ICV 2016: 86.55
UGC Approved Journal No. 48951
SJIF Impact Factor 6.38

ISSN:2320-7817 | eISSN:2320-964X
Special Issue, A 12, March 2018

INTERNATIONAL JOURNAL OF Life Sciences

AN INTERNATIONAL PEER REVIEWED OPEN ACCESS JOURNAL

National Seminar on

Modern Perspectives in Life Sciences (MPLS-2018)

9 & 10 March 2018

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Publisher and Owner: **Dr. Arvind Chavhan,** Published from IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Printed at IJLSCI, 46, Guruvandan, Jawahar Nagar, VMV Road Amravati -444604, India

Managing Editor: **Dr.Arvind Chavhan**, Assistant Professor & Head, Department of Zoology, D.B. College, Bhokar, Nanded, India

Indexing:

Index Copernicus, Google Scholar, OCLC WorldCat, INNOSPACE (SJIF), CiteFactor, ROAD, OAJI, AcademicKeys, J-index.net, ScholarSteer, ResearchBible, DRIJ, DIIF, SIS, SIS, ASI, Directory of Science

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IJLSCI

ISSN:2320-7817(p) | 2320-964X(o)

INTERNATIONAL JOURNAL OF LIFE SCIENCES

EDITORIAL BOARD

International Journal of Life Sciences

ISSN 2320-7817 (p) 2320964X (o)

Special Issue, A12, March 2018

National Seminar on

"Modern Perspectives in Life Sciences" (MPLS-2018)

9th & 10th March, 2018

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March, 2018 : 1 - 8
UGC Approved Journal No 48951

Original Article Open Access

Adsorption studies on water hardness removal by using *moringa oleifera* seed pod husk activated carbon as an adsorbent

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Khati Varada V (2018) Adsorption studies on water hardness removal by using *moringa oleifera* seed pod husk activated carbon as an adsorbent, *Int. J. of. Life Sciences*, Special Issue, A12: 1- 8.

ABSTRACT

Moringa oleifera seed pod husk Activated Carbon was utilized as an adsorbent to remove water hardness ions from hard water. The effect of pH, adsorbent dosage, initial concentrations, contact time, and temperature were investigated using batch adsorption experiments. Characterization of adsorbent was identified by FTIR and XRD techniques. The pH dependence study of the adsorption process revealed that maximum pH for hardness removal was 8 with efficiency of 59%. Temperature study reveals that the adsorption is endothermic as efficiency increases with the increase in temperature. The adsorption of hardness ions on *Moringa oleifera* seed pod husk activated carbon shows that the removal efficiency increases with increase in contact time and also increased as adsorbent dosage increases from 1gm/50ml to 5gm/50m. The study showed that the method is a simple and efficient one to remove calcium and magnesium hardness from hard water solutions and adsorbent had the potential for hard water softening.

Keywords: Activated carbon, adsorption, batch adsorption experiments, *Moringa oleifera* seed pod husk, water hardness removal.

INTRODUCTION

Water hardness is a traditional measure of the capacity of water to precipitate soap. Hardness of water is not a specific constituent but is a variable and complex mixture of cations and anions. It is caused by dissolved polyvalent metallic ions. Hardness of water is due to the presence of high content of calcium and magnesium in addition to sulphate and nitrates. This is the property of water to precipitate soap by formation of complex with calcium, magnesium present in water. Other polyvalent cations also may precipitate soap, but often are in complex form, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. Total hardness is defined as the sum of the calcium and magnesium concentration, both expressed as $CaCO_3$, in mg/L.

The degree of hardness of drinking water As per APHA standard has been classified in terms of the equivalent $CaCO_3$ concentration as follows:

Soft 0-60 mg/L
Medium 60-120mg/L
Hard 120-180mg/L
Very hard >180mg/L

Among the various known forms of water contaminants, Calcium and Magnesium salts are of great apprehension since they lead to water hardness. Water hardness problem is reported to exist in various parts of state, the reason behind is rock type, which is rich in Calcium and Magnesium. These ions dissolve easily in to the groundwater and make them hard. In daily uses, hard water is associated with number of challenges that include scaling in boilers, washing machines and pipes (Seo, 2010), difficult lathering with soap, objectionable spots on sinks and clothes as well as toughening of skin and hair. Hard water is said to cause serious health problems like urolithosis, cardiovascular disorder, kidney problems, anencephaly and cancer (Meena, 2012). Additionally, WHO reports that excess intake of calcium is associated with kidney stones and that of magnesium leads to diarrhea and laxative effect due to change in bowel habit. Calcium and magnesium play vital roles in the structure and functions of the human body. High intake of calcium and magnesium in drinking water could result in symptoms of toxicity such as a kidney stones, gastric and breast cancer, low blood pressure, muscle weakness, confusion and abnormal cardiac rhythm (Yang, 1998). Therefore, the need to purify water which is not suitable for human consumption such as hard water cannot be overemphasized. It is obvious that hard water treatment methods required high capital operations. Hence, finding cheap and effective developed processes remains a major concern. Because of the challenges raised by hardness in water, immediate actions to soften water are to be expected. Water softening by adsorption using agricultural wastes based activated carbon as adsorbent seems to be potential in the sense that the agricultural wastes are locally and cheaply available. For the purpose of removing hardness ions from water, various adsorbent materials have been used such as *Moringa oleifera* (Fahmi, 2011), Peanut hull (El-Sayed, 2010), pumice (Sepehr, 2013) and Phyllanthus emblica (Kannan, 2014).

Earlier studies found that *Moringa Oleifera* is harmless and recommended it for use as a adsorbent in water treatment. The use of *Moringa Oleifera* has an added advantage over the chemical treatment of water because edible. When *Moringa Oleifera* seed powder used as bioadsorbent for removal of fluoride, it was found that the alkali treated seed powder was better than acid treated (Parlikar,2013). When Adsorption Studies for Arsenic Removal Using Activated *Moringa oleifera* leaves is carried out it was found that is an effective and alternative biomass for removing Arsenic from aqueous solution due to high bio-sorption capacity (Sumathi and Alagumuthu, 2014). Various studies were carried out where *Moringa oleifera* seed powder was investigated as a best low cost biosorbent for the removal of toxic heavy metals from wastewater (Ongulu, 2015). Biosorption of Pb^{2+} from aqueous solution by biomass prepared from *Moringa oleifera* bark shows it is promising biosorbent material for the removal of heavy metal ions from wastewater/effluents (Reddy, 2010). Biosorption of Pb2+ from aqueous solution using *Moringa oleifera* pods also gives good results (Adelaja, 2011). Paula *et al*. found that the highest level of metal removal was achieved at pH 5(Paula, 2013).

MATERIALS AND METHOD

***Moringa Oleifera* (Drum Sticks)** - It is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English common name is **drumstick tree** from the appearance of the long, slender, triangular seed-pods. It is a fast-growing, drought-resistant tree, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. It can also be used for water purification and is sometimes used in herbal medicine. Earlier studies have found *Moringa Oleifera* to be non-toxic and recommended it for use as a coagulant. Its use has an added advantage over the chemical treatment of water as it is edible.

Fig 1-*Moringa Oleifera* seed pod

Preparation of *Moringa Oleifera* seed pod husk charcoal - *Moringa Oleifera* pods were collected from locally available trees, for this purpose mature seed pods are selected rather than the immature ones which are preferred for cooking purposes. The pods are sun dried for 3-4 days. The seeds are removed from the pods and their size was reduced by breaking it into small pieces. Then it was packed in an air tight in a cylindrical container with top completely sealed with a cover to prevent the entry of air during the process of charring. The sealed container was heated in furnace by slowly raising the temperature up to 350^0 C for 60 minutes and subsequently washed with distilled water, oven dried and sieved through 100 µ mesh sieve to obtain carbon powder.

Activation of carbon - The resultant charcoal obtained by above procedure was soaked in 2M KOH overnight. It was followed by washing with distilled water till the attainment of neutral pH, and then dried in the hot air oven at 80±5C temperature for 4 hrs to obtain activated carbon. The KOH saved as activating agent to introduce some functional groups and deepening of micropores' depth.

Stock solution as Adsorbates - Synthetic hard water was prepared by dissolving 1.19g of $CaCl_2$ and 1g of $MgSO_4$ were dissolved in a litre of de-ionized water to make a water with hardness of 1214.8 mg/L as $CaCO_3$ and this served as a stock solution (Rolence, 2016).

Batch adsorption experiments - Batch adsorption experiments were conducted to examine adsorption behavior of different adsorbent on water hardness removal under different adsorption condition. Adsorption studies were carried in different conditions namely adsorbent dose, initial concentration, contact time, pH and temperature. The adsorption experiments were conducted in 250 ml conical flasks. In each experiment, a known amount of adsorbent was contacted with 50ml of desired contaminated water with known pH and at a regular interval of time of 60 min. pH of the solution was measured using pH meter and adjusted using 0.1N HCL and 0.1 N NaOH. The solutions were filtered by using Whatman filter papers and filtrates were collected for analysis. In each experiment the conditions were kept constant except for the one in which its effect is studied.

Adsorbent Characterization - The adsorbent was characterized by FTIR analysis. In chemical activation, activating agent is expected to significantly affect the properties of substance. X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy analysis performed to determine the structural and surface properties.

RESULTS AND DISCUSSION

Characterization of Adsorbent

The pH value of activated charcoal was 9.00. pH of charcoal increased due to activation by KOH. The acidic or basic nature of a charcoal or activated charcoal depends on its preparation, inorganic matter and chemically active oxygen groups on its surface as well as the kind of treatment to which the activated carbon was subjected. The pH of the activated carbon affects the adsorptive property of the carbons, as highly acidic or basic carbons are undesirable for processing.

The FTIR technique is an important tool to identify the characteristic functional groups which are vital in adsorption of hardness ions. Fig.2 is FT-IR spectrum for ***Moringa oleifera* seed pod husk** Activated Carbon. Adsorption at 1172.30 and 1113.36cm^{-1} might be due to the vibration of alkoxy group (C–O). The sharp absorption band at 1385.30 cm-1 is ascribed to nitro group (N–O). The region of the spectrum of 1589.13 cm–1 is due to primary amine (N–H). A broad adsorption peak appeared at 3384.61cm-1 is corresponding to the stretching of O–H functional group. C-H deformation is noticed between 876.80 and 825.67 cm-1 supports the presence of aromatic groups in the carbon structure. The peak in around 600-700 cm^{-1} may be attributed to the carbon halogen vibrations (C-X).

Based on the collective FT-IR data, the carbon prepared has high surface heterogeneity which is useful for the multilayer adsorption of hardness. The surface heterogeneity is due to the presence of the functional groups like –C=O, aromatic –C=C, aromatic C-H, hydrogen bonded –OH group. Identified functional groups are likely to account for the adsorption of hardness ions onto the adsorbent surface, hence high efficiency in water softening.

X- ray diffraction pattern of the sample shows some peaks may be due to presence of inorganic and crystalline substance in the carbon. It indicates the crystalline nature of the adsorbent.

Table 1 Effect of pH on Hardness Removal

pH	Residual concentration (mg/L)	Amount adsorbed (mg/L)	% Removal
2	**750**	**225**	**23.07%**
4	**485**	**490**	**50.25%**
6	**425**	**550**	**56.41%**
8	**400**	**575**	**58.97%**
10	**400**	**575**	**58.97%**

Concentration = 975mg/l; Contact time = 60min, Adsorbent dose = 1gm/50ml, Temperature = 30°C.

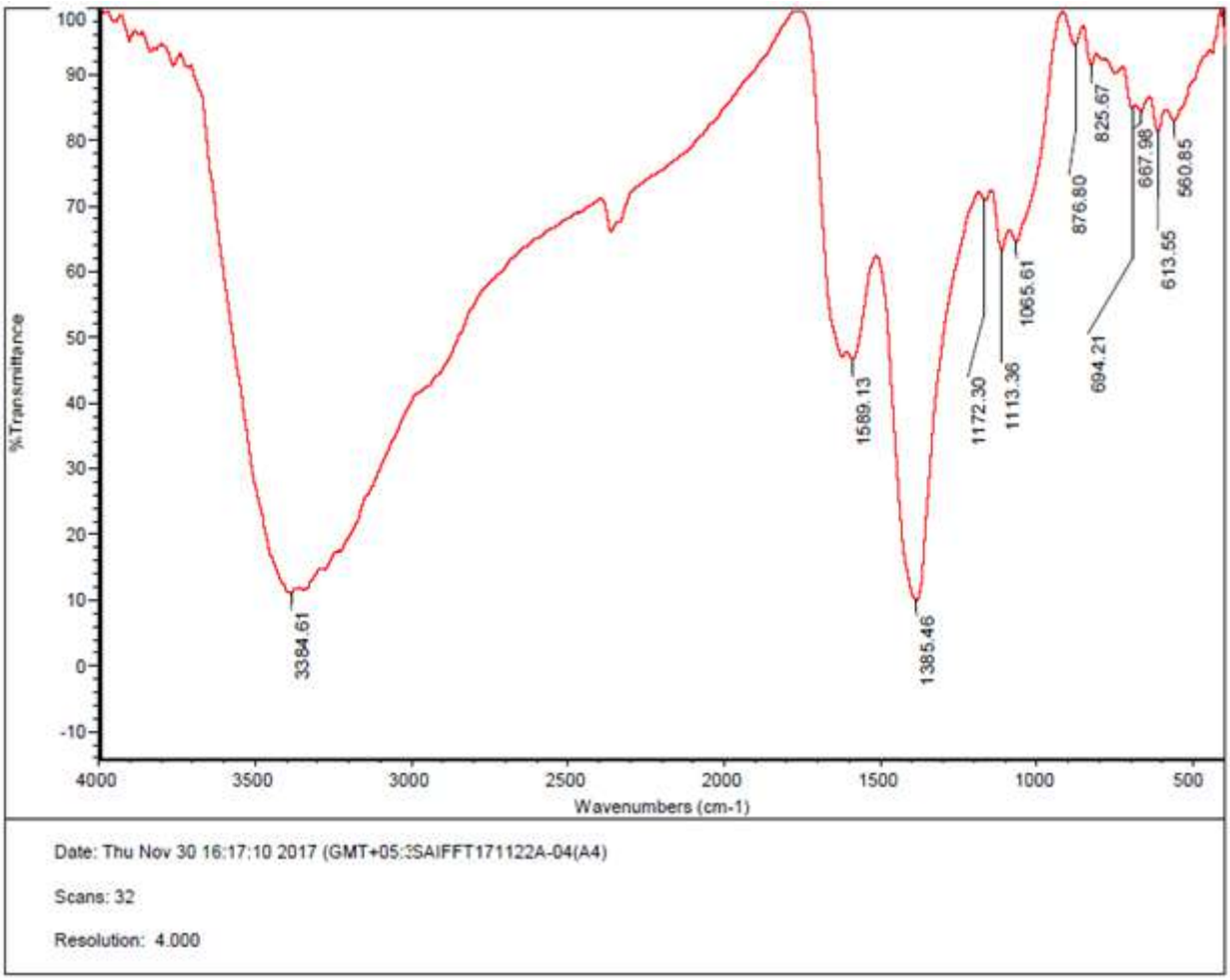

Fig 2- FTIR spectrum of KOH activated *Moringa Oleifera* pod husk

Effect of pH on Hardness Removal

Initially adsorption of hardness increases with increase in pH. This might be due to the increase of hydroxyl ions (OH^-) concentration in the solution that increases negativity of the adsorbents or might be due to that, as pH increases the competition between hydroxonium ions, H_3O and positively charged metal ions on the surface of adsorbent decreases (Jimoh, 2012). At the pH of 6 to 10 hardness removal efficiency was observed to be almost constant. Trend of this nature may be due to presence of nearly equal concentrations of H^+ and OH^- ions in the bulk solution that affect the polarity of adsorbent making it almost neutral to adsorb more ions. Highest removal efficiency was 58.97% that was achieved at the pH of 8 (Table 1).

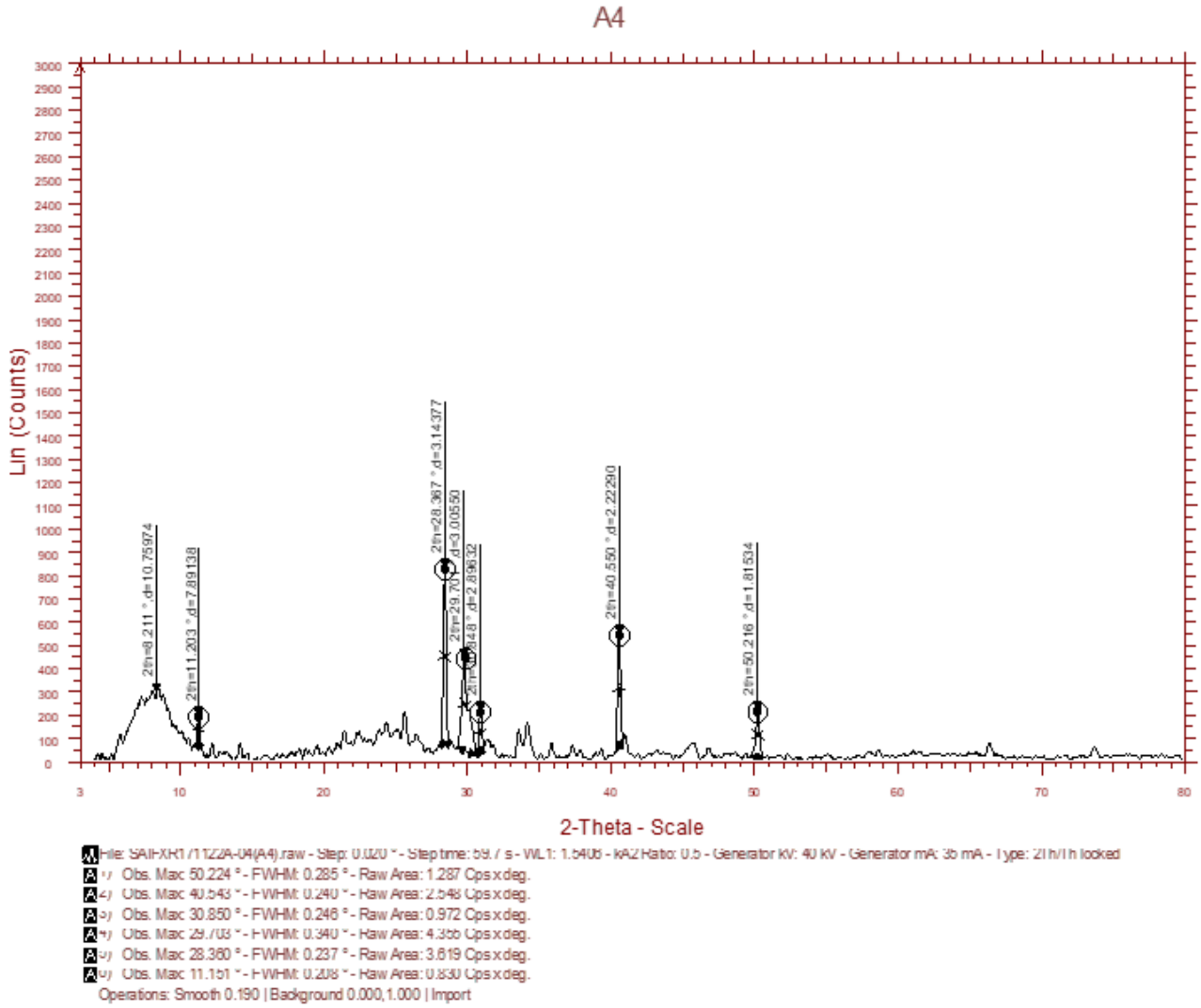

Fig-3 -XRD of KOH activated *Moringa Oleifera* seed pod charcoal

Table 2 Effect of Adsorbent dose on Hardness Removal

Adsorbent dose	Residual concentration (mg/L)	Amount adsorbed (mg/L)	% Removal
1gm	**400**	**565**	**58.54%**
2gm	**360**	**605**	**62.69%**
3gm	**325**	**640**	**66.32%**
4gm	**310**	**655**	**67.88%**
5gm	**300**	**665**	**68.91%**

Concentration = 965mg/l; Contact time = 60min, Temperature = 30°C., pH = 8,

Effect of Adsorbent dose on Hardness Removal

For the powder activated *Moringa Oleifera* pod shell carbon, it was found that the percentage of hardness removal was slowly and steadily increased with the increase of adsorbent dosage. This might be due to that number of active sites increases with increase in amount of adsorbent.

The maximum removal efficiency of hardness being adsorbed was nearly 69% at 5gm dose (Table 2).

Effect of initial concentration on Hardness Removal
For the powder activated *Moringa Oleifera* pod shell carbon, it was found that the percentage of hardness removal was slowly and steadily decreased with the increase of concentration. Removal efficiency is 58.49% around 980mg/lit. Decrease in the value may be due to the available adsorption sites decreases due to adsorption density (Table 3).

Effect of Contact time on Hardness Removal
The effects of contact time on the removal of calcium and magnesium using were activated *moringa oleifera* charcoal as shown in table. The percentage adsorption increased with increase in contact time at constant concentration. Initially adsorption process was rapid and then slowed down after 90mins, the equilibrium times were reached at 120min. Before the optimum time, the removal efficiency increased rapidly due to the abundant availability of active binding sites on the adsorbent surface. After that the removal process became less efficient due to the complete occupation of the surface with the metal ions (Table 4).

Effect of Temperature on Hardness Removal
Effect of temperature on adsorption of the hardness ions onto *Moringa Oleifera* pod shell activated charcoal indicated that adsorption of hardness ions increases with the increase in temperature and reached up to 70% above 50^0C. This may be due to the temperature affects the interaction between the adsorbent and the metal ions which influences the stability of the metal–sorbent complex. Higher temperatures enhance sorption due to the increased surface activities and kinetic energy of the solute. Generally, increase in the temperature increases the rate of adsorbate diffusion across the external boundary layer and in the internal pores of the adsorbent particles and more active sites available with increase in temperature for hardness ions adsorption.

Table 3 Effect of initial concentration on Hardness Removal

Initial concentration (mg/L)	Residual concentration (mg/L)	Amount adsorbed (mg/L)	% Removal
375(mg/L)	133	242	64.66%
475(mg/L)	178	297	62.49%
565(mg/L)	220	345	61.09%
680(mg/L)	276	404	59.47%
980(mg/L)	426	554	58.59%

Contact time = 60min, pH = 8; Adsorbent dose = 1g/50ml, Temperature = 30°C

Table 4 Effect of Contact time on Hardness Removal

Contact time	Residual concentration(mg/L)	Amount adsorbed(mg/L)	% Removal
30min	500	460	47.91%
60min	400	560	58.33%
90min	370	590	61.15%
120min	360	600	62.50%
150min	355	605	63.03%

Concentration = 960mg/l; pH = 8, Adsorbent dose = 1g/50ml, Temperature = 30°C.

Table 5 Effect of Temperature on Hardness Removal

Temperature (^{0}C)	Residual concentration (mg/L)	Amount adsorbed (mg/L)	% Removal
30^0C	500	600	54.54%
40^0C	400	700	63.63%
50^0C	350	750	68.18%
60^0C	330	770	70.00%
70^0C	320	780	70.10%

Concentration = 1100mg/l; pH = 8, Adsorbent dose = 1g/50ml, Contact time = 60 min,

Adsorption isotherms

i) The Langmuir Adsorption isotherms

The Langmuir equation was applied for adsorption equilibrium

$$Ce/qe = 1/Qob + Ce/Qo$$

where, Ce is the equilibrium concentration (mg/L), qe is the amount adsorbed at equilibrium (mg/L) and Qo and b are Langmuir constant related to adsorption capacity and energy of adsorption respectively. The plots Ce/qe as a function of Ce for the adsorption was found linear suggest applicability of Langmuir model in present adsorption system.

ii) The Freundlich isotherm

The Freundlich isotherm is represented by the equation-

$$\text{Log } x/m = \log K + 1/n (\log Ce)$$

Where Ce is the equilibrium concentration (mg/L) and x/m is the amount adsorbed per unit weight of adsorbent (mg/g). Plots of log x/m *vs.* log Ce is linear. Figures 5 show the Freundlich adsorption isotherm for hardness metal ions. The 'K' and 'n' values were calculated from the intercepts and slopes are 7.46 and 1.41 respectively. Value of n and K obtained indicate that the adsorbent is good for uptake of Hardness from aqueous solution.

