic relationships within the tribe *Genisteae*.

Stem oil composition

The essential oils isolated from stems of *C.triflorus* by hydrodistillation (HD) and ultrasound extraction prior hydrodistillation (US-HD) were obtained in 0,014 % and 0,012 % yields (w/w based on dry plant material weight), respectively. A total of 26 and 46 components, listed in Table 1, were identified by GC/MS analysis representing 83,92% and 75,25% of HD and US-HD obtained oils, respectively. Components of both oils were classified according to their functional groups (Table 2) which is useful in determining the pharmacology and toxicology of the essential oil. Acids (52,48%) dominated the HD oil while alcohols (34,12%) majored in US-HD oil. Fatty acids predominated in both oil samples with linoleic acid (28,82%) and palmitic acid (17,34%) as their main components, respectively.While the total essential oil yields of the two samples were similar, the ultrasound pretreatment positively affected their relative content of alcoholic fraction which increased in US-HD oil (34,12% *versus* HD 21,97%), as well as the phenolic which concentrated mainly in US-HD oil (17,18%) than in HD oil (11,21%). The main phenolic contributor was butylatedhydroxytoluene BHT with a higher relative content in US-HD oil (12,31%). This antioxidant compound was previouslyfound naturallyoccurred in phytoplankton and a Tunisian medicinal plant [25,26] and was recently revealed in all parts of *C. triflorus* [18]. The known natural antioxidant phenol, 2,4-bis(1,1-dimethylethyl) was only identified in US-HD oil (1,3%). The same alcoholic terpenoids was present in both oils but in different concentrations. The most important were linalool (4,96%) and vinylguaicol (2,54%) concentrated in US-HD oil whilerelative percentages of Germaniol (5,59%) and α-Terpineol (3,4%) increased in HD oil.However, the sesquiterpeneα-Gurjenene was only identified in HD oil. Esters content was higher 2 fold in US-HD (4,49%) oil than in HD one (2,29%). This was probably related to the possible ultrasonic effect on some components leading to their esterification. Total aldehydes, ketones and hydrocarbons found in similar concentrations in both oils.The presence of some compounds such as aldehydes and esters might be the result of their original presence in leaves, as it might be due the consequence of a degradation process of their precursors compound, linoleic acid. The β-oxidation of this fatty acid may also produce derivative compounds such as the β-ionone and β-Damascenone. Moreover, high operating temperatures used in HD procedure causes peroxidation of insaturated fatty acids leading to their break down and formation of acids in C9 (nonanoic acid identified in the three samples) or in C12 and later alcohols such as 3(Z)-hexen-1-ol present also naturally in plants [27].To our knowledge, the essential oil composition of *Cytisus* species stems has never been reported in the previous literature, except the only study focused on one aroma compound from stems of the well-known medicinal shrub *Cytisusscoparius*because it released one characteristic odour into the airidentified as one kind of thiol called 4-mercapto-4-methylpentan-2-one [28].

Leave oil composition

The essential oils isolated from leaves of *Cytisus triflorus* by two procedures of extraction were obtained in HD 0,042 % and US-HD 0,053 % yields (w/w based on dry plant material weight). The higher essential oil content obtained by

US-HD in a shorter extraction time of distillation (90 mn), in comparison with HD procedure carried out in 180 mn, could be due to the application of ultrasound power before HD. Such increase in the oilyield has previously been reported. Solvent ultrasound assisted extraction of volatile oils from wine and aged-brandies allowed better yields and a shorter extraction time compared to conventional methods [29]. In another combination of US with supercritical fluid extraction (SFE), the procedure conducted to 30% increased oil yield extraction from particulate almonds [30].The same procedure was applied for enhancement of the supercritical extraction from Ginger and allowed both the extraction rate and yield increasing[31].This result may be explained in term of cavitational effects caused by the prior application of ultrasound. In fact, the use of ultrasound enhances the extraction process by increasing the mass transfer between the solvent, in our case the water, and plant material. The mechanic vibrations cause expansion and compression cycles in the physical medium, creating bubbles which collapse and cause cavitations, instantly creating a high local pressure and intense local. These fast changes induce disruptionand thinning of cell membranous, consequently heatingincreasing the mass transfer rate of organic substances from the solid matrix to the solvent [9]. In addition, cavitational bubble collapsecan occur at the surface of the plant membranes causing microfractures. Thisallows for improved waterpenetration into the plant body itself and can also break down cell wall[9,32]. The occurrence of microfracture by ultrasound was demonstrated insoybean flakes [33]and caraway seeds cell wall [34].