It shows that Freundlich model fitted experimental data better than the Langmuir isotherm. The Freundlich curves had good linearity (Correlation coefficient > 0.99) for adsorbent indicates strong binding of ions to the surface of Moringa *oleifera*.

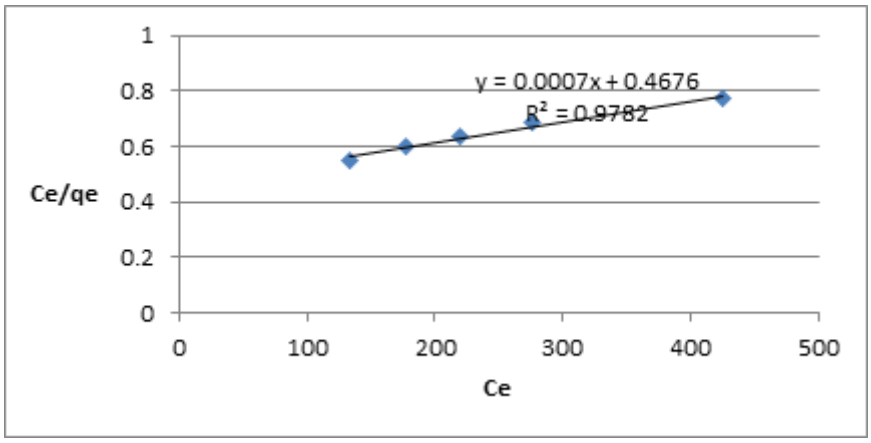

Fig 4: The Langmuir Adsorption isotherms for removal of hardness by *Moringa oleifera*

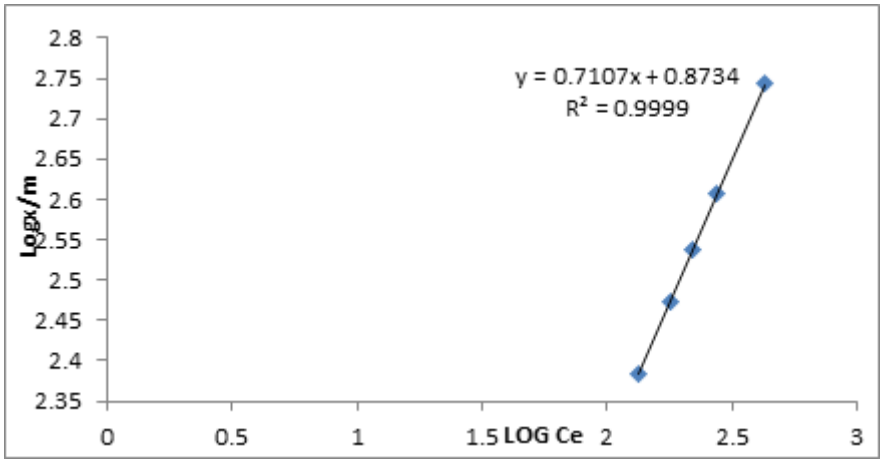

Fig 5: The Frendlich Adsorption isotherms for removal of hardness by *Moringa Oleifera*

CONCLUSION

Activated Carbon was prepared through pyrolysis followed by chemical activation with KOH and used as an adsorbent for removal of hardness. Removal of hardness (Ca^{2+} and Mg^{2+}) by Application of operational conditions such as contact time, adsorbent dose, pH, Temperature and concentration of adsorbate led to increase of hardness removal. Result clearly shows that adsorption of Ca^{2+} and Mg^{2+} on to activated materials was favored. The optimal dose was found to be 5gm and the maximum removal was seen within 150 minutes of contact time. Based on the results obtained in the present study, it is clear that it is effective in water softening. Since the morienga oliefera shells are locally available, especially in regions of Chandrapur district where hardness problem is prevailing, then, this adsorbent is expected to be economically feasible for removal of hardness from groundwater.

Acknowledgement:
I express my enormous gratitude to my guide Dr. Deepali P. Gulwade., Dr. Mrs. Sujata Deo for their invaluable guidance and helpful suggestions. I am thankful to Mr. S. S. Sayare, Sayare Industries, Warora to providing laboratory facilities and 'SAIF cochin' for FTIR and XRD analysis.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:9-16
UGC Approved Journal No 48951

Original Article Open Access

Removal of heavy metal ions using eco-friendly synthesized terpolymer

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Jiwatode Manisha M, Kariya Kiran P and Berad Baliram N (2018) Removal of heavy metal ions using eco-friendly synthesized terpolymer, *Int. J. of. Life Sciences*, Special Issue, A12: 9-16.

ABSTRACT

A novel chelating resin synthesized from phthalic acid, 1,5-naphthalene diamine with formaldehyde by condensation in glacial acetic acid. The structure of chelating resin was clearly elucidated by use of variety of spectral techniques, for example FTIR, 1HNMR and UV-Visible spectroscopy. The average molecular weight of terpolymer resin was determined by non-aqueous conductometric method. The empirical formula and empirical formula weight of the resin were determined by elemental analysis. Scanning electron microscopy was used to established surface features of the chelating resin. The ion exchange behavior of heavy metals, viz. Pb^{+2}, Co^{+2}, Cd^{+2} and Hg^{+2} was evaluated by batch equilibrium method. The study was extended to three variations evaluation of metal ion uptake in the presence of different electrolytes at different concentrations; evaluation of metal ion uptake at different pH; and evaluation of metal ion uptake at different times.

Keywords: Synthesis; Characterization; Metal ion uptake; Distribution coefficient; Batch equilibrium

INTRODUCTION

Ion exchangers have been used commercially on a worldwide basis for almost a century due to diverse applications in many fields such as water softening and deionization (Singh and Saraf, 2009). The presence of heavy metals in environment is a cause of concern due to their acute and long-term toxicity. Lead and mercury are the major hazardous metals present in the environmental wastewater. Thus, removal of trace heavy metals from the environmental area have become of increasing interest and there is a strong need for a reliable analytical procedure that can be applied for the removal and determination of these metals at very low concentrations (Patel *et al.*, 2007). Synthesized a chelating terpolymer resin using an eco-friendly technique and reported for its good binding capacity for Fe^{+2} and Cu^{+2} ions (Hiwase *et al.*, 2010).

Chelating terpolymer resin synthesized from 2,4-di-hydroxy-acetophenone –oxamide –formaldehyde (Butolia *et al*., 2009) selective for Cu^{+2}, Hg^{+2}, Cd^{+2}, Co^{+2}, Zn^{+2}, Ni^{+2}, Pb^{+2} and Fe^{+3}. Ion exchange resin synthesized from 2,4-dihydroxyacetophenone –dithiooxamide – formaldehyde (Rahangdale *et al*., 2008) Phenolic Schiff bases derived from hydroxybenzaldehyde and 4,4' di-amino-di-phenyl ether have been reported as better chelating resin for Cu(II) leading to its separation from a mixture of Cu(II) and Ni(II) ions (Gurnule *et al*., 2003). Salicylic acid and melamine with formaldehyde terpolymer was found to have higher selectivity for Fe^{+3}, Cu^{+2} and Ni^{+2} ions than for Co^{+2}. Zn^{+2}, Cd^{+2} and Pb^{+2} ions. An ecofriendly synthesis of ion exchange resin (Patel and Gurnula, 2016) selective for Fe^{+3}, Cu^{+2}, Cd^{+2}, Zn^{+2} Ni^{+2} and Pb^{+2}.

MATERIALS AND METHOD

1. Chemicals and reagents

The important chemicals like phthalic acid (Chemocart), 1,5-naphthalenediamine and formaldehyde (S.D. Fine chemicals) used in preparation of PANDF terpolymer resin were procured from the market and were chemically pure grade and wherever necessary the purity was tested and confirmed by thin layer chromatography.

2. Synthesis of PANDF terpolymer resin

A mixture of phthalic acid (0.2 mol), and 1,5-naphthalene diamine (0.1 mol) and formaldehyde (0.3 mol) in molar ratio of 2:1:3 in the presence of glacial acetic acid medium was refluxed in an oil bath at 140 ^{0}C ± 2 for 6 h with occasional shaking to ensure thorough mixing. The temperature of electrically heated oil bath was controlled with the help of dimmer stat. The resulting mixture was then cooled, poured into crushed ice with constant stirring and left overnight. The brown coloured resin obtained. The separated terpolymer resin was washed with hot water and ethanol to remove unreacted starting materials and acid monomers. The product so obtained was further purified by reprecipitation technique. For this purpose, the terpolymer resin was dissolved in 8% aqueous sodium hydroxide solution, stirred well, filtered, and reprecipitated by gradual drop wise addition of ice cold 1:1 (v/v concentrated hydrochloric acid/distilled water) with constant and rapid stirring to avoid lump formation. The process of re-precipitation was repeated twice. The purified resin was finally ground well to pass through resin 300 mesh size sieve and kept in a vacuum over silica gel. The yield of these terpolymer resin were found to be 83 %. and the melting point found to be in the range of 383-388 K. The sieved resin was used for further characterization. The reaction sequence of the synthesis of PANDF terpolymer resin is shown in Fig. 1 and the composition determination of terpolymer was examined by elemental analysis. Since PANDF terpolymer resin contains (COOH) group it plays a key role in the ion exchange phenomenon, because of its higher tendency of capturing metal ions.

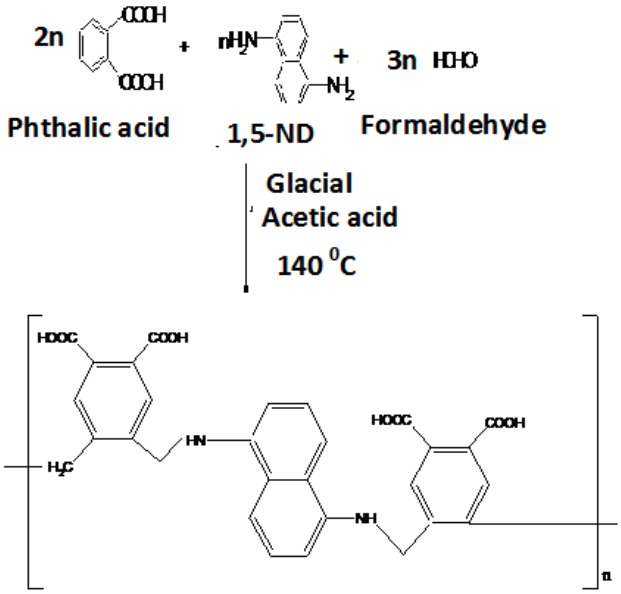

Fig.1. Reaction and suggested structure for PANDF terpolymer resin

3. Characterization of terpolymer resin

a. Physiochemical and Elemental Analysis

The terpolymer resin was subject to microanalysis for C, N and H present in the PANDF were determined by Perkin Elmer elemental analyser. The number average molecular weight $\overline{Mn}$ was determined by conducto-metric titration in DMSO medium using ethanolic KOH as the titrant by using 25 mg of sample. A plot of the specific conductance against the milliequivalents of potassium hydroxide required for neutralization of 100 g of terpolymer was made. Inspection of such a plot revealed that there were many breaks in the plot. From this plot the first break and the last break were noted. The calculations of $\overline{Mn}$ by this method is based on the following consideration. The first break corresponds to neutralization of the more acidic carboxylic group is neutralize and second break in the plot beyond which a continuous increase in conductance is observed

represents the stage at which carboxyl group of repeating units are neutralized completely. On the basis of the average degree of polymerization ($\overline{DP}$) is given by the following relation.

$$\overline{DP} = \frac{\text{Total milliequivalents of base for complete neutralization}}{\text{milliequivalents of base required for smallest interval}} \quad \ldots(1)$$

$\overline{Mn} = \overline{DP}$ x molecular weight of repeating unit

The intrinsic viscosity was determined by using a Tuan-Fuoss viscometer at six different concentration ranging from 3.0 % to 0.5 wt % of resin in DMSO at 32^{0}C. The intrinsic viscosity [η] was determined by using following Huggin's and Kramer's relation:

$$\eta rel = \eta_{sp}/C = [\eta] + K_1[\eta]^2 \cdot C \quad \ldots\ldots\ldots(2).$$

$$\ln \eta_r/C = [\eta] - [K_2[\eta]^2 \cdot C \quad \ldots\ldots\ldots\ldots\ldots(3).$$

b.Spectral and surface analysis

Electronic (UV-Visible) absorption spectra of the terpolymer was recorded at room temperature in the range 185-2600 nm using UV-Visible-NIR Spectrometer Hitachi 330, Perkin Elmer Spectrometer was used for recording FTIR spectrum of the terpolymer resin to identify the linkage and functional groups. The proton NMR spectrum of the PANDF terpolymer resin was recorded in DMSO- d6 solvent using BRUKER AVANCE II 400 NMR Spectrometer. Surface morphology of the terpolymers were studied by Scanning Electron Microscopy (SEM) Jeol 6390LV at Sophisticated Analytical Instrument Facility, STIC Cochin.

4.Ion-exchange properties

The ion-exchange properties of PANDF terpolymer resin was determined by the batch equilibrium method. We studies the influence of various electrolytes, the rate of metal uptake and distribution of metal ions between the terpolymer and solutions.

1.Determination of effect of different electrolytes on Metal ion uptake:

The ion exchange properties of PANDF resin was determined by batch equilibrium method. 25 mg offinely powdered resin was suspended in an electrolyte solution (25ml) of known concentration. The pH of the Solution was adjusted to required value by using either 0.1M HCl or 0.1M NaOH. The suspension was stirred for a period of 24 hours at room temperature. To this suspension 2ml of 0.1M solution of metal was added and the pH was adjusted to the required value. The mixture was again stirred at 25^{0}C for 24 hour and filtered. The polymer was washed and the filtered and washing were combined and estimated for the metal ion content by titrating against standard ethylene diaminetetra acetic acid. A blank experiment was also carried out in the same manner without adding the polymer sample to estimate the metal ion content. The amount of metal ion taken up by the resin in the presence of given electrolyte of known concentration was determined from the difference between the blank reading and the reading in the actual experiments. The experiment was repeated in the presence of several electrolytes of known concentration with four different metal ions such as Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+}.

2. Evaluation of the rate of metal ion uptake:

In order to estimate the time required to reach the state of equilibrium under given experimental conditions, a series of experiments of the type described above were carried out. The metal ion uptake by the chelating resins was estimated from time to time at room temperature at 25^{0}C. It was assumed that under given conditions, the state of equilibrium is established in the 24 hrs. The rate of metal ion uptake is expressed as percent of the amount of metal ion taken up after a certain time related to that in the state of equilibrium.

3. Evaluation of distribution of metal ion at different pH values:

The distribution of each of the metal ions Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+} between the resin phase and aqueous phase was estimated at 25^{0}C using 1M $NaNO_3$solution. The experiment was carried out as described above at different pH values.

4. Distribution ratios of metal ions at different pH :

The distribution of each one of four metal ions i.e. Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{2+} between the polymer phases and the aqueous phase was determined at 25 ^{o}C and in presence of 1 M $NaNO_3$ solution. The experiment was carried out as described earlier at different pH values. The distribution ratio "D"is defined the following relationship.

$$D = \frac{\text{Amount of metal ion on resin}}{\text{Amount of metal ion in solution}} \times \frac{\text{Volume of solution (mL)}}{\text{Weight of resin (g)}}$$

RESULTS AND DISCUSSION

1. Physicochemical studies

The brown coloured resin obtained. The terpolymer resin are found to be soluble in N, N-dimethylsulphoxide (DMSO), concentrated aqueous NaOH and KOH. The synthesized terpolymer was analysed for the % of Carbon, Hydrogen and nitrogen content. Based on the analytical data, the empirical formula of the terpolymer resin was found to be $C_{29}N_2O_8H_{23}.2H_2O$, which is in good agreement with the calculated values of C, H and N. The resin was analyzed for carbon, Hydrogen and nitrogen content C = 60.24 % (F) and 61.81% (cal); H = 4.11 % (F) and 4.79 % (cal); N = 4.20 % (F) and 4.79 % (cal).

The number average molecular weight $\overline{Mn}$ could be obtained by multiplying the $\overline{DP}$ by formula weight of repeating unit (Katkamwar *et al*., 2009). The calculated molecular weight for PANDF resin is 6851.

Viscometric measurement was carried out in DMSO at 32°C. The intrinsic viscosity of PANDF resins determined from both the plots is found to be identical. The intrinsic viscosity [η] was determined by using following Huggin's Eq. (2) and Kramers Eq. (3) which is 0.082 and 0.080, respectively. In accordance with the above relations, the plots of η_{sp}/C and $\ln\eta_r/C$ Vs C were found to be linear giving as slopes K_1 and K_2, respectively. Intercept on the axis of viscosity function gave the [η] value in both the plots (Kushwaha *et al*., 2012)). viscosity obtained from both the plots have been found to be in close agreement with each other.

2 Spectral and surface studies

The UV-visible spectrum of the PANDF [Fig. 2]. UV-Visible spectra of PANDF resins have been recorded in pure DMSO in the region of 200-800 nm. The spectra depicted two characteristic bands in the region of 260-290 band which may be accounted for a π→π* transition and 300 -320 nm may be due to n→π* transition. π→π* transition indicates the presence of aromatic nuclei and n→π* transition indicates presence of –COOH group. The allowed and forbidden transition of π→π* and n→π* transition.

FTIR spectra of PANDF is depicted in (Fig. 3). A band appeared in the region of 3237 cm^{-1} is assigned to the hydroxyl group of –COOH group present in aromatic ring which is involved in an intramolecular hydrogen bonding. The band obtained in the range of 2926 cm^{-1},1499 cm^{-1}, 606 cm^{-1} may be due to –NH- stretching, bending and deformation out of plane vibration of the amine moiety in resins respectively. The presence of methylene bridge ($-CH_2-$) in polymer chain may be assigned due to presence band at 1474 cm^{-1} ,1371 cm^{-1} and 790 cm^{-1} bending, wagging and rocking mode of vibration respectively. The sharp band displayed at 1623 cm^{-1} may be due to the stretching vibration of carbonyl group. The 1,2,4,5 tetra substitution of aromatic benzene ring confirmed by sharp/ medium/ weak absorption bands appeared between 1283-914 cm^{-1}. The >C-O stretch in phenol is ascribed due to the presence of band at 1430 cm^{-1}. ^{1}HNMR spectra of newly synthesized PANDF resins [Fig. 4]. The signal in the region of 7.2-7.76(δ) ppm is assigned to all the protons of aromatic ring.

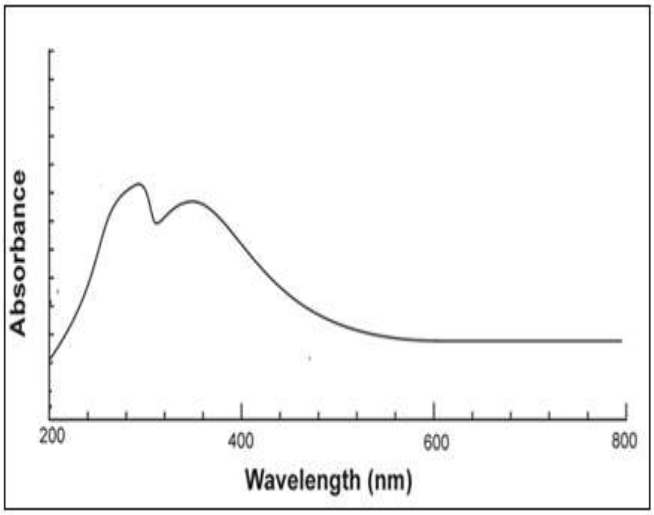

Fig 2. Electronic spectra of PAND terpolymer resin

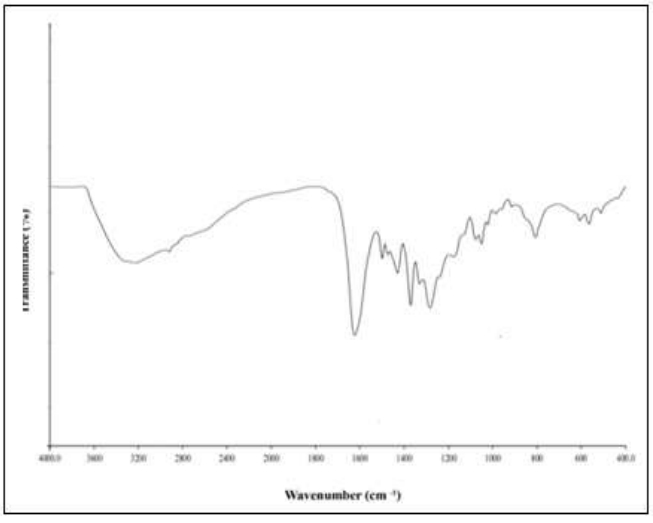

Fig. 3 : IR-spectral data of PANDF terpolymer resin

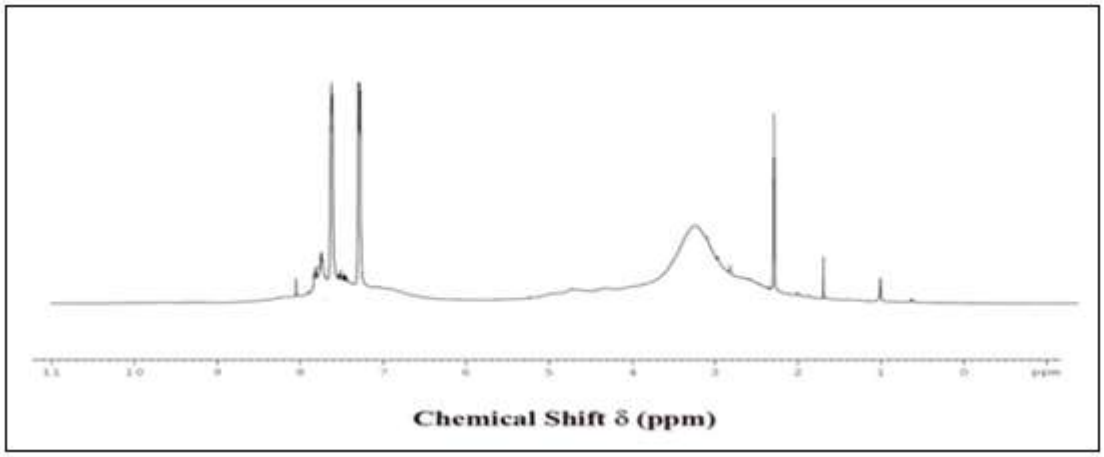

Fig. 4 : NMR-spectral data of PANDF terpolymer resin

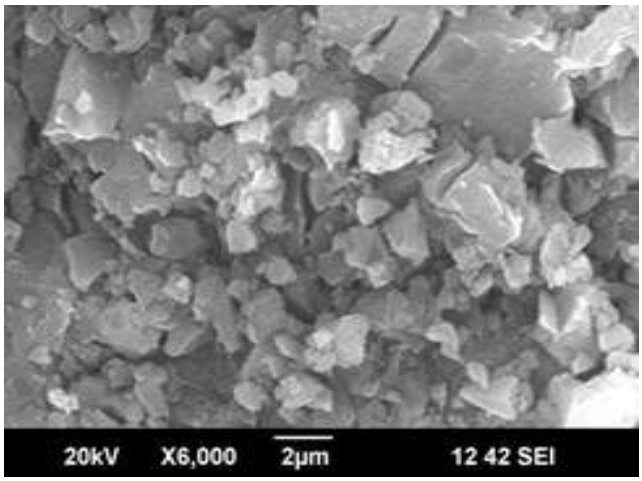

Fig. 5 : SEM image of PANDF terpolymer resin

The signal appeared in the region of 7.9-8.05 (δ) ppm is due to the –NH bridge present in the resin. The methylenic protons of Ar-CH_2-NH- moiety may be recognized as signal appearing in the region 2.51 (δ) ppm.

Surface analysis has found great use in understanding the surface features of the materials. The morphology of the reported resin sample was investigated by scanning electron micrographs at different magnification, which is shown in (Fig.5). It gives the information of surface topology and defect in the structure. The morphology of resin shows a fringed micelle model of the crystalline-amorphous structure. The extent of crystalline character depends on the acidic nature of the monomer. The micrograph of PANDF- shows the presence of crystalline-amorphous layered morphology which is the characteristic of polymer. The monomer have crystalline structure but during condensation polymerization of some crystalline lost into amorphous nature, hence shows higher metal ion exchange capacity.

3. Ion Exchange Properties

With a view to ascertain the selectivity of the terpolymer resin for the selected metal ions, we have studied, the influence of various electrolyte on the selectivity of metal ions, the rate of metal uptake and distribution ratio of metal ions between the terpolymer and solution containing metal ions , are analyzed by using the batch equilibrium method. Data of experimental procedure for EDTA titration is presented in Table.1.

The PANDF terpolymer (Fig. 1.) shows that the group –COOH and –NH contain lone pair of electrons, which can be donated to the metal ion during complex formation. Hence it shows chelating behavior. When the polymer is suspended in metal ion solution, the chelating tendency of terpolymer forms the cyclic complex with metal ion, which absorb the metal ion from the solution to the surface of the polymer. This mechanism of adsorption of metal ion by polymer ligands is known as metal uptake of polymer. As the metal uptake concentration of metal ion in solution decreases, this can be determined by titration with standard EDTA solution. The metal uptake capacity of polymer is different for different metal ion, is known as selectivity of polymer towards the uptake of metal ion. The metal uptake of terpolymer depends on three variables, concentration of electrolyte solution, time and pH of the solution. The chelating behavior of PANDF terpolymer was studies with these three variables by keeping two variable constant at each time.

Polymer + metal ion solution + shaking ⟶
Polymer –metal ion chelate

Batch equilibrium technique developed by Gregor and DeGeiso was used to study of ion exchange property of PANDF terpolymer resin. The result of batch equilibrium study carried out with the terpolymer resin PANDF is presented in Table.2-4. Four metal ions Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{+2} in the form of aqueous metal nitrate solution were used. The ion exchange study was carried out using three experimental variables: a) electrolyte and its ionic strength b) shaking time and c) pH of the aqueous medium. Among three variables two were kept constant and only one was varied at a time to evaluate its effect on metal uptake capacity of the polymer.

3.1. Effect of electrolytes and their concentration on the metal ion uptake capacity

The effect of different concentrations of chloride (Cl^-), nitrate (NO_3^-) and sulfate (SO_4^{2-}) ions in the electrolytes under equilibrium metal–resin interaction conditions were studied. The amount of metal ion taken up by a given amount of terpolymer resin depends on the nature and concentrations of the electrolyte present in (Table.2.). The anions present in the electrolyte are also of vital importance in the metal ion adsorption process. If the electrolyte ligand is capable of forming strong chelates with the metal ions, the availability of metal ions in the solution will decrease (Jadhao *et al*., 2006), Azarudeen and Burkanudeen., 2012). Hence metal ion uptake by the terpolymer decreases. If the anion forms weak chelates with the metal ions, the availability of the metal ions increases in solution. Furthermore, the polymer is capable of breaking the electrolyte ligand–metal chelate bond which leads to increased availability of the metal ions for adsorption. Among the three electrolytes, the sulfate ($SO4^{2-}$) has the capacity to donate more electrons during the complex formation.

Therefore, the sulfate ($SO4^{2-}$) ion-containing electrolyte forms strong and stable complexes, hence the number of metal ions available for polymer adsorption decreases. Compared with the sulfates, the other anions form weak complexes, therefore metal ion uptake increases. However, uptake of other metal ions, for example Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{+2} decreases on increasing the concentrations of chloride (Cl^-) and nitrate (NO_3^-) electrolytes at higher pH. At higher pH, these ligands form strong complexes with the Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{+2}metal ions.

3.2 Evaluation of metal ion uptake at different pH

The distribution of each of the metal ions between the polymer phase and the aqueous phase was determined at room temperature in the presence of 1 M $NaNO_3$ at pH ranging from 1.5 to 6 (Table. 3.). The study was limited to a maximum pH of 6 to avoid any hydrolysis of the metal ion. The metal hydroxide, if formed, will interfere withthe ion-exchange process. The amount of metal ion uptake by the terpolymer at different pH was calculated to optimize the exact pH at which the terpolymer takes most metal ion. The metal ions, for example Co^{2+}, Hg^{2+}, Cd^{2+} and Pb^{+2} have low distribution ratios. The distribution ratio for all the metal ions depends on the stability constants of their complexation during the ion-exchange process. At higher distribution ratios of the metal ions, the stability constants of the ligand and metal complexation will be higher. metal ions $Hg^{2+}$and Pb^{+2} form weak chelates over a wide range of pH. The order of the distribution ratios of the metal ions with the PANDF terpolymer was Co^{2+}>Cd^{2+}>Pb^{2+}>Hg^{2+}.

3.3. Rate of metal ion uptake as a function of time

The study was carried out to determine the time at which a state of equilibrium between the polymer and the metal ion solution is attained. Generally, the rate of metal ion uptake depends upon the ionic size of the metal ions (Table.4.). It is found that Cd^{2+} and Pb^{2+} ions required almost 6 h, Co^{2+} required 5 h and Hg^{2+} required 7 h. Thus, the rate of metal ion uptake follows the order Co^{2+}>Cd^{2+}>Pb^{2+}>Hg^{2+}.

Table 1: Data of experimental procedure for direct EDTA titration.

Metal Ion	Type of titration	pH	Buffer	Indicator	Colour change
Pb^{2+}	Direct	5.5	Hexamine	Xylenol orange	Red-yellow
Co^{2+}	Direct	5.0	Hexamine	Xylenol orange	Red-yellow
Hg^{2+}	Direct	6.0	Hexamine	Xylenol orange	Red-yellow
Cd^{2+}	Direct	5.5	Hexamine	Xylenol orange	Red-yellow

Table 2: Evaluation of the influence of different electrolyte on the uptake of several metal ions of PANDF Terpolymer resins

Metal Ion	Electrolyte Conc. (mol/lit)	Weight of the metal ion (mg) taken up in presence of		
		NaCl	$NaNO_3$	Na_2SO_4
		PANDF	PANDF	PANDF
Co^{2+}	0.01	1.99	1.79	1.70
	0.05	1.19	1.22	1.40
	0.1	1.10	1.50	1.02
	0.5	0.39	0.92	0.79
	1.0	0.35	0.41	0.32
Cd^{2+}	0.01	1.82	1.69	1.62
	0.05	1.40	1.29	1.34
	0.1	0.84	1.20	1.20
	0.5	0.57	0.99	0.70
	1.0	0.34	0.59	0.25
Pb^{2+}	0.01	1.30	1.62	-
	0.05	1.19	1.00	-
	0.1	1.10	0.97	-
	0.5	0.82	0.67	-
	1.0	0.49	0.29	-
Hg^{2+}	0.01	0.70	0.99	-
	0.05	0.21	0.89	-
	0.1	0.19	0.81	-
	0.5	0.14	0.61	-
	1.0	0.12	0.19	-

[a]$[M(NO_3)_2]$ = 0.1 mol/L, Volume electrolyte solution = 25 mL
Volume of metal ion solution =2 mL, weight of resin, Time = 24 h, Room temperature,
pH value : Co^{2+} = 5, (Cd^{2+} ,Pb^{2+}) = 5.5, Hg^{2+} = 6.

Table 3 : Distribution Ratio D[a] Of Different Metal Ions As Function Of The pH Of PANDF Terpolymer Resins

Metal Ion	Terpolymer	Distribution ratio of metal ions [b] at various pH								
		1.5	1.75	2.0	2.5	3	3.5	4	5	6
Co^{2+}	PANDF	-	-	-	39.45	121.40	182.72	219.10	240.74	284.50
Cd^{2+}	PANDF	-	-	-	29.40	40.21	110.26	184.00	212.24	230.40
Pb^{2+}	PANDF	-			31.40	40.00	90.10	130.12	201.01	201.24
Hg^{2+}	PANDF	-	-		-	-	10.43	21.04	41.19	112.2

[a]D = weight (in mg) of metal ions taken up by 1g of terpolymer/ weight (in mg) of metal ions present in 1 mL of solution.
$[M(NO_3]_2]$ = 0.1 mol/L; volume : 2 ml; $NaNO_3$ = 1.0 mol/L, volume =25, time 24 h (equilibrium state) at Room temperature.

Table 4 : Comparision Of the Rate Of Metal Ion Uptake Of PANDF Terpolymer

Metal Ion	Terpolymer	Percentage of the amount of metal ion uptake at different (hr)						
		1	2	3	4	5	6	7
Co^{2+}	PANDF	34.0	50.2	60.3	79.2	92.3	-	-
Cd^{2+}	PANDF	19.4	24.4	42.4	66.2	72.1	76.2	-
Pb^{2+}	PANDF	19.4	29.4	39.4	66.4	74.8	75.0	-
Hg^{2+}	PANDF	-	-	30.6	51.2	61.3	80.5	92.4

[a]$[M(NO_3)_2]$ = 0.1 mol/lit, $NaNO_3$ = 1.0 mol/L, Volume $NaNO_3$ = 25 mL, Volume of metal ion solution =2 mL

CONCLUSION

An eco-friendly terpolymer resin PANDF has been synthesized. The structure of the resin was clearly elucidated by use of a variety of spectral techniques. Empirical formula and average molecular weights were also determined. The surface of the terpolymer resin was found to be more amorphous than crystalline in nature, clearly indicating the suitability of the synthesized resin for ion-exchange applications.

Acknowledgements:
Author is thankful to Head of the Department of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for providing Laboratory facilities.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:17-22
UGC Approved Journal No 48951

Original Article Open Access

Synthesis, Characterization and Thermal Degradation Studies of Terpolymer resin derived from 2,6-dihydroxyacetophenone, ethylenediamine and formaldehyde

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Niley Sanjay N, Kariya Kiran P and Berad Baliram N (2018) Synthesis, Characterization and Thermal Degradation Studies of Terpolymer resin derived from 2,6-dihydroxyacetophenone, ethylenediamine and formaldehyde, *Int. J. of Life Sciences*, Special Issue, A12: 17-22.

ABSTRACT

The terpolymer resin has been synthesized by the condensation of 2, 6-dihydroxyacetophenone, ethylenediamine and formaldehyde in the presence of 2M HCl as catalyst with 1:1:2 molar ratio of reacting monomers. Composition of terpolymer resin was determined on the basis of elemental analysis. The number average molecular weight of the terpolymer was determined by conductometric titration in non aqueous medium. Viscosity measurements were carried out to ascertain the characteristics functions and constants of terpolymer resin. The terpolymer resin was further characterized by UV-Visible absorption spectra, IR and NMR spectra. Thermal degradation curve has been discussed in order to determine its mode of decomposition, order of reaction, activation energy, frequency factor, entropy change, free energy change and apparent entropy change. Kinetic parameters were calculated by using Freeman-Carroll and Sharp-Wentworth methods. The data obtained from Freeman-Carroll method was used to determine various thermodynamic parameters. Synthesized terpolymer shows antibacterial activity.

Keywords: Synthesis, Characterization, Thermal degradation, Antibacterial activity

INTRODUCTION

The use of polymers in all spheres of life has been abundantly increased in recent years. Various workers have pressing demand to synthesize eco-friendly polymers having some biological activities like antimicrobial. The study of the thermal degradation of terpolymer resins have recently became a subject of interest. Polymer additives improve manufacture process and product quality. It can form continuous phase of coating with no deleterious effects on coating, and having better thermal stability (Chauhan *et al*. 2010).

Terpolymer resins, having good thermal stability, have enhanced study of the development of polymeric materials. Terpolymers of substituted acetophenone with formaldehyde/ furfuraldehyde have shown excellent thermal and antimicrobial activity (Chauhan *et al*. 2011). The synthesis of functional terpolymer has gained a lot of attention in recent years due to their applications in adhesives, coating materials, semiconductor, catalyst, flame resistant fibers, ion exchange, and thermally stable and biologically active resins. However, the insolubility and instability of terpolymers have limited their practical applications, but attachment of appro-priate pendant groups or constituents to the polymer backbone cannot only help to improve the processability and stability but also equip it with new functionalities (Vega *et al*. 2006 and Panday and Srivastava, 2002). The research of activated oxime-based monomer and its polymer is focused mainly on maleimide. Soykan and Erol (2003) have reported a synthesis of maleimide monomer N-(4-acetylphenyl) maleimide (NAPMI), and their oxime, carbazone, and thiosemicarbazone derivatives of NAPMI having excellent thermal stability and antimicrobial activity. Such multifunctional poly-mers have been receiving an increasing attention in material science and life science. Hemvichian and Ishida (2002) studied the degradation process of polybenzox-azine in common type (PBA-a) by using TGA and GCeMS. Jadhao *et al*. studied the thermal degradation of terpolymer resins derived from 2,2-dihydroxybiphenyl, Urea, and Formaldehyde (Jadhao *et al*. 2006) Terpoly-mers are useful material in fabrication due to flexibility, chemical inertness as well as being light in weight. Polymers with highly conjugated chain have attracted much attention in the last few years because they are materials of electronics (Singru and Gurnule, 2010, Diaz *et al*., 1999, Suh and Shim, 2000).

MATERIALS AND METHODS

Materials

Solvents like dimethyl formamide and di-methyl-sulph-oxide were used after distillation. 2,6- Di-hydroxy-aceto-phenone ethylene diamine, and Formaldehyde 37% were purchased of market and are from Merck (Maharashtra, India). All other chemicals used were of chemically pure grade.

Synthesis of Terpolymer Resin:

A mixture of 2,6-Dihydroxyacetophenone (0.1 mol), ethylenediamine (0.1 mol) and formaldehyde (0.2 mol) in molar ratio of 1:1:2 in the presence of 2M (200 mL) HCl as a catalyst has been prepared in round bottom flask. The resultant mixture was refluxed over an oil bath at 110ºC ± 2ºC for 5 hrs with occasional shaking to ensure thorough mixing. The temperature of oil bath was controlled electrically with the help of dimmerstat. The resinous black solid mass obtained was immediately removed from the flask as soon as the reaction period was over. The separated terpolymer resin was washed with hot water and ethanol to remove unreacted starting materials and monomers. The properly washed resin was dried, powdered and then extracted with chloroform to remove 2,6-Dihydroxyacetophenone - formaldehyde copolymer which might be present along with 2,6-DHAEDF-I terpolymer and then it is purified. Excellent yield of terpolymer resin was obtained by this reaction [Fig.1].

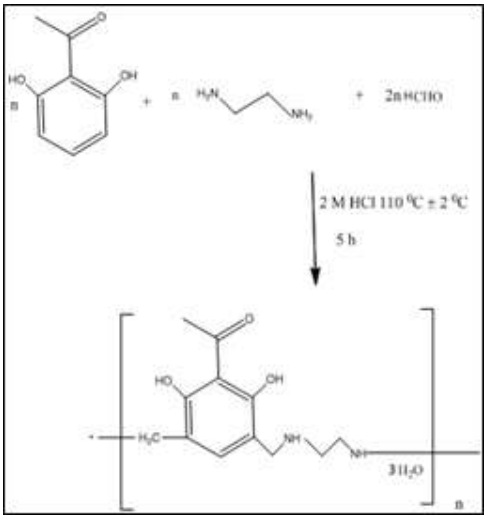

Fig. 1: Reaction and suggested structure of 2,6-DHAEDF-I terpolymer resin

Antibacterial Activities:

Agar diffusion method was used for antibacterial studies. Nutrient agar medium was used for culture of the bacteria. The composition was peptone (10.0 g), sodium chloride (10.0 g), yeast extract (5.0 g) and agar (20.0g) in 1000 ml of distilled water. 20 mg sample was dissolved in 1 ml of dimethylsulphoxide solvent and Ciprofloxacin standard antibiotic of known concentration was used for analysis. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 ^{0}C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hrs. Old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control wells with Ciprofloxacin

were also prepared. All the plates were incubated at 37^0C for 24 hrs and the diameter of inhibition zone were noted. Concentration of samples for antibacterial activity was taken from 100-1000µg/ml. The antibacterial activities of the terpolymer were screened on various bacteria at these concentrations. The synthesized polymer has shown moderate / poor antibacterial activities as compared to standard Ciprofloxacin.

Spectral and elemental analysis:

Electronic absorption spectra of the terpolymer was recorded at room temperature in the range 200-800 nm using UV-Visible-NIR Spectrometer Hitachi 330, Perkin Elmer Spectrometer was used for recording FTIR spectrum of the terpolymer resin to identify the linkage and functional groups. The proton NMR spectrum of the 2,6-DHAEDF-I terpolymer resin was recorded in DMSO-d6 solvent using BRUKER AVANCE II 400 NMR Spectrometer. Intrinsic viscosity (η) was measured in DMSO at 32 ^{o}C using Tuan Fuoss Viscometer. Molecular weight determined by non-aqueous conductometric titration using DMSO. Surface morphology of the terpolymers was studied by Scanning Electron Microscopy (SEM) Jeol 6390LV at Sophisticated Analytical Instrument Facility, STIC Cochin. The elements such as C, N and H present in the 2,6-DHAEDF-I were determined by Perkin Elmer elemental analyser.

Thermogravimetric analysis:

The modes of thermal degradation of the terpolymer 2,6-DHAEDF-I was analysed using thermogravimetric analyser (Diamond TG/DTA thermal analyser) at heating rate of 10^oC/ min in static air atmosphere. Based on the results obtained, the degradation pattern, activation energy (Ea), order of reaction (n), entropy change (ΔS). Free energy change (ΔF), apparent entropy (S*) and frequency factor (Z) were calculated by Freeman-Carroll (Freeman and Caroll, 1958) and Sharp-Wentworth methods (Sharp and Wentworth 1969).

RESULTS AND DISCUSSION

Spectral Analysis

Solubility:

The 2,6-dihydroxyacetophenone ethylenediamine with formaldehyde terpolymer resin was soluble in solvents like N, N-dimethyl formamide (DMF), dimethylsulphoxide (DMSO) and concentrated aqueous NaOH and KOH, whereas resin was insoluble in toluene, xylene and benzene. The elements such as carbon (%C), hydrogen (% H), and nitrogen (% N) contents were analysed for the 2,6-DHAEDF-I terpolymer resin. Based on the analytical data, the empirical formula of the repeating unit for the 2,6-DHAEDF-I resin was found to be $C_{12}N_2O_3H_{16}.3H_2O$.

FTIR Spectra:

The recorded FTIR spectrum of the terpolymer resin is shown in Fig.2. The spectrum shows a broad band at 3282.55 cm^{-1} due to (-OH) stretching of Ar-OH involved in the intramolecular hydrogen bonding. The band at 3150.00 cm^{-1} is due to NH-stretching of amino group, this band seems to be merged with –OH group present in the resin. The pentasubstitution in the benzene ring is established by the presence of medium bands at 813.77 cm^{-1}. The band at 1711.00 cm^{-1} may be assigned to (>C=O) stretching of ketonic group.

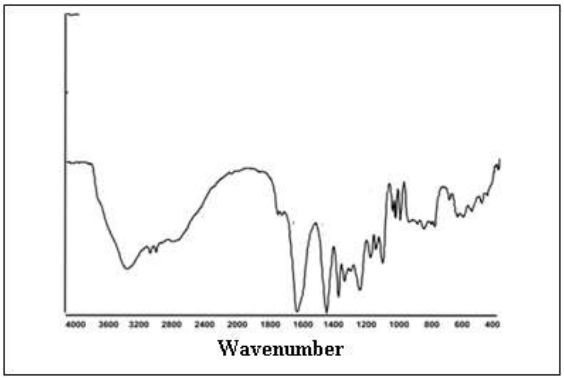

Fig. 2: IR Spectra for 2,6 DHAEDF-I terpolymer resin

^{1}H-NMR:

^{1}H-NMR spectrum of 2,6-DHAEDF-I terpolymer resin shown in Fig.3. The NMR spectrum revels that the signal around 3.57 δ (ppm) are due to the methylenic protons of the Ar-CH_2-NH- linkage. The multiplate signals observed in the range at 6.70 δ (ppm) indicates the presence of aromatic protons. The signal displayed at 11.25δ (ppm) may be due to the phenolic protons (Ar-OH).

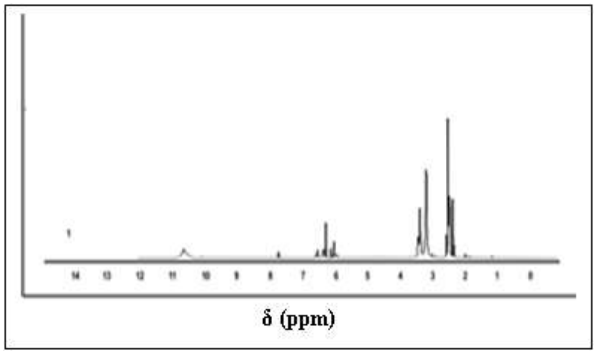

Fig 3: ^{1}H-NMR spectra of 2,6 DHAEDF-I Terpolymer Resin

A weak signal appeared in the region 6.36δ (ppm) is assigned to the protons of –NH- bridge. The methyl proton of the Ar-CO-CH_3 moiety may be identified by the intense peak at 2.51-2.61 δ ppm.

UV-Visible spectra:

UV-Visible spectra of 2,6 DHAEDF-I in DMSO were recorded in the range of 200-800 nm. UV-Visible spectrum of the terpolymer resin is shown in Fig. 4. The spectrum exhibits two characteristic bands at 355 nm and 263 nm. These observed positions for the absorption bands have different intensities. The band at 263 nm intense band which may be accounted for a π→π* transition While the less intense band at 355 nm, is due to n→π* transition.

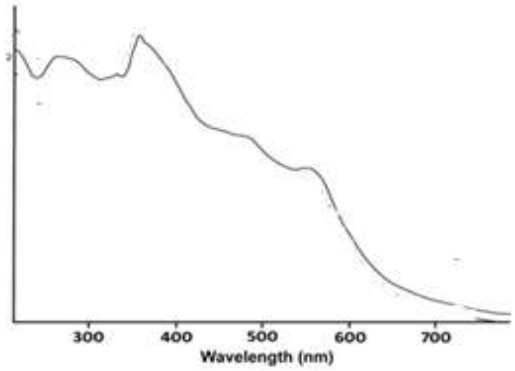

Fig. 4: UV-Visible spectrum for 2,6-DHAEDF-I Terpolymer

Formula for calculating thermodynamic parameters using Freeman-Carroll method.