The composition the two oils is listed in Table 3. A total of 43 compounds were identified by GC-MS analysis, with US-HD 39 and HD 30 volatiles. The main components representing 80,06% and 90,05% of the total volatile composition of both oils, respectively, are given in Table 3.Inversely to the stem oils, the common dominant functional class of both leave oils was alcohols (Table 4)with major monoterpenoidic compounds, which is in agreement with our previous study in where essential oil of leaves was extracted by another procedure, steam distillation [19].Alcoholic fraction of the essential oil obtained by hydrodistillation without pre-treatment represented 72,93 % (Linalool 41,62%, α-Terpineol 12,77% and Geraniol 8,79%), followed by phenols (9,53%) majored by butylatedhydroxytoluene (2,99%). The concentration of ketones (4,44%) wastwo fold higher than acids (2,93%) with as main contributors Dihydroactinidiolide (1,01%) and fatty acids (1,63%), respectively.The identified alcoholic and phenolic fractions were made up of oil isolated from ultrasonic pretreated leaves (61,32% - 8,62%, respectively) and show similar main constituents to those of the HD oil but in lesser concentrations. Acids (5,02%) were dominated by fatty acids (4,52%) which were majored by palmitic acid (3,06%). The ultrasonic pretreatment of both stems and leaves affected positively the concentration of this common saturated fatty acid which was higher in US-HD oils than in HD ones. Among the determined ketones (3,89%), β-Damascenone(0,97%) was the major contributor. Volatile hydrocarbons were selectively extracted. HD oils werecharacterised by linear alcaneHeinecosane (0,22%), whereas US-HD oil was marked by cyclic alcanesNaphtalene compounds (0,59%) and linear alcaneeicosane (0,2%). Terpene-richessential oils are commonly found in the secretion of glandular trichomes of different species [23].The higher terpenoidic content of leave essential oil obtained by application of two extraction methods could partially justify the use of the leaves of *C. triflorus* in algerian popular medicine. Alcohols and phenols are more potent antimicrobial agents [35] and the former have important ecological functions in plants as allelochemicals [36].

The main component Linalool is one of target compounds of the sample oils due to its pharmacological and ecological roles.In the HD one, the linalool oxide/linalool ratio was 0.02 while it decreased to 0,016in US-HD oil. That would indicate that linalool oxidation occurred more upon HD process during 3 hours. As shown in Table 3, numerous valuables bioactive components were extracted by US-HD (Geraniol 7,92 %, α-Terpineol 10,81 %, eugenol 0,91 %) although their proportions were lesser than HD ones. Similarly to that found with stem oils, in comparison with HD oil, US-HD oil was found richer in the antioxidant 4-Vinylguaïcol (2,82 %), Mellol (5,45 %) and phenol, 2,4-bis(1,1-dimethylethyl) which was only present in US-HD obtained oil.Moreover, similarly to stem essential oils, some other compounds were detected only in US-HD oil such as sesquiterpeneFarnesylacetone (0,25%) and Naphtalene derivatives (0,59%). They probably were isolated with the application of the ultrasound prior hydrodistillation.

The impact of ultrasound pretreatment on leave and stem EO composition could be related to difference in the histological structure of both plant materials. As reported byRiera*et al.*, 2004 [37], the rheological nature of the seed structure (hardness, compactness) may have a direct impact on the capability of ultrasound to improve extraction of lipids compounds from the plant cells. In fact, variation in the extraction yield as well as in component yield may result from structure, rheology or the compositional differences resulting in varying degrees of susceptibility to ultrasound shock waves and like-lihood that cavitation bubble will contact with the plant surface causing micro-jetting. In conclusion, the results from this study provided useful data on anatomical features of hairs and the profiles of leave and stem essential oils of *Cytisus triflorus*L'Hérit. Their chemical composition might be connected with ecological properties as well as pharmacological potential of the plant particularly its leaves.In the case of stem, US-HD seemed to be more efficient than HD for extraction ofmore numerous valuable compounds such as antioxidants, with reduction in extraction time, while HD in the case of leave provided better results in term of extraction yield oil as well as valuable target compounds such as Linalool, Geraniol and BHT.Although that, the US-HD might be more selective than conventional HD by using the suitable extraction conditions. Additional stirring could be used to prolong sufficient contact of the plant material with water. This is often necessary to achieve high levels of extraction [9]. A sono-extraction reactor has been developed for that purpose [10]. Therefore, further studies will be carried out in order to improve the ultrasound pre-treatment extraction parameters that could significantly enhance the recovery of target compounds. More attention should also be given to other parts of *Cytisustriflorus*and some possible application could be proposed to maximize the value of this plant species.