Entropy Change (ΔS)

Intercept= $\frac{\log kR}{h\Phi Ea} + \frac{\Delta S}{2.303R}$ [1]

Where, k=1.3806x10^{16}erg/deg/mole
R=1.987cal/deg/mole
(8.314J/K/mol)
h = 6.625 x 10^{-27} erg sec
ϕ = 0.166
ΔS= change in entropy

Free energy change (ΔF)

ΔF = ΔH - TΔS [2]

Where, ΔH = Enthalpy change = Activation energy
T = Temperature in K
ΔS = Entropy change {from [1] used}

Frequency Factor (Z)

$$B_{2/3} = \frac{\log Z E_a}{R\Phi} \quad \textbf{[3]}$$

$$B_{2/3} = \log 3 + \log[1 - 3\sqrt{1-\alpha}] - \log\ p(x) \quad \textbf{[4]}$$

Where, Z = Frequency factor
B = Calculated from equation [4]
log p(x) = Calculated from Doyle's graph
α =degree of transformation [α= w/W_c]

Apparent entropy (S*)

$S^* = 2.303 \log \frac{Zh}{kT^*}$ [5]

Where,
Z = from relation [3]
T* = Temperature at which half of the compound is decomposed from it total loss.

Table 1 shows thermal degradation behavior of the terpolymer and Fig.5 shows TGA-DTA curve. Thermal activation energy plot and Freeman Carroll plots for 2,6-DHAEDF-I terpolymer resin are shown in Fig 6. and Fig. 7 respectively. Kinetics parameter such as entropy change (ΔS), free energy change (ΔF), apparent entropy (S*) and frequency factor (Z) were calculated based on the thermal activation energy the expression shown in equation [1], [2], [3] and [4]. Using the Freeman-Carroll and Sharp-Wentworth methods, the kinetic parameters were calculated and present in table.2. The activation energy values calculated from FC and SW are in good agreement with each other. The low frequency factor value predicts that the degradation reaction is slow and no other possible reason can be given (Jacobs and Eompkins, 1955 and Ozawa, 1985). This is further supported by negative value of the entropy change which suggests more ordered structure for activated terpolymer than reactants. However, a few points do not fall on straight line in Fig 6. which show that the reaction does not obeys the first order kinetics perfectly.

Antibacterial Activity

In order to explore antibacterial activity of 2,6-DHAEDF-I resin have been tested for antibacterial activity against *Bacillus substilus*, *Escherchia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and terpolymer shows moderate antibacterial activity against *P. aeruginosa, Bacillus substilus*, *Escherchia coli*, *Staphylococcus aureus* shows poor antibacterial activity (Table 3).

Table 1: Thermal degradation behaviour of 2,6 DHAEDF-I Terpolymer Resin

Terpolymer	Temperature Range (^{0}C)	Stage of Decomposition (DTA Peak)	Species degraded	% mass loss	
				Found	Calc.
2,6 DHAEDF-I	R.T –317 ^{0}C	First	Loss of $3H_2O$ molecule	11.65	12.05
2,6-DHAEDF-I	317 – 557 ^{0}C	Second (Exo, b)	Loss of side chain (-CH_2-NH-CH_2-CH_2-NH-,$COCH_3$,2OH,-CH_2) and partial degradation of aromatic nucleus	91.66	91.72

Table 2: Results of thermogravimetric analysis of 2,6 DHAEDF-I

Terpolymer	Decomposition Temp. (^{0}C)	Half Decomposition Temp. (^{0}C)	Activation Energy kJ/mole		Kinetic Parameters by FC				
			FC	SW	ΔS (J)	ΔF(kJ)	Z (S^{-1})	S* (J)	n
2,6 DHAEDF-I	317^{0}C	437	31.94	33.30	-165.73	44.11	39.11	-30.26	0.90

Table 3: Relative antibacterial activity of 2,6-DHAEDF-I terpolymer

Sr. no.	Terpolymers	Concentration screened (µg/mL)	Diameter of inhibition zones in mm			
			E. coli	*P. aeruginosa*	*S .aureus*	*B. subtilis*
1	2,6 DHAEDF-I	100	NF	NF	NF	NF
		250	NF	NF	NF	NF
		500	NF	NF	NF	NF
		1000	4.0	4.0	4.0	4.0
2	Ciprofloxacin (Standard)	100	23	01	21	15
		250	26	03	25	19
		500	28	08	27	22
		1000	31	14	34	25

***NF-** Not found.

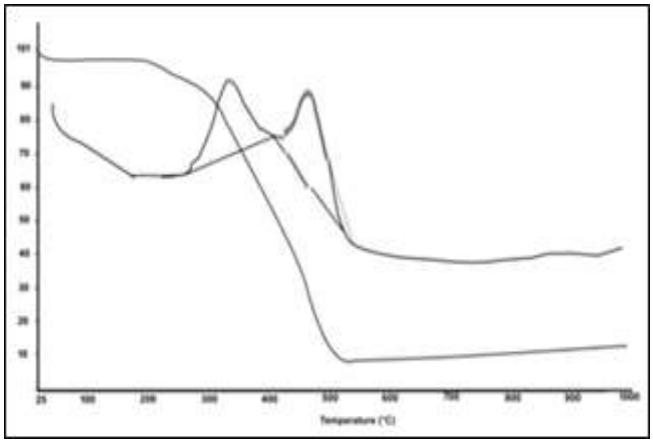

Fig. 5: 2,6 DHAEDF-I TGA-DTA curve

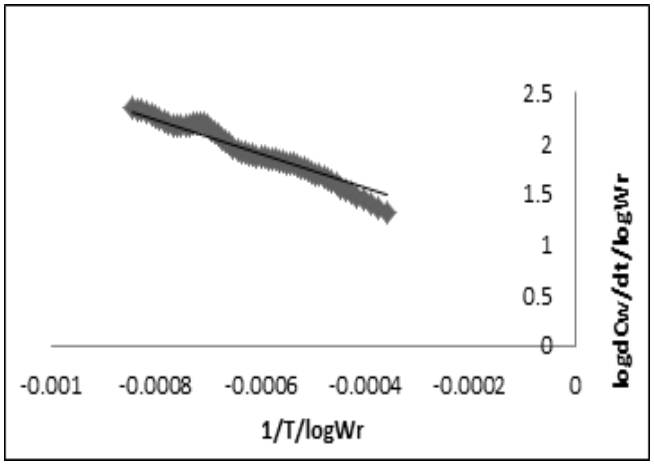

Fig. 6: Thermal Activation Energy Plot of 2,6 DHAEDF-I Terpolymer Resin.

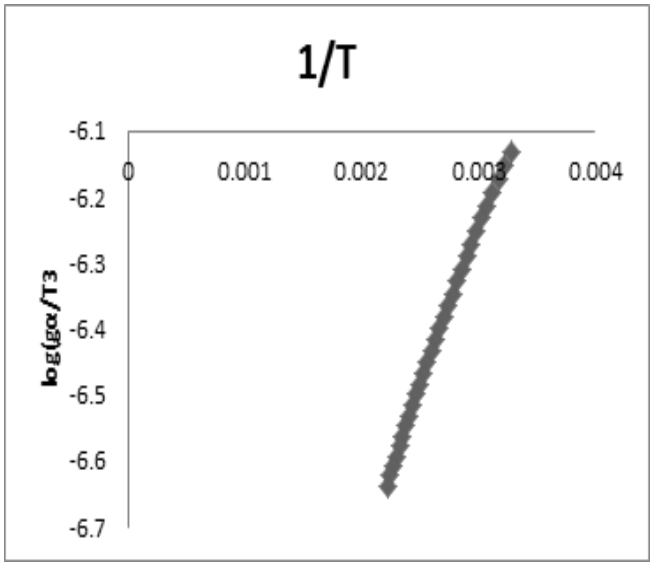

Fig. 7: Freeman-Carroll plot of 2,6-DHAEDF-I-I Terpolymer Resin

CONCLUSION

Terpolymer involving 2,6-dihydroxyacetophenone, ethylenediamine and formaldehyde was synthesized in the presence of hydrochloric acid as a catalyst by condensation reaction at 110± 2 ^{0}C for 5 hours. The spectral characterizations of the terpolymer confirm the linear structure. TGA curve shows that the terpolymer resin had good thermal stability. The activation energy calculated for the resin by Freeman-Carroll and Sharp-Wentworth methods was found to be in good agreement with each other. The low frequency factor and the negative entropy values calculated from Freeman–Carroll method suggested that the thermal decomposition would be a slow reaction. The thermal degradation kinetics indicate that 2,6 DHAEDF-I terpolymer shows one step degradation after loss of water molecule. The 2,6 DHAEDF-I terpolymer resin shows moderate/poor antibacterial activity against all bacteria screened and no antifungal activity againt *C. albicans* and *A. niger*.

Acknowledgement:
Author is thankful to Head of the Department of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for providing Laboratory facilities.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:23-28
UGC Approved Journal No 48951

Original Article Open Access

Synthesis, characterization and biological activities of Schiff base and it's transition metal complexes

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Mahendra Pratap Singh (2018) Synthesis, characterization and biological activities of Schiff base and it's transition metal complexes, *Int. J. of. Life Sciences*, Special Issue, A12: 23-28.

ABSTRACT

Biivalent transition metal Mn(II), CO(II), Ni(II) and Cu(II) complexes of 3,4-{[-benze 1-sulphacetamide] imino] methyl} furan complexes derived from heterocyclic furfural and aniline sulphacetamide, have been synthesized and characterized by elemental, magnetic moment and electrochemical such as electronic spectra, IR Spectra and ESR Spectral studies. The ligand and metal complexes were screened for biological activity against Gram (+) and Gram (-) bacteria by the agar well diffusion method.

Keywords: Schiff base, Transition Metal Complexes, Spectral Studies, Antibacterial Studies, hetrocycles.

INTRODUCTION

Compound containing hetrocyclic structures have high degree of binding affinity to biological system (Rajiv et al, 2011). The metal complexes of Schiff bases showed higher biological activity in composition to Schiff bases. A large number of Schiff base transition metal complexes have been synthesized, which showed antibacterial, antimalerial, antileukemia activity, DNA binding, DNA cleavage, anticancer and antioxidant activities (Ebrahimpour et al, 2015; Rosu et al, 2010; Tabassum et al, 2013; Wu et al, 2015). Schiff base containing hetrocycles possess hypnotic activity (Vachala et al, 2012). In past few decades, researchers have focused towards transition metal complexes of heterocyclic aromatic Schiff bases having, nitrogen, oxygen and sulphur donar atoms are due to their therepentic importance (Sridhar et al, 2001; Valverde et al, 2005). Its complexes have also been found to be active against HIV and tumor cells, as good anti-tubercular, anti-inflammatary, anticoagulant, anticonvulsant and as chemotherepentic agents in cancer and infectious disease research (Malathy et al, 2015; Osowole et al, 2012; 2010a; 2011b; 2012c). According to the search material, no work has been carried out on this ligand and its transition metal complexes. I herein report the synthesis, characterization and biological activity of new Schiff

base ligand 3,4{[-benzene 1-sulphacetamide] imino} methyl} furan and its bivalent transition metal Mn(II), CO(II), Ni(II) and Cu(II) complexes. In present study, metal co-ordination with ligand, takes place via imino nitrogen and the oxygen of sulphacetamde >C=O group. Ligand and all complexes are characterized by elemental, magnetic moment and spectral data studies.

METHODS AND MATERIALS:

All the chemicals used were of analytical and GR-grade and purchaged either from BDH, E-Merk, S.D.Fine's and Sisco chemical industries, Bombay. The solvents and liquid reagents were carefully purified by distillation, while solid reagents and metal salts were used as such. The complexes were recrystallized with the solvents, depending upon its solubilities, and its purity was checked by TLC.

Physical measurements:

Magnetic susceptibility measurements of the prepared complexes were carried out on EG & G model 155 VSM at room temperature. The infrared spectra of the Schiff base, 3,4- {[-benzens 1-sulphacetamide] imino] methyl} furan and its complexes were recorded on Perkin Elmer Spectrometer in the FT-IR region using KBr Pellets. The electronic spectra in solution EtoH/DMF were recorded on ELICO SL 171 Spectrophotometer at room temperature. Mass spectra of the prepared Schiff base ligand was taken on MASPEC System (MSW/9629) using 200^{o}C intel temperature. The ESR spectra of Cu(II) complex was recorded on a varian X-band spectrometer E_4. An elemental analysis of carbon, hydrogen and nitrogen was done on at RSIC, Chandigarh. Estimation of sulphur in ligand and complexes were determined by standard method (Weicher, 1965) and estimation of halogen was estimated by Volhard's method (Weicher, 1965a). Metal content of complexes were determined by standard methods (Flascuke, 1954; Kalthof et al, 1963; Tradwell, 1968; Vogel, 1961; Wathrich, 1965).

Synthesis of 3,4- {[-benzene 1-sulphacetamide] imino] methyl} furan (SB_1) $C_{13}H_{12}N_2O_4S$

An equimolar solution of the titeled Schiff base was prepared by refluxing equimolar solution of aniline-sulphacetamide (10mmol, 2.14g in 25ml alcohol) and 2-furfuraldehyde (10mmol, 0.96ml in 25ml alcohol) on water bath for 3-4 hour and then cooling, a yellow solution resulted, was stirred for 2-3 hour. The solvent was slowly evaporated. A solid colored powder is obtained and washed with ethanol and dried in vaccum. Light yellow crystals were obtained.

Synthesis of metal complexes

The metal chloride (5mmol) in 25ml ethanol was added slowly to a solution of the Schiff base ligand (10mmol in 25ml ethanol). The resulting mixture was stirred for 30 minutes and then refluxes for 2-3 hour on water bath. The product was cooled and solvent was slowly evaporated, washed with ethanol, acetone and ether, dried in vacuum. The different colored crystals of different complexes with metal salts and ligand in 1:2 metal: ligand molar ratio have been isolated as $[Mn(SB_1)_2Cl_2]$, $[CO(SB_1)_2Cl_2]$, $[Ni(SB_1)_2Cl_2]$ and $[Cu(SB_1)_2Cl_2]$.

RESULTS AND DISCUSSION

The all prepared metal complexes were stable in dry air. They are soluble in DMF/DMSO but insoluble in most organic solvents. They were characterized with the help of physic-chemical methods such as magnetic susceptibility measurements, electronic spectra, IR spectra, ESR (Cu complex only) and elemental analysis. The analytical data and magnetic moment values are listed in table-1

Table-1 Analytical data and magnetic moment values of synthesized ligand and it's transition metal (II) complexes.

S.N.	Compound	Colour	Percentage of Elements (Found) Calculated							μ(B.M.)
			C%	H%	O%	N%	S%	Cl%	M%	
1	SB_1	Light Yellow	52.89 53.02	4.38 4.46	21.80 21.74	9.54 9.58	10.98 10.96	- -	- -	
2	$Mn(SB_1)_2Cl_2$	Yellowish Brown	43.58 43.64	3.85 3.92	18.02 17.94	7.96 7.92	8.98 8.96	9.92 9.88	7.82 7.78	5.74
3	$CO(SB_1)_2Cl_2$	Pink	43.54 43.50	3.90 3.86	17.78 17.82	7.80 7.81	8.90 8.94	9.84 9.86	8.36 8.26	4.5
4	$Ni(SB_1)_2Cl_2$	Greenish Brown	43.60 43.67	3.75 3.72	17.74 17.80	7.86 7.70	8.86 8.90	9.80 9.82	8.26 8.15	2.98
5	$Cu(SB_1)_2Cl_2$	Bluish Green	43.25 43.18	3.85 3.82	17.75 17.72	7.72 7.70	8.86 8.84	9.26 9.15	8.78 8.76	1.90

Table-2 Electron Spin Resonance Values of Cu (II) Complexes.

Parameter	G	g_{II}	$g_{\perp}$	g_{av}	G	A_{II}	$A_{\perp}$	Aav	λ/Δ_{II}	$\lambda/\Delta_{\perp}$	μ_{eff}
Complex $Cu(SB_1)_2Cl_2$	2.11	2.25	2.16	2.19	1.562	208	80	122.66	0.0309	0.0788	1.896

Magnetic measurements:

Complex of metal Mn(II) showed 5.74 BM. At room temperature, which is slightly lower than spin only value of 5.92 BM for high spin octahedral Mn(II) complex. CO(II) complex showed 4.5 BM, which is higher than the spin-only value of 3.89 BM, due to orbital participation. Therefore CO(II) complex is expected to have octahedral geometry, can be explained on the basis of octahedral symmetry involving a high degree of orbital contribution due to three-fold degeneracy of the ^4T_1g ground state. The magnetic moment values of Ni(II) complex is slightly higher than spin only moment of 2.83 BM. The magnetic moment values of Cu(II) complex is found in the range 1.90 BM at room temperature. According to found data, the Cu(II) complex can be assigned as distorted octahedral geometry (Figgish BN et al, 1960; Prabhu V et al, 1995) around Cu(II) ion as well as the presence of one unpaired electron on the metal ion. The geometry of all the complexes are further substantiated on the basis of spectral studies.

Electronic spectral studies

The Mn(II) complexes have a half-filled d-shell due to d^5 configuration and are spherically symmetric. Being thus, it is unaltered by operations of the octahedral group and belongs to ^6A_1g. The bands, observed in this Mn(II) complex, are assigned to the transitions and energies in terms of Racah Parameters (Tomilso, 1969). The Mn(II) complex shows four bands 19840, 20600, 25100 and 26420 cm^{-1} due to the $^6A_1g \rightarrow {}^4A_1g$, $^6A_1g \rightarrow {}^4T_2g$, $^6A_1g \rightarrow {}^4Eg$ and $^6A_1g \rightarrow {}^4A_1g$ transitions, respectively. The transitions, energies and calculated values for various parameters B=803, C=2514, 10Dq=8833 and β%=7.0, β=0.93, F^2=1161, F^4=71.81, f=1103, λ=196, h=1.0 and π/B=2.17. Due to these values, the band fit into the Tanabe Sugano matrix. All the values of different ligand field parameters are in close agreement with those of known octahedral Mn(II) complex.

The electronic spectra of synthesized CO(II) complex showed three bands on 8050, 17200 and 19800 cm^{-1}, which may be assigned to the transitions $^4T_1g \rightarrow {}^4T_2g$ (F) ν_1, $^4T_1g \rightarrow {}^4A_2g$ (ν_2) and ^4T_1g (F)$\rightarrow {}^4T_1g$ bands closely resemble with a spectra of the other distorted octahedral CO(II) complexes (EI-Sonavati AZ, 1992). Using free ion value of B=971 CM^{-1}, The value of spectral parameters in CO (II) are as follows 10Dq=9150, B=857, β= B'/B= 0.88, β% = 12% and Π/B=1.22, C=1725, f=1015.65, h=0.500, F^2=1103.45, F^4=48.81 and π=21716.The value of ν_3/ν_1 is 2.45 and this value go down in the usual range (2.00-2.80) observed for the majority of octahedral CO(II) coordination compound (Patel P and Bhattacharya PK, 1993).The electronic spectra of synthesized Ni(II) complex have been analyzed by NSH Hamiltonian theory (Donini JC, 1977). To distinguish projected normalized parameters DQ, DS and DT were employed than the lower case Dq, Ds and Dt. The Ni (II) complex shows bands at 8200, 10460, 14100, 16670 and 25640 cm^{-1} due to the $^3B_1g \rightarrow {}^3B_2g$ (νB), $^3B_1g \rightarrow {}^3Eg$ (νE), $^3B_1g \rightarrow Eg$ (Sh), ^3A_2g (F)$\rightarrow {}^3T_1g$ (F) ν_2 (F)$\rightarrow {}^3T_2g$ (F)$\rightarrow {}^3T_2G$ (P) ν_3 transitions, respectively, suggesting, tetragonal structure of Ni (II) complex. The ratio, DT/DQ gives the amount of distortion, have been calculated and indicate that the present complex somewhere between square planar and octahedral structure. Since the limiting value of DT/DQ for a square planar complex is 0.4226 (Dhakarey R, 1980). The calculated electronic spectral parameters of Ni(II) complex summarized as Dq^{xy}=1045, Dq^z=590, Dt=256.28, Ds=480, -DS=3370, DQ=22546, -DT=3498, Dq^L=22541, DQ^z=30823, DQ_A=14270, DQ_E=26684, -DT/DQ=0.15, dσ=-1205, dπ=-70.05, Δ_1=155.86, Δ_2=9709.31, Δ_3=3217.95, Dq^E=1121.20 and Dt/Ds=0.54.

The Cu(II) complex possess two or more bands, which may be assigned to the transition $^2Eg \rightarrow {}^2T_2g$, a characteristic of the distorted octahedral Cu(II) complex (Lever ABP, 1968). The newly prepared Cu(II) complex with SB_1 ligand containing overlapping band. Each of the resolved spectra of Cu(II) complex show four bands at 11800, 13100, 15440 and 17300 cm^{-1}. Hence, the interpretation of the spectrum was carried out assuming that Cu(II) complex is tetragonaly distorted in D^{4th} symmetry. The band assignment was carried out considering the spin orbit interaction. Considering the electronic spectra of the synthesized one $Cu(SB_1)_2Cl_2$ complex exhibited band at 15440 and 17300 cm^{-1}, may be due to the $\Gamma=7^a$ (^2Eg) → $\Gamma 6^b$ (^2T_2g) and $\Gamma 7^a$ (^2Eg) → $\Gamma 7^c$ (^2T_2g) transitions. According to conventional theory, the spin orbit splitting of these bands should amount to

about 3/2λ in first order (Liehr, 1960). The energy separation between the ^2T_2g state bands (1858 cm^{-1}) is higher than that of the free ion value (1245 cm^{-1}). The large value of λ indicate the slight molecular distortion (Ortolano, 1964) in the complex formation, besides the spin orbit coupling. Two other band appeared at 11800 and 13100 cm^{-1} were correspond to $\Gamma 7^a$ (^2Eg) → $\Gamma 6^a$ (^2Eg) and $\Gamma 7^a$ (^2Eg) → $\Gamma 7^b$ (^2T_2g) transitions. Here, the root for d^9 complexes in first order becomes E [$\Gamma 7^b$ (^2T_2g)] = 4 Dq-2Ds+Dt, correspond to 10 Dq. From this equation λ can be easily calculated. The other parameters Dq, Ds and Dt were determined by using λ = -1240cm^{-1} and founded as 10Dq=13100, Ds=2197, Dt=602 and ν_4-ν_3=1860. All the above information indicated the distorted octahedral (Osowole et al, 2012a) geometry around Cu(II) metal ion.

Infrared Spectral studies:

The most important IR-spectra of the Schiff base and its metal(II) complexes were recorded in KBr and listed in table-4. The ligand exhibits intense frequency due to νC=N as sharp band at 1645 cm^{-1}, which consistent with the iminic absorption of free shiff bases (widad et al. 2014). In all complexes, these band shifted to upper wave numbers and was observed at 1649-1653 cm^{-1} indicating the involvement of azomethine nitrogen in the coordination with the metal ion (Kumar LS 2011). The band of νC=O in the lower region 1680-1709 cm^{-1} in the metal complexes with respect to Schiff base ligand at 1718 cm^{-1}, showing the confirmation of carbonyl oxygen involvement in coordination with the metal ion (Anacona et al, 2015; Cherchiaro et al, 2004; Murukan et al, 2007). It is also confirmed by the presence of a new band in the complexes at 537-547 cm^{-1} due to M-N and 434-446 cm^{-1} due to M-O respectively (Ferraro, 1971; Nakemato, 1970; Sonmez et al, 2006; Sonmez and Sekerci, 2003).

The medium bands at 1265 cm^{-1} assigned to νC-O-C group of furan ring. This band unaffected in the complex formation, which clearly shows the noninvolvement in chelation with all metal(II) ion. The ligand has strong band at 1334 cm^{-1} and at 1150 cm^{-1} due to -SO_2 group (Bellamy LJ, 1962). On complexation, these bands are unaffected with metal (II) ion, indicating the non-participation in coordination. Another lower frequency band at 290-330 cm^{-1} was also appearance in complexs due to M-Cl (Ferraro JR, 1971; Nakemato S, 1970).

The IR-spectra of bivalent metal Mn(II), CO(II), Ni(II) and Cu(II) complexes shows the bidentate nature of the ligand with oxygen of sulphacetamide >C=O oxygen and Schiff base linkage nitrogen acting as donar sites.

ESR Spectra of Cu (II) complex:

The ESR spectra of $Cu(SB_1)_2$ Cl_2 complex has been recorded at room temperature with two "g" values computed by Peisach and Blumberg's method (Vachala SD et al, 2012). The complex Cu (SB_1) Cl_2 showed g=2.11, g_{II}=2.25, g⊥=2.16, gav=2.19, G=1.562 respectively, which showed g_{II} > g_1 > ge for Cu(II) complex of sulphacetamide, which implies 3d unpaired electron of Cu(II) ion occupied the dx^2-y^2 orbital as the ground state. It would be characteristic of axial symmetry (Niswander RH et al, 1975) i.e., tetragonal distorted octahedral conformation (Bai LJ et al, 1982; Nohria L et al, 2001). The gs in a D^{4th} symmetry should be g_{II}=ge+8λ/Δ_{II}, and g_I=ge+2λ/Δ_I (ge=2.0023) listed in table-2, that for its complex λ/Δ_{II} < λ/Δt indicating the ^2Eg labile lies below the ^2B_2g level. It is seen from data that reduction in Δg_{II} values may be due to an increase in λ or a decrease in λ or a combination of both. An increase in Δg_{II}, Δ and or a decrease in λ will lead to decrease in Δg_{II}, Δg_I and Δav and increase in covalency of coordination bonding (Devies MB 1993; Prabhu V and Venkappaya D 1995) from metal to ligand and opposite it, shows increasing ionic character of the coordination bonding (Kivelson D and Neiman R 1961). The exchange coupling constt. (G < 4) indicated considerable exchange interaction between Cu(II) ions (Sonmez M and Hacryusufoglu 2006). Therefore, from the esr spectra of this complex, confirmed covalent character of the metal ligand band.

Table-3 Antibacterial study for ligand and their Metal Complexes

Sr. No.	Compounds	Diameter of growth of Inhibition Zone (mm)#			
		B.cereus	***S.aureus***	***E.coli***	***A.niger***
1	SB_1(L)	12	16	15	11
2	Mn $(SB_1)_2Cl_2$	16	18	20	14
3	CO $(SB_1)_2Cl_2$	20	17	17	15
4	Ni $(SB_1)_2Cl_2$	15	14	16	12
5	Cu $(SB_1)_2Cl_2$	18	17	19	16

Table-4 Characteristic Infrared Spectral Data of SB_1 ligand and it's Metal (II) Complexes

S.N.	Compound	νC=N	νC=O	νC-O-C	νM-N	νM-O	νSO_{2Sym}	νSO_{2Asym}
1	SB_1	1645s	1718s	1263s	-	-	1152m	1332m
2	$Mn(SB_1)_2Cl_2$	1653s	1709m	1262m	537s	434s	1153m	1330m
3	$CO(SB_1)_2Cl_2$	1659s	1705m	1263m	540m	438m	1150m	1329m
4	$Ni(SB_1)_2Cl_2$	1649s	1697m	1264w	543m	440m	1154w	1332s
5	$Cu(SB_1)_2Cl_2$	1652s	1680m	1262m	547s	446m	1153m	1330m

Biological Activity:

The microbial culture was procured from Microbial Type Culture Collection (MTCC). All synthesized compound was dissolved in DMSO. Biological activity of the synthesized Schiff base ligand and it's four metal complexes have been done by agar well diffusion method against 2 Gram (+) bacteria (*Bacillus cereus* and *S. aurens*) and one Gram (-) bacteria (*E.Coli*). All microbial culture was prepared by taking 10mg of compound in DMSO. An agar medium was taken into each petri dish with a same concentration of 20 ml. All synthesized compound was prepared with different concentration (100, 50, 40, 30, 20, 10, >μg mL^{-1}) in DMSO. These different concentrated compounds were swapped with 100 μL inocula of the test microorganisms tanking more than 15 minutes for adsorption. The petri disc incubated at 37°C for 48 hours. The microbial activities against synthesized complexes were writing down by the measurement of growth of Inhibition Zone with zone reader (Hi Antibiotic zone scale). DMSO was used as positive and negative control respectively. DMSO used as negative control for fungal strains. The procedure was performed triplicates for each microorganism (Aneja KR et al, 2011) and the inhibition growth was recorded. The minimum inhibitory concentration of each compounds was recorded as decreasing concentration range of 400 to 3.12 μg/Ml. The decreasing concentration of 100 μL volume was taken into wells in the agar petri dish, which have already seeded with same volume of standardized inoculums (10^6 cfu/mL), and recorded comparing with negative control and data have been in table-3.

CONCLUSION

The present study of Schiff base ligand (SB_1) -3,4{[-benze 1-sulphacetamide] imino] methyl} furan and it's metal Mn(II), CO(II), Ni(II) and Cu(II) Complexes have Octahedral geometry for Mn(II) and CO(II), distorted octahedral geometry for Ni(II) and Square planner geometry for Cu(II) complexes respectively. The synthesized ligand and all these metal(II) complexes are found to be active against Gram (+) bacteria (B. cereus and S. aureus), Gram (-) bacteria (*E.Coli*) and fungi (A. niger). Ligand (SB_1) has less reactive than it's all complexes with respect to Gram (+) *B.cereus* bacteria and fungal *A.niger*. But in complexes, Ni(II) complex is found to be less reactive against Gram (+), Gram (-) bacterial and fungal activity with respect to other metal(II) complexes.

Acknowledgements:

I am thankful to Editor IJLSCI for reviewing my manuscript. Also, thankful to provide facility to analyze the given above studies by IIT Chennai, Ranbaxy Badodara, RSIC Chandigarh and medical college lab Agra for providing bacteria. At the last but not least thankful to Singh PK and Chavhan PR for Valuable support.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :29-34
UGC Approved Journal No 48951

Original Article Open Access

Removal of Chlorpyrifos pesticide from wastewater using RPHF-I

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Maskey Sudhirkumar M (2018) Removal of Chlorpyrifos pesticide from wastewater using RPHF-I, *Int. J. of. Life Sciences*, Special Issue, A12: 29-34.

ABSTRACT

In the pesticide especially chlorpyrifos is currently one of the most widely applied pesticides in the all over the India. In this article, the copolymer RPHF-I using for effective adsorbents of chlorpyrifos pesticide from wastewater. Chlorpyrifos (CPS) pesticide may appear as pollutants in water sources, having undesirable impacts to human health because of their toxicity, carcinogenicity and bioaccumulation to tissue. The aim of the present study is an attempt to synthesize new group of copolymer RPHF-I with higher efficiency of chlorpyrifos removal from aqueous solution. In batch method experiment is carried out to remove the chlorpyrifos. The adsorption capacity of the copolymer was studied as the function of solution pH, concentration of chlorpyrifos and contact time of adsorption. The adsorptive applicability of copolymer was tested by Langmuir isotherm and Freundlich isotherm. The adsorption capacities were found to be 92.23% for pesticide removal. The copolymer RPHF-I can be successfully used as an efficient material for removal of pesticide from aqueous environments and can have a variety of potential environmental pollution control.

Keywords: Chlorpyrifos Pesticide, Copolymers, Batch Experiments, Adsorption, Environmental Pollution.

INTRODUCTION

As India is a tropical country, it suffers severe losses in agriculture due to pests. This necessitates the use of pesticides to protect our crops against the attack of several pests. Pesticides are the chemicals that kill or destroy the pests (Aktar et al. 2009). They are also poison chemicals have adverse effect to human, animal and microorganism by accumulating in food and water. As per an estimate, worldwide nearly 10,000 deaths occur annually, owing to the use of chemical pesticides. At present, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides with an annual production of 90,000 tons (Tijani et al. 2007). In the pesticide especially chlorpyrifos

(O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphoroth-ioate) is hazardous to humans, they affect the central nervous system, the cardiovascular system, and the respiratory system(Muller et al. 2000). It was introduced in 1965 by Dow Chemical Company. According to Dow, chlorpyrifos is registered for use in nearly 100 countries and is annually applied to approximately 8.5 million crop acres (Rathod et al. 2017). Chlorpyrifos exposure may lead to acute toxicity at higher doses. Persistent health effects follow acute poisoning or from long-term exposure to low doses, and developmental effects appear in fetuses and children even at very small doses (Rauh et al. 2017).) For this study is an attempt to synthesize and characterize new copolymer with chlorpyrifos adsorbent properties for their removal from environment, specifically chlorpyrifos from contaminated water. In the present investigation, copolymer RPHF-I was synthesized by using resorcinol (R), Phenylhydrazine (PH) and Formaldehyde (F) in 1:1:2 molar ratios of the reacting monomers. The new copolymer was characterized by Elemental analysis, NMR, TGA and SEM. The newly obtained copolymer have been proved to be a very good adsorbent which can be successfully used for removal of Chlorpyrifos contaminated water which can then be used for safe potable purpose.

MATERIALS & METHODS

All chemicals used were of analytical grade. Resorcinol, Phenylhydrazine, Formaldehyde (37%) procured from Merck, India. Double distilled water was used for all the experiments.

Synthesis and Purification of RPHF–I Copolymer:
The copolymer (RPHF-I) was synthesized employing the method published earlier (Rahangdale et al. 1993; Maskey et al. 2015). The purity of newly synthesized and purified copolymer sample has been tested and confirmed by TLC. The yield of Copolymer resin was found to be 82%. The proposed structure of RPHF-I with reaction scheme has shown in fig.1**.**

Characterization of copolymer:
Characterization of surface modified copolymer was carried out by techniques like Elemental Analysis, NMR, SEM and TGA. The scanning was carried out at Sophisticated Analytical Instrumental Facility (SAIF) Punjab University, Chandigarh and SAIF Cochin.

Batch Experiment:
Batch equilibrium studies were conducted with different parameters such as pH, agitation time, initial concentration of Chlorpyrifos solution and effect of adsorbent doses. The systems were agitated on rotary shaker at 200 rpm, filtered through Whatmman no.42 filter paper and filtrate was analyzed for Chlorpyrifos concentration using UV-Visible Spectrophotometer. From experimental data, the applicability of Langmuir model and Freundlich model was judged. Linear regression coefficient (R2) and isotherm constant values were determined from the model.

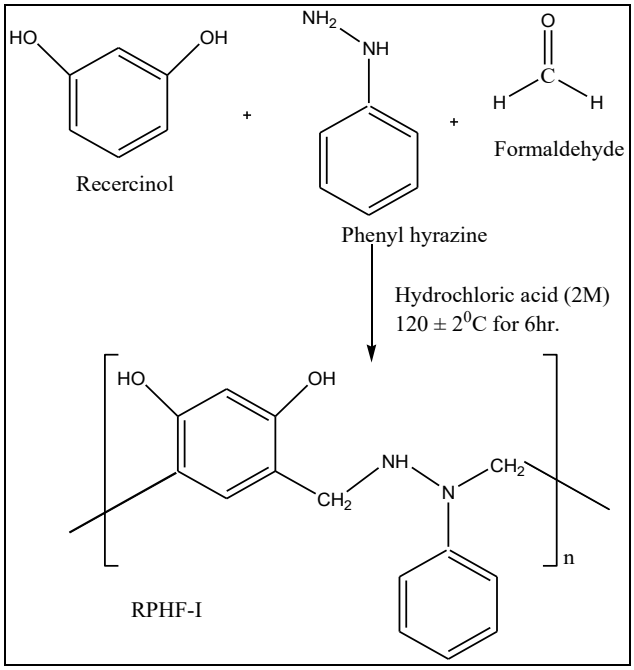

Fig.1:Reaction Scheme and Structure of Copolymer (RPHF-I)

RESULTS AND DISCUSSION

Elemental analysis was analyzed the percentage of carbon, hydrogen, nitrogen and oxygen content in Copolymers. Elemental analysis data of Copolymers are shown in table 1, it is found that the determine values of the percentage elements are in good agreement with the calculated values. The elemental analysis data suggest the empirical formula and the empirical formula weight for the repeating unit of Copolymers (Campbell et al. 1989).

The ^{1}H NMR spectrum of RPHF-I Copolymer is reprented in **fig.2**. The chemical shifts (δ) ppm observed have been assigned on the basis of the literature (Pretsch et al. 2000). The signals in the region at 6.2-7.2

(δ) ppm may be assigned to the protons in the aromatic ring. The medium singlet at 2.5 (δ) ppm may be due to methylene proton of Ar–CH_2 bridge. A singlet observed in the region 3.5(δ) ppm is corresponding to methylene proton of Ar–CH_2–N moiety. A signal observed at 4.1 (δ) ppm is attributed to proton of amines i.e. Ar-NH moiety. A singlet observed in the region 1.2(δ) ppm may be due to the protons in–NH linkage. The signal at 8.1(δ) ppm is assigned due to phenolic–OH group involved inintramolecular hydrogen bonding.

Table 1:- Results of elemental analysis of Copolymers

Name of Copolymer	Carbon (%)	Nitrogen (%)	Oxygen (%)	Hydrogen (%)	Empirical formula of repeated unit	Empirical formula weight
RPHF-I	59.80 (Cal.)	9.96 (Cal.)	11.29 (Cal.)	4.94 (Cal.)	C14H14N2O2	242
	59.10	9.12	11.29	4.12		

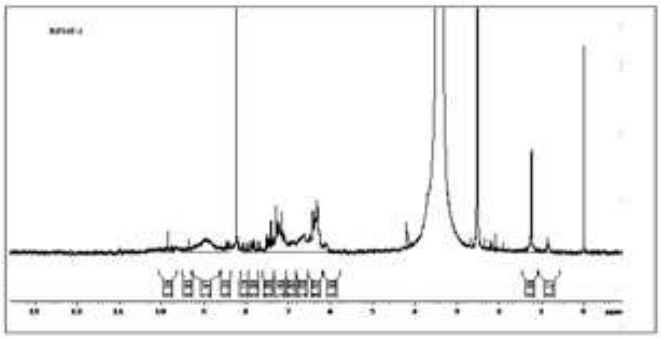

Fig. 2: ^{1}H NMR spectrum of RPHF-I

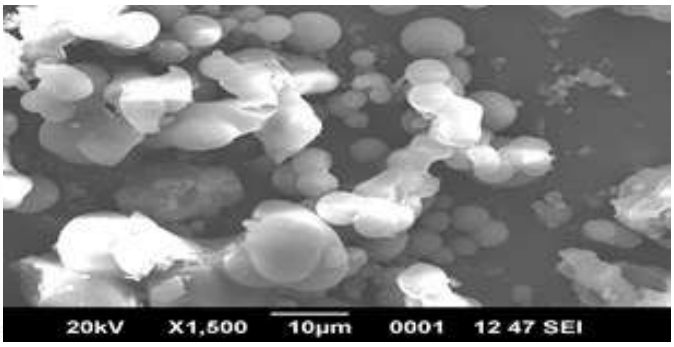

Fig.3: SEM image of RPHF-I

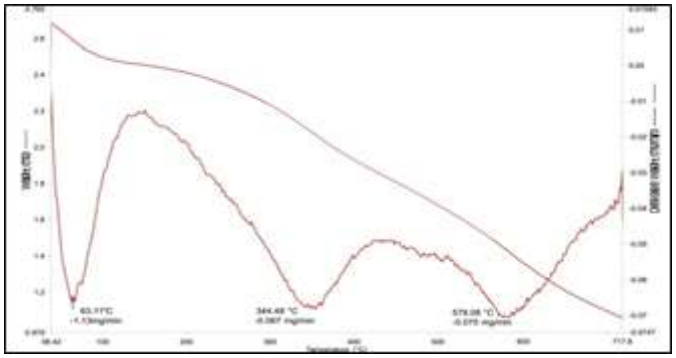

Fig. 4:- TGA of RPHF-I

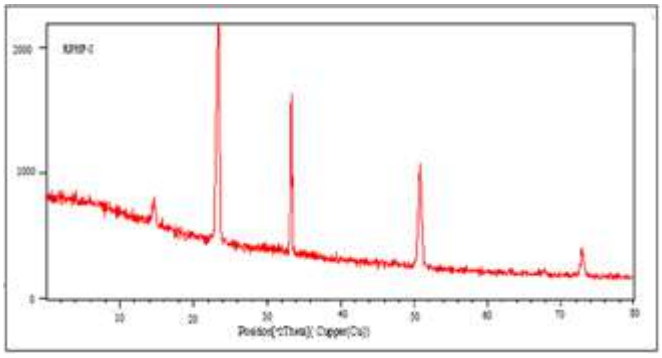

Fig.5:-XRD of RPHF-I

Fig.3 indicates the SEM images of RPHF-I obtained using an accelerating voltage of 20 KV at (x 1500, x10000) magnification. At such magnification, SEM images clearly revealed that wide variety of microspheres are presents on the surface RPHF-I. Microspheres were formed in the form of large beads with uniformity and monodispersity (Lai et al. 2007). The microspheres are globular size with diameter 2.86 µm, 1.98 µm and 2.86 µm. The image also showed RPHF-I is crystalline states. The microspheres presented in the Copolymer surface may be responsible for the swelling behavior and reactivity of active sites buried in the polymer matrix and also responsible for better adsorption of metal ion (Wang et al. 2007).

The TGA curve of RPHF-I shown in fig.4. it can be seen from figure that three consecutive weight loss steps were observed in RPHF-I. The first weight loss was about 50 to 150^{0}C. The derivative peak observed at temperature 63.11^{0}C with a weight loss of 4 %which may be due to the removal of water molecule (Learmonth et al. 1964). When temperature was raised

to the range 300-350^{0}C,the second derivative peak appeared at 344.480C with 20 % weight loss of material which may be due to the elimination of –OH groups attached to the aromatic nucleus of polymeric sample. In the third stage, the weight loss in the temperature range of 500 to 700^{0}C.The derivative peak observed at temperature 578.08^{0}C with a weight loss of 48 % which may be due to the elimination of –CH_2 bridges and the aromatic nucleus (Horowitz et al. 1963). After 700^{0}C, the TGA curve almost flatten due to the only residue remains behinds.

The X-ray diffractograph of RPHF-I has shown in fig.5. In this spectrum a high intense sharp peaks at 2θ= 21^0,33^0 and 50^0 show crystalline nature of Copolymer. The spectrum also contains low intense peaksat 2θ= 15^{0}and 72^0 indicate semicrystalline nature. Thus it can be concluded that RPHF-I Copolymer exhibits crystalline and semicrystalline nature (Cullity et al. 1978; Klug et al. 1974).

Adsorption of Chlorpyrifos (CPS) on RPHF-I

Effect of pH on Chlorpyrifos removal:

The adsorption capacities of RPHF-II towards Chlorpyrifos were determined using various pH values of solution in the range of 1.0 to 10. From **fig.6**. It is evident from this figure that maximum at pH 5 the adsorbents i.e. RPHF-I remove 93.23% of Chlorpyrifos ion.

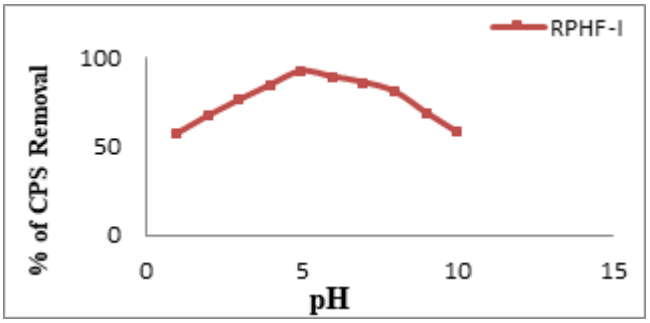

Fig.6: Effect of pH on CPS removal

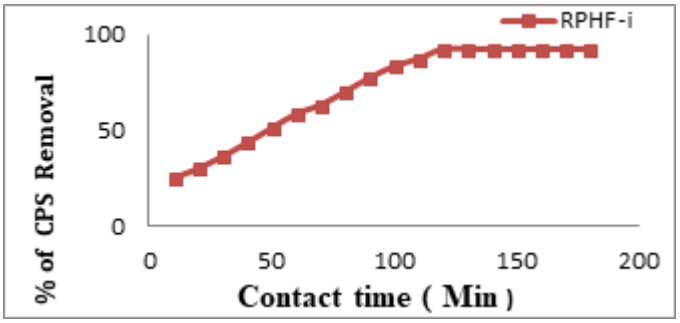

Fig.7: Effect of Contact time on CPS removal

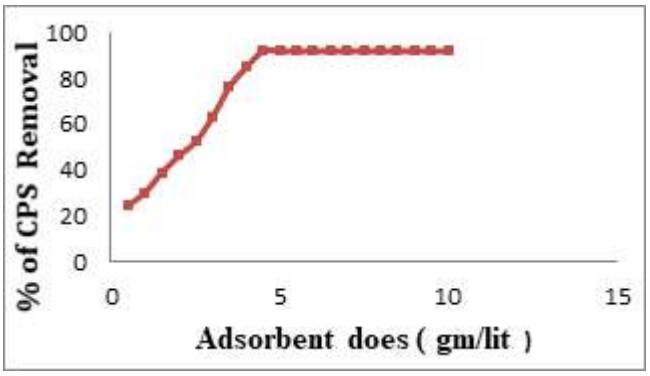

Fig.8: Effect of Adsorbent dose on CPS removal

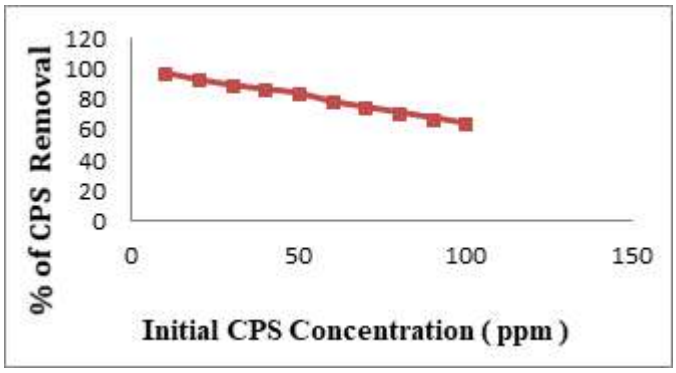

Fig. 9: Effect of initial concentration of CPS removal

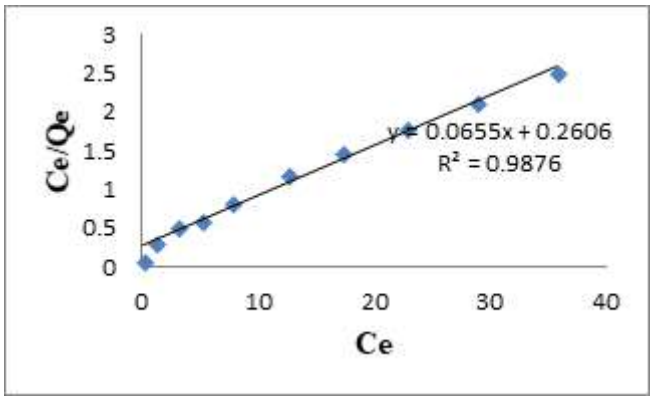

Fig 10: Langmuir isotherm for the adsorption

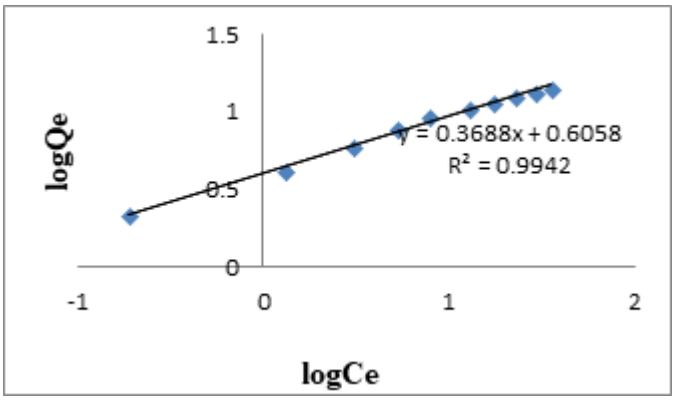

Fig . 11: Freundlich isotherm for the adsorption

Effect of contact time on Chlorpyrifos removal:
From fig.7 It can be seen that Chlorpyrifos removal efficiency of RPHF-I increased from 20% to 93.23% when contact time was increased up to 140 min. Thus optimum contact time for RPHF-I was found to be 140 min.