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Table1: Chemical composition of stems essential oils(% of total volatiles)

No.	Compound	RT	HD	US-HD
1	1-octen-3-ol	20.97	-	0.19
2	Hexanoicacid	22.31	1.35	0.27
3	1-hexanol, 2-ethyl	23.56	-	0.17
4	Benzylalcohol	24.05	-	0.1
5	Benzeneacetaldehyde	24.21	-	0.1
6	Linalool	27.62	1.43	4.96
7	Mellol	28.29	0.34	0.38
8	Hexanoicacid, 2-ethyl-	29.58	0.82	0.1
9	Methyl salicylate	32.48	-	0.23
10	(+),α-Terpineol	32.54	3.4	1.82
11	Cis-Geraniol (Z)	34.03	1.29	0.59
12	Trans- Geraniol (E)	35.33	4.3	1.54
13	Pelargon	36.91	0.74	0.2
14	Vinylguaicol	38.11	0.2	2.54
15	Eugenol	39.96	0.61	0.27
16	Benzene, 4-ethenyl-1,2-dimethoxy	40.52	-	0.52
17	1,2,4-trimethoxybenzen	40.69	-	0.31
18	β-Damascenone	41.05	0.77	0.48
19	benzen, 1,2-dimethoxy-4-(2-propyl)- (Dihydromethyleugenol)	42.06	-	0.76
20	Cis-Geranylacetone	44.03	0.1	0.15
21	α-Gurjenene	44.99	1.10	-
22	Bht	46.34	10.4	12.31
23	Phenol, 2,4-bis(1,1-dimethylethyl)	46.81	-	1.30
24	Lauric acid	49.21	2.32	0.30
25	Tridecane	51.02	0.2	-
26	Benzophenone	51.26	-	0.56
27	Tetradecane	53.01	1.16	
28	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl (Styrene)	54.19	-	0.18
29	2,6-diisopropylnaphtalene	54.61	-	0.30
30	2,6-diisopropylnaphtalene	54.79	-	0.22
31	Octanal,2-(phenylmethylene)-	55.56	0.46	0.32
32	Anthracene, 1,2,3,4-tetrahydro	56.68	-	0.15
33	Myristic acid	56.54	2.2	0.59
34	Anthracene	56.77	-	0.38
35	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	58.88	-	0.48
36	Hydroxyfarnesylacetone	58.96	0.81	0.21
37	7-acetyl-6-ethyl-1,1,4,4- tetramethyltetralin	59.19	-	0.13
38	Isobutylphthalate	59.55	1.45	2.24

39	Pentadecanoic acid	59.98	1.43	0.78
40	Palmitic acid, methyl ester	61.79	0.84	0.44
41	Dibutylphtalate	62.71	-	1.58
42	Palmitic acid	63.59	15.62	17.34
43	1,19-eicosadiene	67.97	3.68	-
44	phytol	67.61	-	0.58
45	1,3,12-nonadecatriene	68.42	-	4.25
46	Linoleic acid	68.73	28.82	7.56
47	9,12,15-octadecatrien-1-ol (Z,Z,Z)-	68.90	-	6.61
48	Linoelaidicacid (isomere de linoleicacic)	69.47	-	0.28
49	docosane	70.34	-	0.16
50	Hexacosane	92.17	-	0.32

RT: retention timeHD: Oil obtained by hydrodistillation

US-HD: Oil isolated from stems pre-treated by ultrasound prior hydrodistillation

Table 2. Relative composition (%) of *C. triflorus* stems oils by functional class

Functional class	HD	US-HD
Alcohols	10,76	16,94
Phenols	11,21	17,18
Acids	52,48	27,32
Esters	2,29	4,49
Ketones	1,68	1,4
Aldehydes	0,46	0,42
Hydrocarbones	5,04	5,96
Total	83,92	75,15

Table 3:Chemical composition of *Cytisus triflorus* leaves essential oils (% of total volatiles)

N°	Compound	RT	HD	US-HD
1	3-Hexen-1-ol, (Z)	11,64	0.48	0,93
2	1-Octen-3-ol	20,47	2,97	2,88
3	Benzyl alcohol	23,82	0,43	-
4	Cis-Linalool oxide	25,76	0,53	0,31
5	Trans-Linalool oxide	26,69	0,33	0,17
6	Linalool	27,63	41,62	29,43
7	Mellol	28,20	4,36	5,45
8	Octanoic acid	32,07	0,66	t
9	(+)-, α-Terpineol	32,50	12,77	10,81
10	Cis-Geraniol (Z)	33,94	2,68	2,34
11	Trans-Geraniol (E)	35,25	6,11	5,58
12	Pelargon (nonanoic acid)	36,59	0,64	0,28
13	4-Vinylguaicol	37,92	1,73	2,82