Effect of Adsorbent dose on Chlorpyrifos Removal: From **fig.8** the RPHF-I dose increased from 0.5 to 7.0 gm/lit, there was increased of removal efficiency of Chlorpyrifos from 21.30 % to 93.23% It is also seen from the figure that a further increase of RPHF-I i.e. 7.0 gm/lit does not affect the percentage of Chlorpyrifos removal. The optimum dose of RPHF-I for the maximum removed percentage of Chlorpyrifos was 7.0 gm/lit.

Effect of initial Chlorpyrifos Concentration:
The percentage of adsorption with different Chlorpyrifos concentration was studied by varying Chlorpyrifos ion concentration from 10 to 100 mg/lit keeping other parameters such as pH of solution, adsorbents dose, contact time optimum. The results are show in **fig.9.** From the figure, it is observed that percentage of Chlorpyrifos removal was found to decrease from 93.23% to 58.45% as initial concentration started from 10 to 100 mg/lit for RPHF-I.

Adsorption Isotherm
In order to establish the most appropriate correlation for the equilibrium data in the design of adsorption system, two common isotherm models were tested Freundlich and Langmuir models.

Langmuir isotherm:
The isotherm data have been linearized using the Langmuir equation and is plotted between Ce/Qe versus Ce shown in fig.10. . The Langmuir constant 'Qm' which is measure of the monolayer adsorption capacity of RPHF-I is obtained as 15.38. The Langmuir constant 'b' which denotes adsorption energy, is found to be 0.250. The high value (0.987) of regression correlation coefficient (R2) indicates good agreement between the experimental values and isotherm parameters and also confirms the monolayer adsorption of Cr(VI) onto the RSF-I. The dimensional parameter 'RL' which is a measure of adsorption favorability is found to be 0.091(0< RL < 1) which confirms the favorable adsorption process for Chlorpyrifos on RPHF-I adsorbent (Khattri et al. 1999).

Freundlich isotherm:
The Freundlich equation suggests multilayer adsorption. Sorption energy exponentially decreases on completion of the sorption centers of and adsorbent. Therefore, the parameters of k_f and 'n' were be estimated from the intercept and slope of the plots between $\log Q_e$ against $\log C_e$. Freundlich isotherms are shown in fig.11. The k_f value of both the adsorbents i.e. RPHF-I was found to 4.0271 mg, which indicate that dominance of adsorption capacity. The Freundlich exponent 'n' was 2.717 for RPHF-I which is reflects the favourable adsorption. The value of R2 was found to 0.994 for RPHF-I (Arivoli et al. 2007).

CONCLUSION

Copolymer RPHF-I successfully synthesized with using monomers Resorcinol Phenylhydrazine and Formaldehyde in the molar ratio of 1:1:2 in the presence of 2M HCl as a catalyst. RPHF-I copolymer has been characterized using elemental analysis, NMR, TGA, SEM and XRD. This copolymer is proved to be an excellent adsorbent for Chlofirofis pesticide. The optimum parameters for efficient application of the RPHF-I copolymer under present investigation are adsorbent dose 7.0 g, pH 5 and contact time is 140 min. The adsorptive applicability of copolymer was tested by Langmuir isotherm and Freundlich isotherm. The adsorption capacities were found to be 92.23% for pesticide removal.

Acknowledgement:
Authors are highly thankful to Principal, Science College, Congress Nagar, Nagpur for providing necessary laboratory facilities. Authors are also thankful to Director SAIF Punjab University, Chandigarh and SAIF Cochin University, Kerala for their help in scanning of material.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :35-38
UGC Approved Journal No 48951

Original Article Open Access

Pharmacological profile and phytochemical investigation of *syzygium caryophyllifolia* leaf extracts

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Jogi Pravin S, Deshmukh Umesh B and Shaikh Shadma (2018) Pharmacological profile and phytochemical investigation of *syzygium caryophyllifolia* leaf extracts, *Int. J. of Life Sciences*, Special Issue, A12: 35-38.

ABSTRACT

Present research work was subjected to deals with phytochemical study of extracts of newly identified plant *Syzygium caryophyllifolia* from Chandrapur forest region and also tested for antimicrobial activity against gram positive, gram negative and fungi. Extracts of leaves of plant was prepared in ethanol and ethyl acetate and tested for various biologically active constituents like alkaloid, terpenoid, flavonoid, tannine, saponnin, glycosides, steroids etc. The study revealed the presence of some biologically active component in the plant extract and also suggested that the leaves of *Syzygium caryophyllifolia* have promising biological activity against microorganism.

Keywords: *Syzygium caryophyllifolia*, Mediciinal Plants, Phytochemical

INTRODUCTION

It is well known that plants and animals are the biggest source of biologically active medicinal compounds for mankind. Plants are natures "chemical factories" providing richest source of organic chemical on the earth. Most of the medicinal plants from this forest are used in traditional medicine to cure various sicknesses and diseases. Indian forest is rich in variety of medicinal plants, most of plants species have high potential abilities in ayurveda, unani, siddha, traditional medicines. Only very few have been studied phytochemically and pharmacologically for their potential medicinal values. Forest of Chandrapur is known for their biodiversity in flora and fauna and also having variety of medicinal plants. The indigenous system of medicine namely Ayurveda, Unani and Siddha have been in existence in several centuries (Jogi and Akkewar, 2012). *Syzygium cumini* is one of the well know plant used for various disease particularly diabetes. All parts including roots, leaves, stem, fruits and flowers have been used in curing various diseases (Reynestson et al., 2005).

It is widely distributed throughout India and ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus.

During an ethno-botanical exploration of Chandrapur District of Maharashtra State, a plant was noted 7 km away from Chandrapur City in Lohara Village which the local people call as "ChotaJambhul". The flowering twigs of the plant were collected and after referring to the pertinent literature (Almeida, 1996), it was identified as *Syzygium caryophyllifolia* (Lamk.) DC family Myrtaceae. This variety named as *Syzygium caryophyllifolia* (Lamk.) DC till not studied for chemically and pharmacologically. This paper deals with phytochemical screening and biological study of ethanol and ethyl acetate extract of leaf of *Syzygium caryophyllifoli.*

MATERIALS & METHODS

Plant Collection: The present work was carried out at Department of chemistry, J.M.V. Chandrapur, Gondwana University, Gadchiroli. The plant named *Syzygium caryophyllifolia* was collected from Chandrapur forest region near Lohara village. Their botanical identity of plant was determined and authenticated from literature available in Department of Botany, J.M.V. Chandrapur. The leaves of *Syzygium caryophyllifolia* was thoroughly washed with water and dried under shade for about ten days. The dried plant sample was ground well into a fine powder in a mixture grinder. The powder was stored in an air sealed polyethylene bag at room temperature before extraction.

Preparation of Extract: The powdered plant material was extracted using Soxhlet apparatus with organic solvents ethanol and ethyl acetate. The extracts were concentrated. The extracts were stored in air tight glass container at 40 C.

Antimicrobial Screening of Extracts: The microorganism used in the study: Gram-negative E-coli, Gram-positive S-aurous and Nizer fungus Aspergillus were obtained from stock culture in the Department of Microbiology, J.M.V. Chandrapur. Susceptibility test were carried out. The modified agar well diffusion method (Garrod et al, (1981), Trease and Evans, 1989) to test the antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar. The culture was prepared in triplicate and incubated at 37^{0}C for 24 to 72 h. 0.2 ml of the broth culture of the test organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10mcg/disk, 30mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37^o C for 24 h and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

Phytochemical Analysis: The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannine, Saponin, Flavonoid, Phlobatannin, Anthraquionone, Reducing Sugar, Glycoside and Cardiac glycoside (Sofowara. 1993), Herborne, 1973), Okwu, 2001), Rahilla et al., 1994).

Alkaloid: About 0.2 g of the extracts was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitated indicates the presence of alkaloids.

Tannine: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allow to cool. Equal volume of $CHCl_3$ was added to the filtrated. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour indicates the presence of anthraquinones.

Glycoside: The extracts was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drop of Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.

Reducing Sugars: The extracts were shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for minutes. An orange red precipitate indicates presence of reducing sugar.

Saponin: About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Flavonoids: Extracts of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Phlobatannins: The extracts (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCl solution. Red precipitated show the presence of Phlobatannins.

Terpenoids (Salkowski test): 0.2 g of extracts was mixed with 2 ml Chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

Cardiac glycosides: Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H2SO4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brow ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

RESULTS

Phytochemical screening of ethanol and ethyl acetate extract of *Syzygium caryophyllifolia* is shown in table 1. The susceptibility of test microorganism to the crude extracts of *Syzygium caryophyllifolia* is shown in table 2.

Table-1. Phytochemical tests of various extracts of plant Syzygium caryophyllifolia.

Chemical composition	**Ethanol Extract**	**Ethyl acetate extract**
Alkaloid	+	+
Tannine	-	-
Anthroquinone	-	-
Glycoside	-	-
Reducing sugar	-	-
Saponine	-	-
Flavonoid	-	+
Phlobatannins	-	-
Terpenoid	+	+
Cardiac glycosides	+	+

Key to symbols: - = Absent, + = present

Table-2. Antimicrobial activity of various extracts of *Syzygium caryophyllifolia*.

Extracts	Microorganism		
	Gram + (S aureus)	Gram – (E coli)	Nizer fungus Aspergill
Ethanol extract	-	-	+++
Ethyl acetate extract	+++	-	+++

Key to symbols: - = Inactive (inhibition zone <5 mm); + = slightly active (inhibition zone 5-10 mm); ++ = moderately active (inhibition zone 10-15 mm); +++ = highly active (inhibition zone >15 mm).

DISCUSSION

The qualitative analysis of extracts from leaf of plant *Syzygium caryophyllifolia* showed the presence of phytochemical constituents such as alkaloid, terpenoid , and Cardiac glycoside. The results are summarized in table 1 and 2. The above results indicates that, the leaves of plant investigated are rich in alkaloid, terpenoid, Cardiac glycoside and flavonoids. Ethanol and Ethyl acetate extracts showed the presence of cardiac glycoside. All extracts have showed absence of anthraquinone. Extracts of leaf were tested against Gram positive S-aurous and gram negative E-coli. Extracts also tested for antifungal activity against Aspergillus Niger and showed the inhibition of growth. Ethyl acetate extract was found to be highly sensitive against Gram positive S-aurous and Aspergillus Niger (with zone of inhibition above 13 mm means highly sensitive). Ethyl acetate extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. Ethanol extract also showed antibacterial activity against Aspergillus Niger. The inhibitory activity of these extracts confirmed the potential use of the plant in the treatments of microbial induced ailments.

The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure of bioactive compounds.

CONCLUSION

This plant is rich in presence of alkaloid, terpenoid and other biologically active class of natural products. The plant studied here can be seen as a potential source of

useful drugs. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure of bioactive compounds.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:39-43
UGC Approved Journal No 48951

Original Article Open Access

Synthesis of Supported Metal Nanoparticles: Future Scope

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Nagpure Atul S (2018) Synthesis of Supported Metal Nanoparticles: Future Scope, *Int. J. of. Life Sciences*, Special Issue, A12: 39-43.

ABSTRACT

Octahedral Molecular Sieves (OMS-2) material was prepared by redox method and was employed as catalyst support for the preparation of heterogeneous OMS-2 supported metal catalysts. All the materials were systematically characterized using various techniques such as X-ray diffraction (XRD), N_2 adsorption-desorption, Inductively Coupled Plasma-Optical Emission Spectrometry **(**ICP-OES), Transmission Electron Microscopy (TEM), etc. Highly dispersed supported metal nanoparticles were synthesized using ion-exchanged method. Characterization studies confirmed that metal particles are homogeneously distributed over OMS-2 support.

Keywords: Heterogeneous catalyst, metal nanoparticles, ion-exchange method, catalysis.

INTRODUCTION

Heterogeneous catalysts widely used in numerous catalytic transformations such as dehydrogenation, hydrogenation, hydrogenolysis, oxidation, dehydration, carbon-carbon bond formation, ammonia formation, Fischer–Tropsch Synthesis, etc (Suib *et al*., 1997; Chen *et al.*, 2001; Amin *et al.*, 2000; Suib *et al.,* 2000). Manganese containing octahedral molecular sieves (OMS-2) materials was exploited as efficient heterogeneous catalysts support for synthesis of several supported metal catalysts. OMS-2 materials possess various important features such as highly porous nature, good adsorption-desorption property, ion-exchange capacity, moderate surface acidity-basicity, etc (Suib *et al*., 1997). Moreover, doping of other metal predominantly divalent or trivalent cations in OMS-2 changes its electronic, structural as well as catalytic properties (O'Young *et al.,* 2002). The metal-doped OMS-2 material was found to be an excellent heterogeneous catalysts for oxidation of 2-propanol (O'Young *et al.,* 2002), oxidative dehydrogenation of ethanol (O'Young *et al.,* 2002), supercritical water oxidation of pyridine (Abraham *et al*., 1999), phenol (Abraham *et al*., 1995), ammonia (Gloyna *et al*., 1998),

etc. In recent time we have reported efficiency of OMS-2 supported Ru nanoparticles catalyst for selective hydrogenolysis of biomass-derived 5-hydroxymethylfurfural (HMF) to 2,5-dimethylfuran and selective oxidation of HMF to 2,5-furandicarboxylic acid (Nagpure *et al*., 2014). In the current study we have revealed the usefulness of K-OMS-2 material for the preparation of highly dispersed Cu metal nanoparticles.

MATERIAL AND METHOD

Chemicals

All chemicals utilized were of reagent grade and used without any purification. $KMnO_4$ (99%), $MnSO_4.H_2O$ (99%), $CuCl_2{\cdot}2H_2O$ (99%), HNO_3 (70%) and $NaBH_4$ were obtained from Loba chemicals, Mumbai, India.

Preparation of materials

Preparation of catalyst support (K-OMS-2)

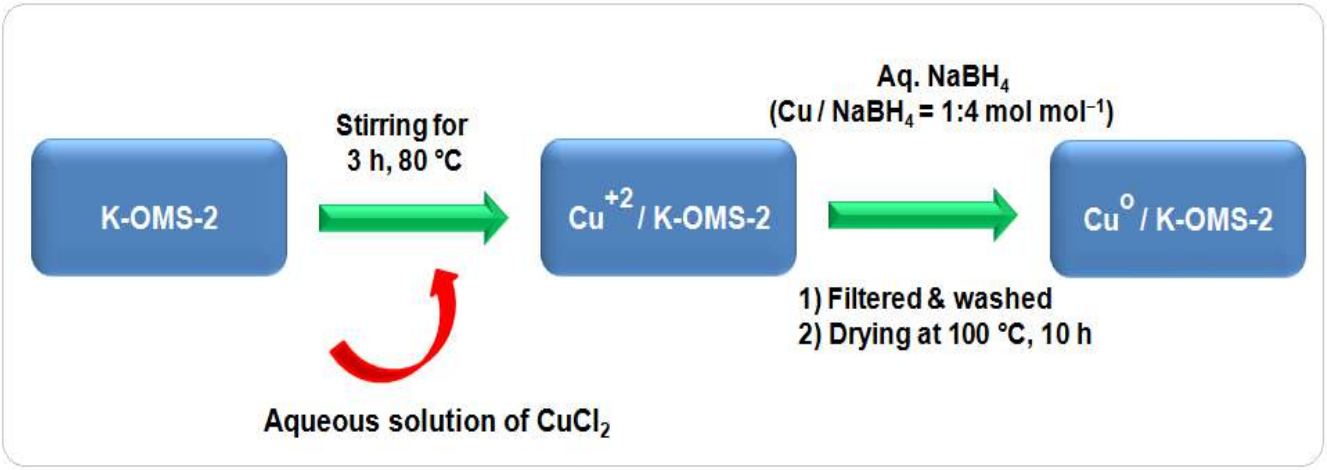
Support K-OMS-2 material was prepared according to the earlier reported literature (Newsam *et al*., 1994). In a typical synthesis method, $KMnO_4$ (5.89 g) was added in distilled water (100 mL) and the obtained suspension was added drop by drop to a solution containing mixture of $MnSO_4$ (8.8 g in 30 mL water) and concentrated HNO_3 (3 mL) under continuous stirring at room temperature. The resulting black precipitated was reflux for 24 h (at 100 ºC). Afterward, the material was washed with distilled water until the pH become neutral. Finally, the sample was dried for 12 h (at 100 ºC) and was calcined for 3 h (at 350 ºC) to get K-OMS-2 material.

Preparation of Cu catalysts supported on K-OMS-2

Cu catalysts supported on K-OMS-2 were prepared by ion-exchange method (Scheme 1) according to previous literature (Nagpure *et al*., 2015). In a typical synthesis procedure, K-OMS-2 material (1.96 g) was dispersed in 50 mL of deionized water in a 100 mL round-bottomed flask. To it, aqueous solution of $CuCl_2{\cdot}2H_2O$ (Cu amount was calculated for desired Cu loading) was added drop by drop under constant stirring and the obtained slurry was stirred for 3 h (at 80 °C). The solution was cooled to room temperature. Subsequently, $NaBH_4$ ($Cu/NaBH_4$ = 1:4 mol mol^{-1}) in water was added to the above solution with stirring at room temperature for 1 h to get Cu in metallic state. The mixture was filtered and washed until no chloride ions were detected (confirmed by $AgNO_3$ test). After all, the sample was dried in an oven at 100 °C for 10 h. The prepared catalysts were designated as 2wt% Cu / K-OMS-2 and 5wt% Cu / K-OMS-2.

Material Characterization

All the materials were systematically characterized by several physico-chemical characterization techniques such as X-ray diffraction (XRD), N_2 sorption, Transmission Electron Microscopy (TEM) and Inductively Coupled Plasma-Optical Emission Spectrometry **(**ICP-OES).



Scheme 1. Preparation of Cu / K-OMS-2 catalyst by ion-exchange method.

RESULTS AND DISCUSSION

Structural characteristics of the materials

X-ray diffraction (XRD)

The XRD patterns of K-OMS-2 and as synthesized K-OMS-2 supported Cu catalysts are depicted in Figure 1. The XRD peaks of Cu catalysts are in good agreement with the reported data (*JCPDS card 29-1020*) of cryptomelane K-OMS-2 material (Chen *et al.*, 2001; Amin *et al.*, 2000; Suib *et al., 2000*). This result signifies that cryptomelane structure of K-OMS-2 remained intact even after Cu exchanged. Importantly, the XRD peaks intensity of Cu catalysts enhances with the increased in the Cu content, demonstrating that the Cu metal assisting the crystallization. No additional peaks were detected pertaining to the metallic Cu or Cu oxides (CuO/Cu_2O), indicating that Cu nanoparticles are highly dispersed on K-OMS-2 support.

***N_2* physisorption**

N2 adsorption-desorption isotherm of as synthesized K-OMS-2 and Cu catalysts are given in Figure 2. All the samples showed a characteristic type II sorption, which can be attributed to the microporous nature of the samples (Nagpure *et al.*, 2016). The Brunauer–Emmett–Teller (BET) surface area values for samples are given in Table 1. The BET surface area for K-OMS-2, 2wt% Cu / K-OMS-2 and 5wt% Cu / K-OMS-2 was found to be 96, 78 and 65 m^2/g, respectively.

This result shows decreased in BET surface area of samples with increased in Cu content of the catalyst. This may be due to the blockage of pores by the Cu particles in the framework.

Table 1. Chemical composition and structural characteristics of materials

Catalyst	BET surface area (m^2/g)	Total pore volume [a] (cm^3/g)	Cu content [b] (wt%)	Average Cu particle size [c] (nm)
K-OMS-2	96	0.13	--	--
2 wt% Cu / K-OMS-2	78	0.11	1.9	2.2
5 wt% Cu / K-OMS-2	65	0.09	4.7	3.3

[a] Total pore volume at $P/P_0 = 0.899$. [b] Estimated by ICP-OES. [c] Measured by TEM.

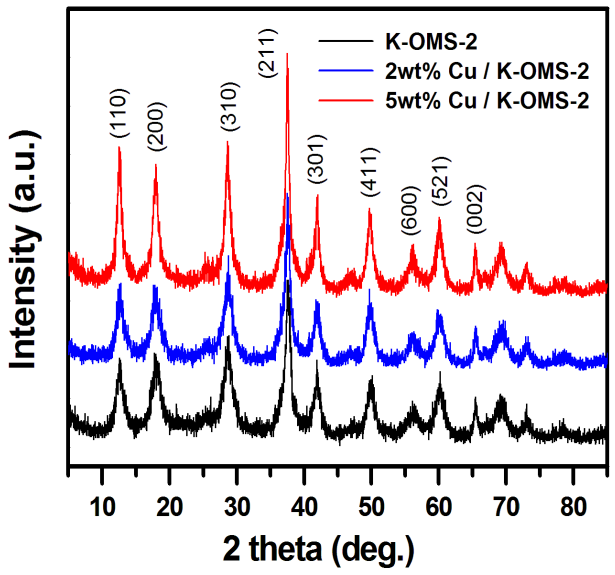


Figure 1

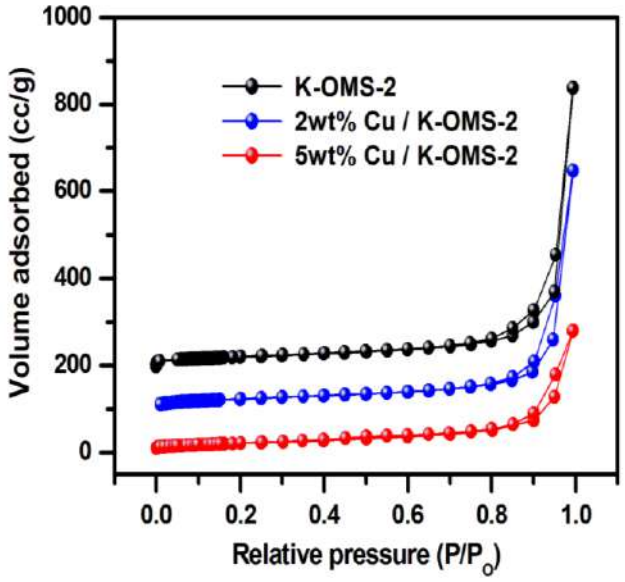


Figure 2

Figure 1. XRD patterns of K-OMS-2 and Cu catalysts.
Figure 2. N_2 adsorption-desorption isotherm of K-OMS-2 and Cu catalysts.

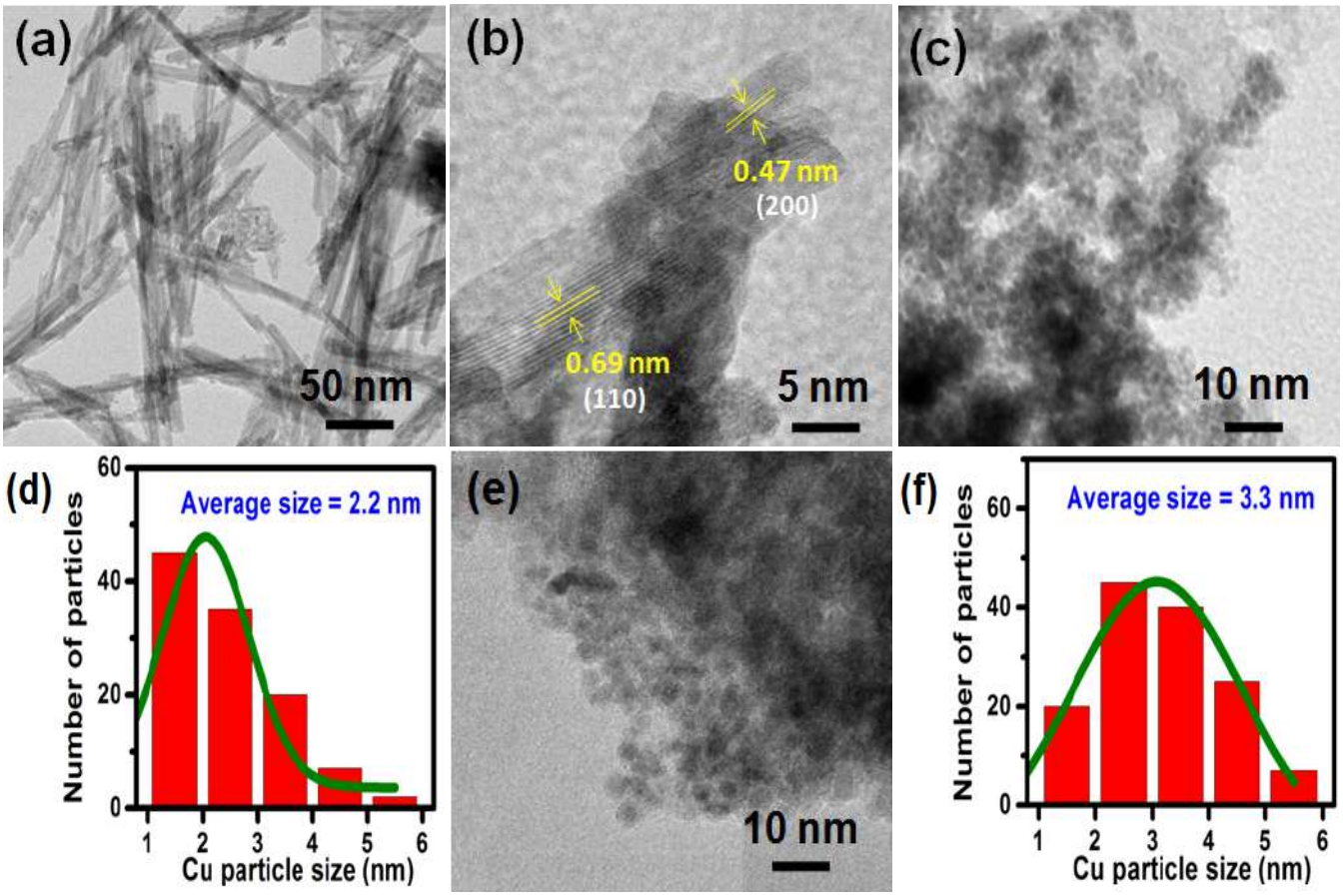


Figure 3. TEM images of K-OMS-2 (a and b), TEM images and the Cu nanoparticles size distribution for 2wt% Cu / K-OMS-2 (c and d) and for 5wt% Cu / K-OMS-2 (e and f).

Transmission electron microscopy (TEM)

TEM images of K-OMS-2 material and K-OMS-2 supported Cu catalysts are given in Figure 3. The TEM micrograph of K-OMS-2 is consist of well-defined lattice planes, confirming the good crystallinity of K-OMS-2 material (Figure 3a, b). The lattice fringes spacing of 0.47 and 0.69 nm in K-OMS-2 material are the characteristic of (200) and (110) crystal planes, respectively, which are related to the planes of the cryptomelane structure (Suib *et al.*, 1997; Chen *et al.*, 2001; Amin *et al.*, 2000; Suib *et al.,* 2000). It can be seen that the Cu nanoparticles are homogeneously distributed throughout the K-OMS-2 support. The average particle size of Cu nanoparticles in 2wt% Cu / K-OMS-2 and 5wt% Cu / K-OMS-2 catalyst was found to be 2.2 and 3.3 nm, respectively (Figure 3c, d, e, f). Therefore, it can be concluded that the K-OMS-2 support play a vital role for the stabilization of Cu nanoparticles, hence leading to smaller Cu particles.

CONCLUSIONS

Octahedral Molecular Sieve (**K-OMS-2**) material was synthesized by redox method and was used for preparation of well dispersed Cu metal nanoparticles catalysts by ion-exchange method. All the materials were studied by various physico-chemical characterizations techniques such as XRD, N_2 sorption, ICP-OES and TEM. Highly efficient Cu nanoparticles catalysts would be promising heterogeneous catalyst.

Acknowledgement:

Dr. Atul S. Nagpure acknowledges Dr. Satyanarayana V. Chilukuri (Chief Scientist, CSIR-National Chemical Laboratory, Pune-411008, INDIA) for his significant guidance and discussion.

Conflicts of interest:

The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:44-48
UGC Approved Journal No 48951

Original Article Open Access

Soil fertility status under Rice based cropping systems in Gadchiroli Tehsil, Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Satpute GD, Rewatkar SB, Gupta RG (2018) Soil fertility status under Rice based cropping systems in Gadchiroli Tehsil, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A12: 44-48.

ABSTRACT

The present investigation was undertaken the study of soil fertility status under rice cropping in different villages of Gadchiroli Tehsil of Gadchiroli District (M.S). Ten villages are taken under the study. These villages are Gadchiroli, Gogaon, Amirza, Ambeshiwani, Dongarrgaon (Tukum), Lanzeda, Navegaon, Porla, Potegaon, Yewali. The soil samples were taken from 0-15 cm with the auger before and after the rice cultivation during the year 2014-15 and 2015-16. The pH values of soils in all the villages slightly acidic in nature varied from 5.99 to 6.11. The mean values of EC were found a range varying from 0.05 to 0.13ds/m. with the safe limit. Organic carbon was found in low range. The fertility status of soil could be evaluated using nutrient index method and fertility indicator. Evaluated nutrient index of soil using organic carbon, the soil samples showed low level.

Keywords: soil fertility, rice crop, Gadchiroli tehsil, Maharashtra.

INTRODUCTION

Soil, the source of life, is the most vital and valuable natural resource which is not renewable quickly. Soil fertility is a dynamic natural property that can change through the impact of natural and human derived factors (Kavitha et al., 2015). Having detailed knowledge about soil fertility is a prerequisite for assessing the long-term impact of new intensive rice production technologies on paddy soils (Dobermann et al., 1997). An assessment of the soil fertility status by using a soil index could provide key information to improve strategies and effective techniques for the future to achieve sustainable agriculture. Soil Fertility Index (SFI) values can be used to develop fertility maps and make recommendations based on soil spatial variability in fertility management. The analysis, which allows the identification of the main limiting factors for agricultural production, and enables decision makers to enhance high quality crop

management, can increase land productivity (Rabia, 2012).

Soil fertility is defined as 'a measure by its capacity to support population of plants and animals above ground, flora and fauna below ground'. In other words, soil fertility is 'the state of a soil with respect to its ability to supply elements essential nutrients for plant growth without toxicity effect. Concentrations of any element'. This definition has not been true for all soils and as a result, the productivity of such soils is impaired. Productivity therefore is a characteristic of a soil to adequately support plants growing on it. (Abdullahi, (1997).

In India, low fertility of soils is the major constraint to achieving high productivity goals (SLUSI, 2010). In many parts of the country, soil fertility fluctuates throughout the growing season each year due to alteration in the quantity and availability of mineral nutrients through the addition of fertilizers, manure, compost, mulch, and lime in addition to leaching (Denis et al. 2017).

The fertility status of soils can be evaluated using nutrient index methods and fertility indicators. (RaviKumar and Somashekar, 2013), evaluated the nutrient index of soils using organic carbon, available P and available K concentrations as a measure of soil fertility in Varahi River basin, India. Similarly, fertility status of soils of several micro watersheds in Karnataka has been mapped and the nutrient status of these areas is well documented (Vishwanath, 2008; Pulakeshi et al., 2012; Vidyavathi, 2012). However, the fertility status of soils of Gadchiroli area yet not studied. Hence, we have chosen Soil fertility status under rice based cropping systems in Gadchiroli Tehsil, Maharashtra.

MATERIAL AND METHODS

The study was carried out in 10 villages namely Gadchiroli, Gogaon, Amirza, Ambeshiwani, Dongarrgaon (Tukum), Lanzeda, Navegaon, Porla, Potegaon, Yewali. The soil samples were taken from 0-15 cm depth with the help of auger of 20 sites were randomly selected in each form; soil sampling was done in a zigzag pattern within each field and mixed thoroughly following a standard procedure for soil sampling and sample preparation (Andreas and Berndt, 2005). All the collected samples were air dried in shade, crushed gently with pestle and mortar, and then sieved through 2.0 mm sieve to obtain a uniform soil sample. The samples were analyzed for physicochemical properties by using standard methods of analysis for soil pH, electrical conductivity (EC) and soil organic carbon (OC).

RESULTS AND DISCUSSION

Soil pH

The measure of soil pH is an important parameter which helps in identification of chemical nature of the soil (Shalini et al., 2003), as it measures hydrogen ion concentration in the soil to indicate its acidic and alkaline nature of the soil. It follows from 0 to 7 are diminishing acidic, 7 to 14 increasing alkaline and 7 is neutral.

In the ten samples of Gadchiroli Tehsil, the variation of pH in June 2015 is from 6.00 to 6.11. The variation of pH in Dec. 2015 is from 6.00 to 6.08. The variation of pH in June 2016 is from 6.03 to 6.11. The variation of pH in Dec 2016 is from 5.99 to 6.11 were found.

Electrical Conductivity (EC)

The electrical conductivity (EC) is the measure of the soluble salts present in the soil and is affected by cropping sequence, irrigation, land use and application of fertilizers, manure, and compost (Singh et al., 2016). High value of electrical conductivity represents higher degree of salinity. Excessive amount of dissolved salts in soil solutions causes hindrance in normal nutrient uptake process by either imbalance of ions uptake, antagonistic effect between nutrients or excessive osmotic potentials of soil solution or a combination of the three effects (Rahman et al., 2010).

In the ten samples of Gadchiroli Tehsil, the variation of EC in June 2015 is from 0.05 to 0.13. The variation of EC in Dec. 2015 is from 0.07 to 0.12. The variation of EC in June 2016 is from 0.06 to 0.11. The variation of EC in Dec 2016 is from 0.08 to 0.12 were found.

Organic Carbon (OC)

Organic carbon has a vital role in agricultural soils It supplies plant nutrients, improves oil structure, improve water infiltration and retention, feeds soil micro flora and fauna, and enhance the retention and cycling of applied fertilizer (Johnston, 2007).

In the ten samples of Gadchiroli Tehsil, the variation of Organic carbon in June 2015 is from 0.28% to 0.53%. The variation of Organic Carbon in Dec. 2015 is from

0.23% to 0.45%. The variation of Organic carbon in June 2016 is from 0.26% to 0.53%. The variation of Organic carbon in Dec. 2016 is from 0.29% to 0.48%. In the Gadchiroli Tehsil, all the ten samples were in low range, as shown in Table No. 6.

Table 1: Method were used for analyzed soil parameter

Sr. No.	Parameters	Method	References
1.	Organic carbon	Combustion	Walkley and Black, (1934)
2.	pH	Water extract (1:2.5)	Rhoades and Oster (1986)
3.	Electrical Conductivity	Water extract (1:2.5)	Rhoades and Oster (1986)

Table 2: Soil samples collected from cultivator around of Gadchiroli Tehsil

Sr. No.	Name of village	Survey No.	Samples Code
1	Gadchiroli	237/3	Gd-1
2	Gogaon	441 [3079]	Gd-2
3	Amirza	354 [39]	Gd-3
4	Ambeshivani	136 [78]	Gd-4
5	Dongargaon (Tukum)	40 [23]	Gd-5
6	Lanzeda	196/ A/1 [3141]	Gd-6
7	Navegaon	158 [102]	Gd-7
8	Porla	296 [299]	Gd-8
9	Potegaon	30 [35]	Gd-9
10	Yewali	424 [419]	Gd-10

Table 3: Showing the result of pH of paddy soil of Gadchiroli Tehsil

Sr. No.	Sample Code	pH				Mean
		(June-2015)	(Dec-2015)	(June-2016)	(Dec-2016)	
1	Gd-1	6.08	6.01	6.07	6.05	6.05
2	Gd-2	6.07	6.06	6.06	6.07	6.06
3	Gd-3	6.11	6.05	6.08	6.07	6.07
4	Gd-4	6.01	6.00	6.08	6.09	6.04
5	Gd-5	6.00	6.01	6.07	6.06	6.03
6	Gd-6	6.10	6.08	6.03	6.01	6.05
7	Gd-7	6.08	6.02	6.05	6.02	6.04
8	Gd-8	6.09	6.05	6.07	6.07	6.07
9	Gd-9	6.02	6.00	6.07	6.05	6.03
10	Gd-10	6.03	6.01	6.11	5.99	6.04

Table 4: Showing the result of EC of paddy soil of Gadchiroli Tehsil

Sr. No	Sample Code	EC				Mean
		(June-2015)	(Dec-2015)	(June-2016)	(Dec-2016)	
1	Gd-1	0.05	0.8	0.06	0.09	0.07
2	Gd-2	0.08	0.07	0.07	0.09	0.07
3	Gd-3	0.08	0.09	0.08	0.07	0.08
4	Gd-4	0.09	0.10	0.11	0.09	0.09
5	Gd-5	0.12	0.10	0.11	0.08	0.10
6	Gd-6	0.10	0.12	0.11	0.12	0.11
7	Gd-7	0.12	0.11	0.11	0.12	0.11
8	Gd-8	0.08	0.08	0.06	0.07	0.07
9	Gd-9	0.07	0.08	0.09	0.10	0.08
10	Gd-10	0.13	0.12	0.11	0.11	0.11

Table 5: Showing the result of organic carbon of paddy soil of Gadchiroli Tehsil

Sr. No.	Sample Code	OC (%)				Mean
		(June-2015)	(Dec-2015)	(June-2016)	(Dec-2016)	
1	Gd-1	0.28	0.23	0.26	0.29	0.26
2	Gd-2	0.43	0.41	0.46	0.43	0.43
3	Gd-3	0.47	0.41	0.49	0.43	0.45
4	Gd-4	0.43	0.40	0.45	0.39	0.41
5	Gd-5	0.32	0.27	0.33	0.27	0.29
6	Gd-6	0.53	0.45	0.53	0.48	0.49
7	Gd-7	0.35	0.33	0.36	0.33	0.33
8	Gd-8	0.43	0.41	0.45	0.39	0.42
9	Gd-9	0.42	0.38	0.37	0.38	0.38
10	Gd-10	0.37	0.35	0.36	0.36	0.36

Table 6: Tabulated formats of range of soil organic carbon

Range of OC	Name of Samples	No. of Samples
Low Range (< 0.50 %)	Gd-1, Gd-2, Gd-3, Gd-4, Gd-5, Gd-6, Gd-7, Gd-8, Gd-9, Gd-10	10
Middle Range (0.50 to 0.75%)	Nil	Nil
High Range (> 0.75 %)	Nil	Nil

$$\text{Nutrient Index} = \frac{\text{No .of samples in low range X 1 + No of samples in medium range X 2 +No. of samples in high range X 3}}{\text{Total number of samples}}$$

$$\text{Nutrient Index} = \frac{10 \text{ X } 1 + 0 \text{ X } 2 + 0 \text{ X } 3}{10}$$

$$\text{Nutrient Index} = 1.00$$

Table 7. Nutrient index with range and remarks

Nutrient Index	Range	Remarks (OC)
I	Below 1.67	Low
II	1.67-2.33	Medium
III	Above 2.33	High

Nutrient index:

In order to compare the level of fertility of one area with those of another it is necessary to obtain a single value for each nutrient. Here the nutrient index introduced by Parker et al., (1951) is useful. The percentage of samples in each of the three classes, low medium and high is multiplied by 1, 2 and 3 respectively and divided by total number of samples to give the index.

CONCLUSION

The physico-chemical properties of soil were analyzed for 10 villages of Gadchiroli tehsil of Gadchiroli district. The parameter such as pH, Electrical Conductivity and Soil Organic Carbon were undertaken for study. The pH values of soils in all the villages slightly acidic in nature. The values of EC with the safe limit. Organic carbon was found that the samples in low range, the fertility status of soil could be evaluated using nutrient index method, the soil samples showed low level.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:49-54
UGC Approved Journal No 48951

Original Article Open Access

CCPPAC: A Surface Modified Material for Removal of Heavy Metal

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Hunge Sudhir, Rahangdale Pralhad, Lanjewar Mamata (2018) CCPPAC: A Surface Modified Material for Removal of Heavy Metal, *Int. J. of Life Sciences*, Special Issue, A12: 49-54.

ABSTRACT

Environment is deteriorating day by day due to industrial pollution, toxic chemicals leads to the accumulation of heavy metals contamination in the waste water. In view of their toxicity, non-biodegradability and persistent nature their removal becomes an absolute necessity. Hexavalent chromium metal is one of the carcinogenic pollutant in the environment and is frequently present in wastewater from various industrial units. The present research article reports the characterization and use of chitosan-coated activated carbon derived from the bark of *Pongamia pinnata* (CCPPAC*)* as a potential adsorbent after for removal of hexavalent chromium from aqueous solution. SEM analysis proved the mesoporous nature of the material under investigation. The batch experiment was carried out to study the effect of significant process parameters such as pH, contact time, adsorbent doses and initial Cr(VI) concentration. The maximum adsorption efficacy for Cr(VI) removal by CCPPAC was found at pH 4.5, 5 gm/lit of adsorbent dose and 140 min contact time. Under optimum condition, 96% Cr(VI) was removed from aqueous solution. This investigation verifies that CCPPAC, a mesoporous material can be successfully used as an excellent sorbent material for removal of hexavalent chromium from contaminated water and thus can be applied in wastewater treatment.

Keywords: Adsorption, Bio-sorbent, Chitosan, Hexavalent chromium, *Pongamia pinnata* bark.

INTRODUCTION

The pollution of water emerged as one of the most significant environmental problems of recent times. Pollution of water has its origin mainly in urbanization, industrialization and increase in human population observed during the past one and half century. Several industries like sugar factories, dairies, paper and pulp, tanneries, metal

plating, fertilizer industries etc. releases substantial quantities of toxic heavy metal in water. The removal of heavy metal contaminants from aqueous solutions is one of the most important environmental concerns because metals are biorefractory and are toxic to many life forms (KaYrabulut *et al*. 2014). Metals, which are significantly toxic to human beings and ecological environments, include chromium, copper, lead, mercury, cadmium, nickel, iron etc. (Bowen, 1979).

Chromium(VI) is one of the most toxic and carcinogenic form for bacteria, plants and animals. Chromium and its compound are widely used in the chromplating, leather tanning, metal processing, wood preservatives etc. (Fiol *et al*, (2000), Gupta, *et al*, (2003) The maximum concentration limit for chromium discharge into inland surface water is 0.1mg/l and it should not exceed to 0.05mg/l in potable water. Several technologies have been developed to remove carcinogenic chromium(VI) from water and waste water. The most common methods include chemical precipitation, ion exchange, ultra-filtration, solvent extraction, sedimentation, reverse osmosis, dialysis and adsorption etc. (Hunge *et al*. (2014). However, these conventional methods have certain major disadvantages such as incomplete removal and high operating cost. Amongst all of these, adsorption onto commercial activated carbon is well-established and effective technique. However, it is highly expensive since most of the activated carbon materials are obtained from non-renewable sources like coal, lignite, peat etc. It is a growing need to derive the activated carbon from cheaper and locally available waste materials. Several research workers used different low-cost adsorbents from agriculture waste such as coconut coir pith, sawdust, rice husk, cotton seed hulls, sugarcane bagasse, peanut hull etc. for the removal of Cr(VI) from contaminated water. The present investigation, studies were carried out for the removal of Cr(VI) from aqueous solution using activated carbon derived from bark Pongamia pinnata belong to Rhamnaceae family which is an extremely drought hardy and native fruit of India. It is useful as food, fodder, nutrient and medicine. Pongamia pinnata having tremendous medicinal properties, attributed by adverse group of secondary metabolites such as alkaloids, flavonoids, terpenoids, saponin, pectin, triterpenoic acids and lipids. It is extensively used in Ayurveda, Unani and Haemeopathic medicine. Chitosan{2-acetamido-2-deoxy-β-D-glucose-(N-acetylglucosamine)} is a deacetylated polymer of chitin and is usually prepared by its deacetylation with strong alkaline solution. It has excellent physicochemical properties. It is environmental friendly and bioactive material which is slightly soluble at low pH. It is soft and has tendency to form a gel in aqueous solution (Govindarajan *et al* (2011), Nomanbhay, *et al*. (2005). The composite sorbent was characterized by FTIR and Scanning Electron Microscopy (SEM) studies. Batch isothermal equilibrium method was conducted at 303K to evaluate the efficiency of newly synthesized bio-sorbent for removal of Cr(VI) from the aqueous solution. Experiments were carried out to study the effect of pH, adsorbent dosage, contact time and initial Cr(VI) concentration. The newly synthesized composite has been proved to be very good adsorbent which can be successfully used for removal of carcinogenic hexavalent chromium from aqueous solution.

METHODS AND MATERIALS:

Chemicals

All the chemicals used in the investigation were of either analytical or chemically pure grade and procured from Merck (Mumbai, India).

Preparation of Activated Carbon from the bark of *Pongamia pinnata* (PPAC)

The bark of *Pongamia pinnata* tree was collected from the local area. The bark was cut into small pieces, washed with tap water to remove the sand particles and then treated with formaldehyde to avoid release of any colour of bark into aqueous solution. Then, it was washed several times with deionized water and sun dried for 6 days. After drying, the bark was subjected to pyrolysis process for carbonization using Muffle Furness at 800-900^{0}C for 7 to 8 hrs so that volatile constituents were removed and residue was converted into a char. The char was then subjected to microwave activation in microwave oven at 360 W for 30 min (Hunge SS *et al* (2014) The resulting activated carbon particles were ground and sieved in 120-200 mm size. This activated carbon was then washed with double distilled water and dried at 105^{o}C for 3 hrs and stored in airtight bottle.

Preparation of Chitosan Gel

Chitosan was procured from Otto Chemical, Mumbai (India). 30 g of chitosan was added into 1000 ml of 10% oxalic acid with constant stirring. The mixture was warmed at 40 – 45^{0}C for proper mixing. The chitosan-oxalic acid mixture was formed as a whitish viscous gel.

Surface coating of PPAC with Chitosan Gel

500 ml of Chitosan gel was double diluted with distilled water and warmed to 40 -45^{0}C. 300 g of PPAC was slowly added into diluted chitosan gel and shake mechanically using rotary shaker for 24 hr. The chitosan coated PPC(CCPPAC) was then washed with deionized water and dried. The process was repeated 3 times to form thick coating of chitosan on the PPAC surface. The coated chitosan was 30 to 35% by weight. Oxalic acid was quantitatively neutralized (Caiesoughlin *et al (*1990)by 0.5% sodium Hydroxide solution. The solid CCPPAC was filtered, washed with deionized water, dried and stored in air tight container.