14	Eugenol	39,81	2,20	0,91
15	(E)-isieugenol	40,42	2,61	2,46
16	β-Damascenone	40,99	1,13	0,97
17	Naphtalene, 1,2-dihydro-1,1,6-trimethyl-	41,79	-	0,22
18	Geranylacetone	44,03	0,41	0,42
19	(E)- β-Ionone	45,24	1,00	0,77
20	3-Buten-2-one,4-(2,26-trimethyl-1-Cyclohexen-1-yl	45,38	0,30	0,77
21	ButylatedHydroxytoluen (BHT)	46,17	2,99	2,25
22	Phenol, 2,4-bis(1,1-dimethylethyl)	46,87	-	0,18
23	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-	47,28	1,01	0,84
24	Megastigmatrienone	49,22	0,27	0,11
25	Lauric acid	49,84	0,35	0,27
26	Megastigmatrienone	50,98	0,13	0,26
27	Diisonaphtalene	52,99	-	0,13
28	Diisopropylnaphtalene	54,81	-	0,24
29	Octanal, 2-(phenylmethylene)-	55,20	-	t
30	Myristic acid	56,63	-	0,30
31	Hydroxyfarnesylacetone	58,85	0,19	0,27
32	1,2-(Benzenedicarboxylic acid, bis(2-methylpropyl)	59,57	-	0,22
33	Benzoic acid, 2-hydroxy-, phenylmethyl ester	59,96	-	0,32
34	Farnesylacetone	61,28	-	0,25
35	Pentadecanoic acid, 14-methyl-, methyl ester	61,81	-	0,1
36	Palmitic acid	63,01	1,05	3,63
37	Eicosane	64,24	-	0,2
48	Phytol	67,45	0,32	0,36
39	Linoleic acid	68,26	0,23	0,32
40	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	69,18	0,33	3,06
41	9,12,15-Octadecatrienoic acid, methyl ester	69,75	-	-
42	Tricosane	72,72	-	-
43	Heinecosane	73,51	0,22	-

RT: retention time

HD: Oil obtained by hydrodistillation

US-HD: Oil isolated from leaves pre-treated by ultrasound priorhydrodistillation

Tr: Trace (0.1%)

Table 4: Relative composition (%) of *C.triflorus* leaves oil samples by functional class

Functional class	HD	US-HD
Alcohols	72,93	61,32
Phenols	9,53	8,62
Acids	2,93	5,02
Ketones	4,44	3,89
Aldehydes	0,0	0,0
Esters	0,0	0,42
Hydrocarbones	0,22	0,79
Total	90,05	80,06

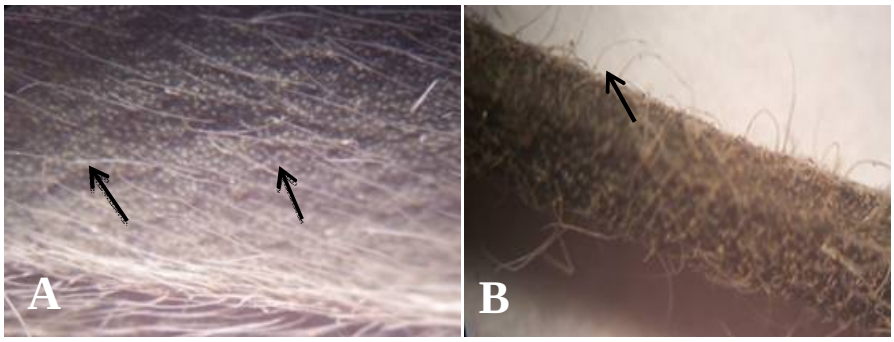
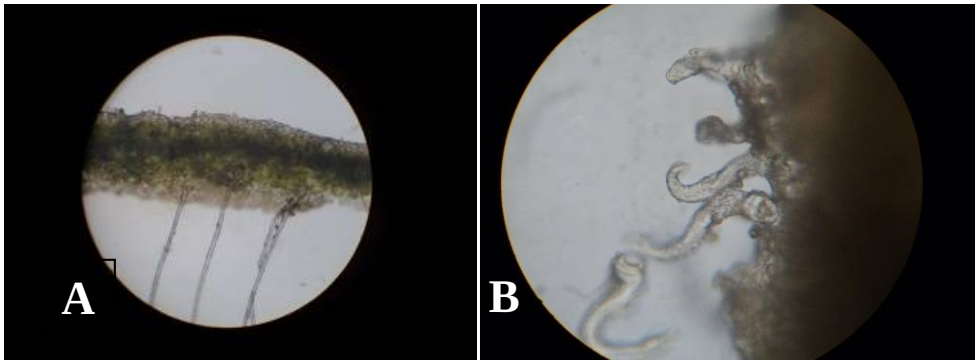


Fig. 1. Surface of epidermis of *Cytisustriflorus*.

A. Part of the abaxial surface of the leaf with visible non-glandular and glandular trichomes (arrows). **B.** Part of the surface of the stem with visible non-glandulartrichomes (arrow).



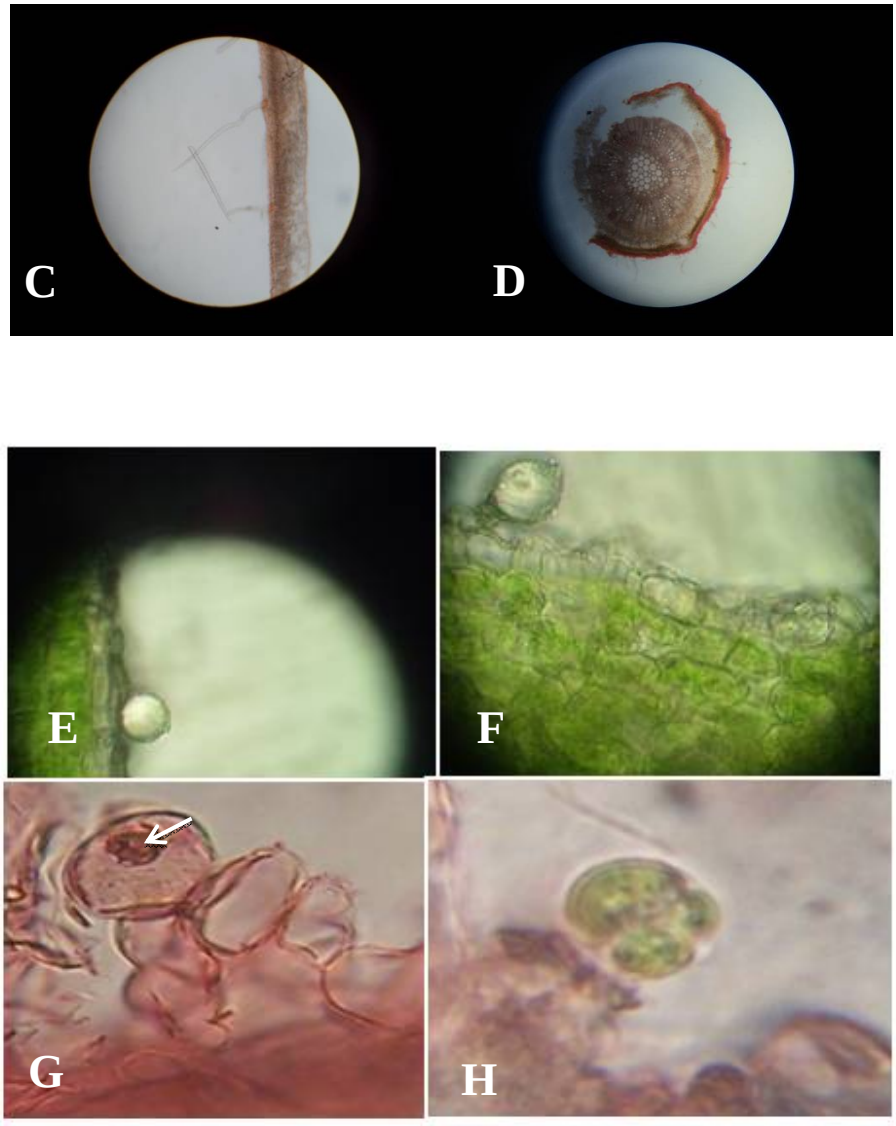


Fig. 2.Light micrographs of hand cross-sections of *C. triflorus* leaf and stem.

A. Non-glandular trichomes of leaf without staining (40x). **B.**hooked Non-glandular trichomes of stem without staining (100x)**. C.**Cross section of leafstained with Sudan III (40x). **B.** Cross section of stem stained with Sudan III (40x). **E-F**.Sessile glandular trichomes on the epidermis of leaf (cross sections without stainig; 100x, 400x respectively).**G.** Leaf capitate glandular trichomestained with Sudan III (note the subcuticular space filled with a large secreted material, arrow) (400x). **H.** Stem capitate glandular trichome stained with Sudan III (400x).