Characterization of CCPPAC

Characterization of CCPPAC was done by FTIR (Fig.1) and SEM (Fig.2)

Adsorption Studies

Working standards were prepared by progressive dilution of stock solution of Cr(VI). Removal of Cr(VI) using CCPPAC was carried out by batch equilibrium method. The influence of various parameters such as effect of pH, contact time, adsorbent dosage and initial Cr(VI) concentration were studied, taking 25 mg/l of initial Cr(VI) concentration and 5 g/l of adsorbent dose. The effect of adsorbent doses was studied by varying them from 0.5-10g/l. The effect of initial Cr(VI)concentration was studied by changing concentration from 10-100mg/l with adsorbent dose of 5g/l at 30^{0}C. The residual concentrations were measured using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Characterization of CCPPAC

FTIR spectrum of CCPPAC is shown in **Fig.1**. The band at 3445.27cm^{-1} indicates presence of the free hydroxyl group stretching of chitosan molecules. The band at 2662.41cm^{-1} is due to the C-H bond stretching of aldehyde (C-H=O Group). The shifting of band from its standard value (2889.21.cm^{-1}) due to the involvement of chitosan in composite formation. The absorption at 1633.30cm^{-1} is due to the C=O stretching mode of the amido (CONH) group of chitosan. The two characteristic bands appeared at 1005.85cm^{-1} and 911.73cm^{-1} (skeletal vibration involved in C-O-C stretching) prove the presence of saccharide structure (Peniche *et al* (1969)**.** The peaks at 532.07cm^{-1} and 464.47cm^{-1} corresponds to N-H bending.

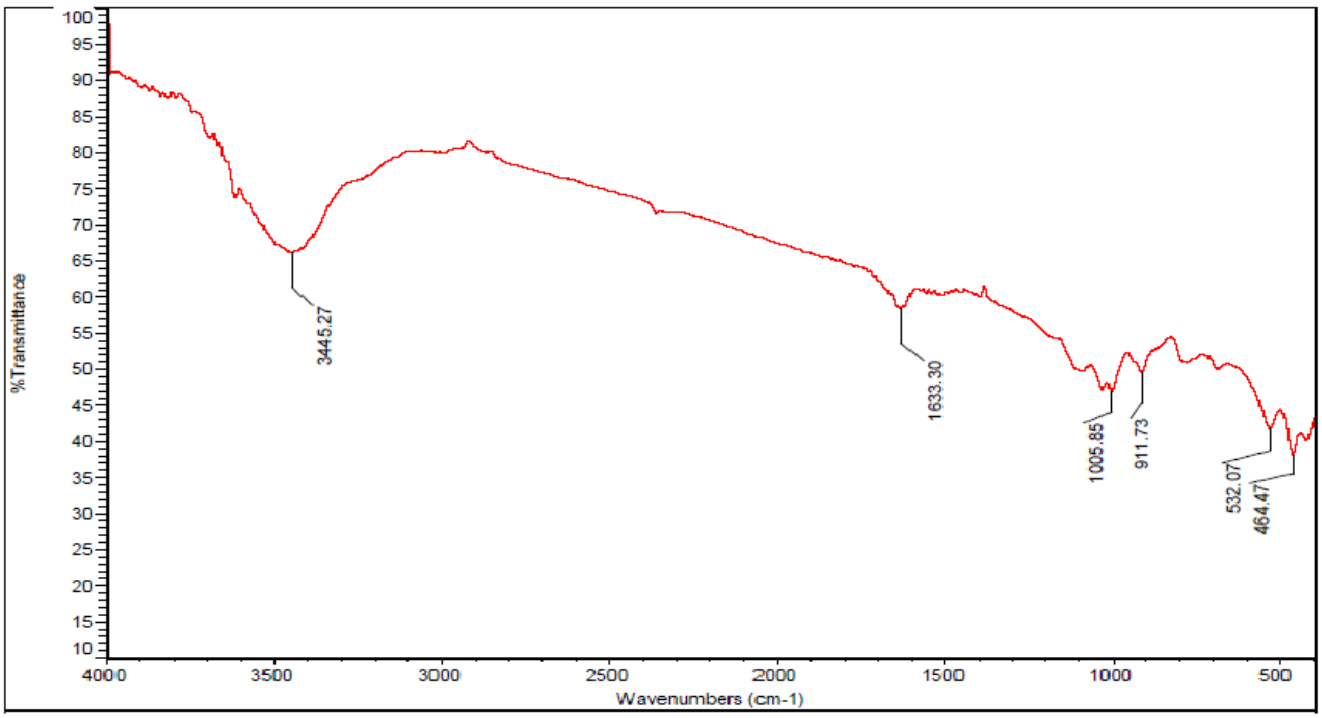

Fig.1 FTIR Spectrum of Chitosan Coated *Pongamia pinnata* Activated Carbon (CCPPAC)

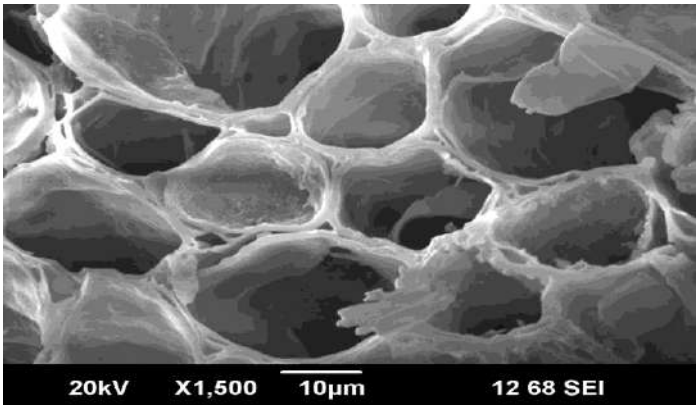

Fig.2 SEM image of Chitosan Coated *Pongamia pinnata* Activated Carbon (CCPPAC)

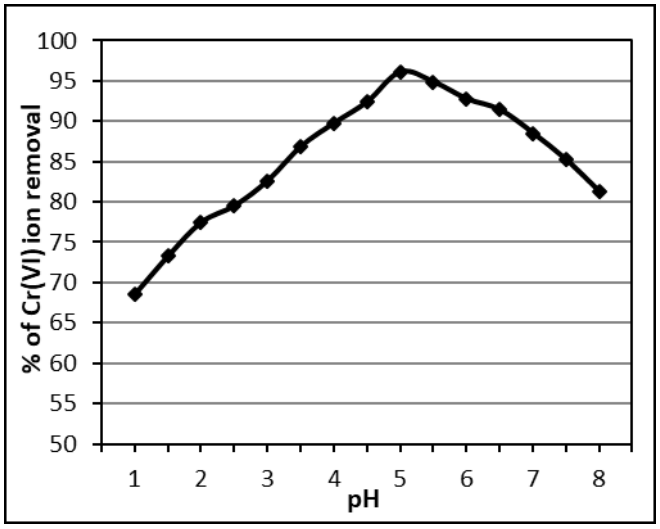

Fig.3 Effect of pH on Cr(VI) removal by CCPPAC

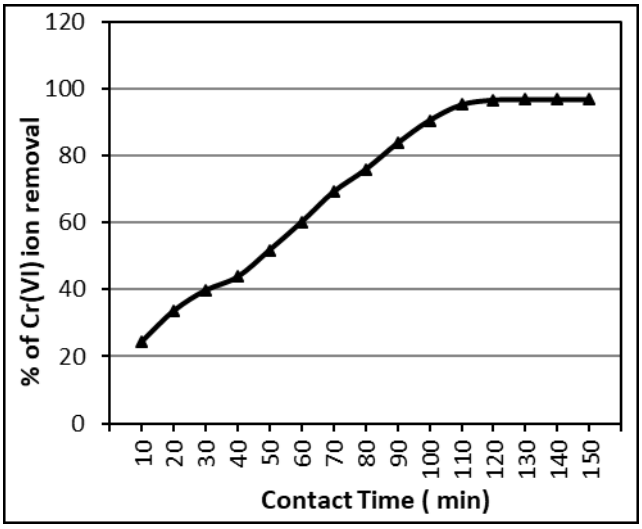

Fig.4 Effect of Contact time on Cr(VI) removal by CCPPAC

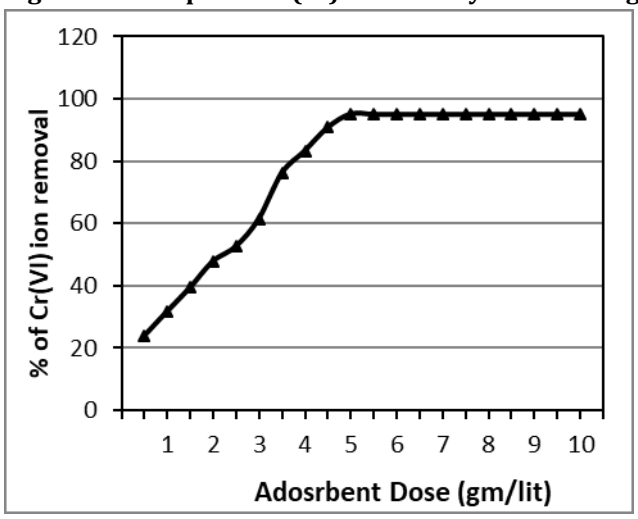

Fig.5 Effect of Adsorbent dose on Cr(VI) removal

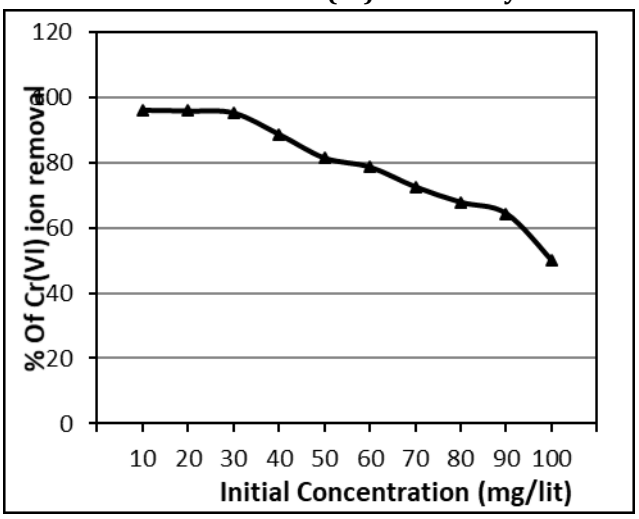

Fig.6 Effect of initial concentration of Cr(VI) removal

Fig.2 represents SEM micrographs of CCPPAC. SEM image has been obtained using an accelerating voltage of 20kV at X1500, magnification. High magnification SEM micrographs clearly reveal that the wide varieties of pores are present on the surface of CCPPAC accompanied with fibrous structure. It can also be noticed that there are holes and cave type openings on the surface of the adsorbent, which would have created more surface area available for adsorption. The size of holes and caves was found to be in the range 1- 10µm.

Effect of pH

The effect of pH on the adsorption of Cr(VI) by CCPPAC was studied at pH 1 to 8. From **fig.3** it is clear that the removal of Cr(VI) increases with an increase in pH from 1.0 to 5.0 and it is optimum at 4.5. The percent of adsorption increases from 60 to 96 as pH was increased from 1 to 5. The percentage of adsorption decreases steadily to 83% when pH increased above 5.0 and it was further decreased to 70% as pH was raised to 8.

Effect of Contact Time

Adsorption experiments were conducted as a function of contact time and results have shown in **Fig.4**. It can be observed that Cr(VI) removal ability of CCPPAC increased with increase in contact time before equilibrium was reached. Other parameters such as dose of CCZMAC, pH of solution and initial concentration were kept optimum. It can be seen from fig.4 that Cr(VI) removal efficiency increased from 25 to 96% when contact time was increased from 10 to 160 min. Optimum contact time for CCPPAC was found to be 140 min. Cr(VI) removal efficiency remained nearly constant after 130 min i.e. equilibrium time.

Effect of Adsorbent Dosage

Fig.5 shows the effect of dosage on the removal of Cr(VI) which was studied by varying the amount of CCPPAC from 0.5 to 10g/l while keeping other parameters (pH, contact time and initial concentration) constant. It is clear from the figure that percentage removal of Cr(VI) increased with the increase in CCPPAC doses and it was found to be maximum i.e. 95% at the dose of 5g/l. This is due to availability of more surface area. It indicates that by increasing the CCPPAC dosages, the adsorption efficiency for Cr(VI) removal increases. After 5g/l dose of CCPPAC, the adsorption efficiency remain constant because the maximum adsorption set in and amount of Cr(VI) present in the solution bounded to adsorbent remains nearly constant after this dose.

Effect of initial metal ion concentration

The effect of initial metal ion concentration on the percentage removal of hexavalent chromium by CCPPAC has shown in fig.6. It can be seen that the percent removal of Cr(VI) decreases with the increase in initial Cr(VI) concentration. In this study, the experiment was performed to study the initial concentration effect in the range 10-100mg/l .The adsorbent dose was maintained 5g/l. The result shows the decrease in removal from 96 to 49%. This can be justified by the fact that adsorbent have limited number of active sites which are saturated beyond certain concentration of adsorbate.

CONCLUSION

- The activated carbon derived from the bark of *Pongamia pinnata* tree and surface was successfully coated with chitosan and characterized employing FTIR and SEM studies.
- The newly developed CCPPAC high porous structure and excellent surface area.
- CCPPAC was most effective for Cr(VI) removal. At pH 4.5, 96% of Cr (VI) was removed from aqueous solution. Adsorption was found to pH dependent. Above pH 5.0, decline in Cr(VI) removal was noticed.
- The increase in percent removal capacity for Cr(VI) was observed with increase of adsorbent dose and contact time. Maximum removal is 95% for 5.0 g/l dose and 130 min. of contact time.
- The Chitosn coated activated carbon under present investigation can be successfully employed for Cr(VI) abatement from contaminated water and thus can be used for water/ wastewater treatment.

Acknowledgments

Authors are highly thankful to Dr. R.B. Kharat, Ex-Director, Institute of Science, Nagpur for their valuable guidance, moral support, timely help and constant encouragement during the course of this investigation. Authors also thanks to Scientist In-charge, SAIF, STIC, Cochin University, Cochin for FTIR and SEM analysis of the sample. Authors are also thankful to Managing Director of Deenee Chemical Laboratory (DCL), Chandrapur for access atomic absorption spectrophotometer and UV-Visible spectrophotometer and other instrumental facilities as and when required.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:55-57
UGC Approved Journal No 48951

Original Article Open Access

Effect of temperature on molecular interaction of extract Brayophallum leaves

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Bedare GR and Dhote AB (2018) Effect of temperature on molecular interaction of extract Brayophallum leaves, *Int. J. of. Life Sciences*, Special Issue, A12: 55-57.

ABSTRACT

Presence of electron donor and electron acceptor group changes the relative strength of intermolecular interaction present in the liquids. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Extraction of brayophallum leaves in water was carried out using soxhelt extractor and its ultrasonic velocity, density and viscosity were measured at different temperatures. The data obtained from ultrasonic propagation parameters such as ultrasonic velocity, adiabatic compressibility, acoustic impedance, free length and their variation with temperature is useful in understanding the nature of molecular interaction in terms of physical parameters.

Keywords: ultrasonic, adiabatic compressibility, molecular interaction, extraction, brayophallum

INTRODUCTION

The ultrasonic study of liquid is most important in understanding the nature and strength of molecular interactions. The biological activity of drug molecules and the activation energy of the metabolic process basically depend on the type and strength of the intermolecular interactions. From the literature, the nature and degree of molecular interactions in different solutions depend upon the nature of solvent, the structure of solute molecule and extent of solutes taking place in the solution (Vasantharani *et al*. (2009), Kaur and Juglan (2015), Kolhe and Bhosale (2017), Dhote and Bedare (2017). Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and the bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids Edoga *et al*. (2005), Mann (1978).

In the present study we were extracted Bryophyllum leaves using soxhelt extractor in the solvents water. The ultrasonic velocity, density and viscosity of waterextract is measured at different temperatures. From experimental data acoustic parameters were calculated and effect of temperatures on molecular interaction of water extract of brayophallum leaves was predicted.

METHODS AND MATERIALS:

The leaves of Brayophyllum leaves were collected. The powdered plant samples were extracted successively with water using Soxhlet apparatus at 55-85 °C for 8-10 hour in order to extract the polar and non-polar compounds. For each solvent extraction, the powdered pack material was air dried and then used.

The ultrasonic velocity (U) in liquid mixtures which prepared by taking purified AR grade samples, have been measured using an ultrasonic interferometer (Mittal type, Model F-81) working at 2MHz frequency and at temperature 303K. The accuracy of sound velocity was ±0.1 ms-1. An electronically digital operated constant temperature water bath has been used to circulate water through the double walled measuring cell made up of steel containing the experimental solution at the desire temperature. The density of pure liquids and liquid mixtures was determined using pycknometer by relative measurement method with an accuracy of ±0.1Kgm-3.

RESULTS AND DISCUSSION

Using the experimental data of ultrasonic velocity (U), density (ρ), viscosity (η), various acoustical parameters such as adiabatic compressibility (βa), intermolecular free length (L_f), Acoustic impedance (Z) at different temperatures were calculated by the following equation

$\beta a = (U2\rho)-1$ ….. (1)

$Lf = K\beta a$ ½ ….. (2)

$Z = U \rho$ …… (3)

$\tau = 4/3\eta\beta a$ …… (4)

The decrease in ultrasonic velocity and density with increase in temperature shows decreasing cohesive forces. When temperature increases it has two opposite effects namely increase in molecular interaction and destruction of structure formed previously. When the thermal energy is more than the interaction energy, it causes the destruction of previously formed structure Godhani *et al*. (2012). Thus, the increase in temperature favors the increase in kinetic energy and volume expansion and hence, results in the decrease in density and ultrasonic velocity. It shows that with increasing temperature less interaction between the constituent of Brayophyllum leaves in water solvent.

Adiabatic compressibility is a measure of intermolecular association or dissociation or repulsion. The adiabatic compressibility should beindependent of temperature and pressure for unassociated and weakly associated molecules and determines the orientation of the solvent molecules around the liquid molecules (Singh *et al*., 1991). The structural arrangement of the molecule affects the adiabatic in the present investigation it shows that with increase in temperature adiabatic compressibility increases indicating that there is a weak interaction between content of Brayophyllum leaves in water. The free length is the distance between the surfaces of the neighboring molecules.

Generally, when the ultrasonic velocity increases,the value of the free length decreases and vice versa. The increase in intermolecular free length indicates the weak interaction between the solute and solvent molecules with increasing temperature.

Table 1:Acoustic parameters of extract of Brayophyallum leaves at different temperatures

Temperature K	Ultrasonic velocity (m/s)	Density (Kg/m^3)	viscosity $\eta x10^{-3}$ (NSm^{-2})	Adiabatic compressibility $\beta x10^{-10}$	Intermolecular free length	Acoustic Impedance $Zx10^4$	Relaxation Time $T x x10^{-11}$
301.15	1785.50	1057.4	0.9868	3.068	0.0110	185.626	4.288
303.15	1715.50	1053.1	0.9373	3.225	0.0112	180.720	4.0303
308.15	1695.45	1049.8	0.8608	3.313	0.0114	177.983	3.464

Acoustic impedance decreases with increasing temperatures indicates weak interaction in the solution. As temperature increases, excitation energy increases and hence relaxation time decreases.

CONCLUSION

With increasing temperature ultrasonic velocity of extract of brayophallum leaves decreases while adiabatic compressibility, free lengthincreases while acoustic impedance and relaxation time decreases which shows weak molecular interaction in the solution.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:58-62
UGC Approved Journal No 48951

Original Article Open Access

Synthesis of 5-((benzylidene amino)methyl)-4-(substituted phenyl),6-methyl, 3,4-dihydropyrimidin-2(1H)-ones

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Dharamkar RR and Meshram YK (2018) Synthesis of 5-((benzylidene amino)methyl)-4-(substituted phenyl),6-methyl, 3,4-dihydropyrimidin-2(1H)-ones , *Int. J. of. Life Sciences*, Special Issue, A12: 58-62.

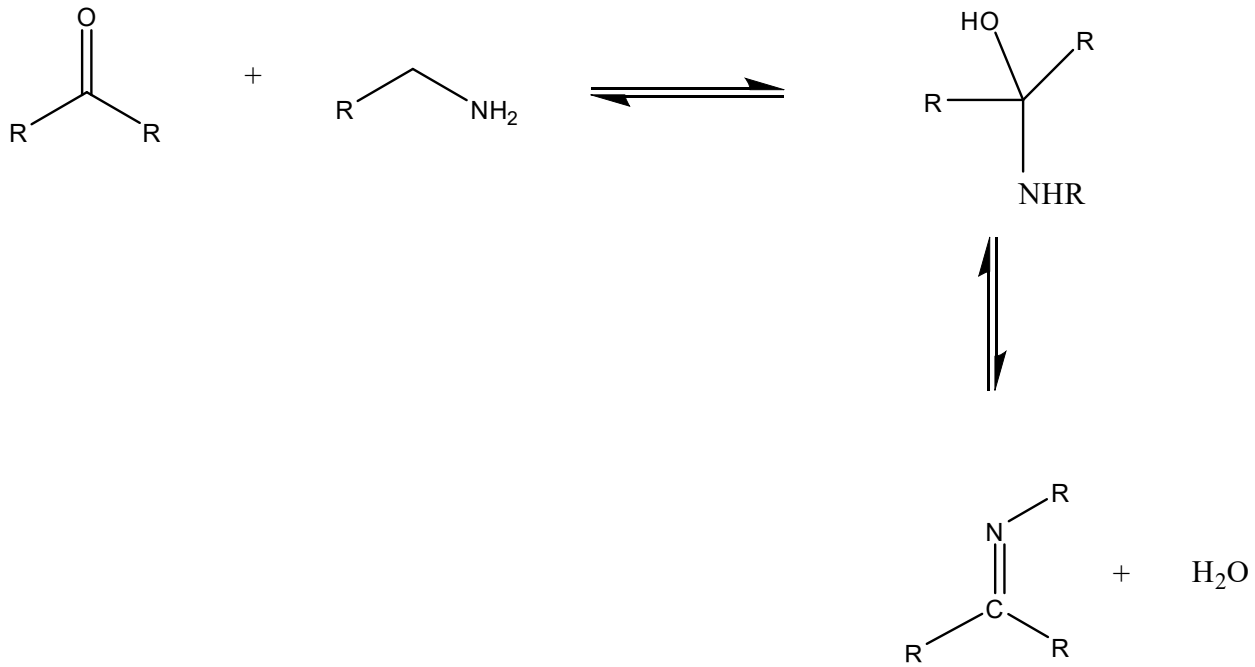
ABSTRACT

A Simple and Economic Synthesis of 5-((benzylidene amino)methyl)-4-(substituted phenyl)-6-methyl, 3,4-dihydropyrimidin-2(1H)-ones by using 5-(aminomethyl)-4-(substituted phenyl)- 6-methyl- 3,4-dihydropyrimidin -2(1H)-ones and benzaldehyde in presence of ethanol. When banzaldehyde is react with primary amine i.e. 5-(aminomethyl)-4-(substituted phenyl)- 6-methyl- 3,4-dihydropyrimidin-2(1H)-ones in presence of alcoholic medium it gives formation of corresponding Schiff bases. The newly synthesized compounds were well characterized by IR, 1H NMR and mass spectral studies.

Keywords: Benzaldehyde, Ethanol, Schiff bases, 5-(aminomethyl)-4-(substituted phenyl)- 6-methyl- 3,4-dihydropyrimidin-2(1H)-ones etc.

INTRODUCTION

Over the last two decades there has been rapid progress in synthetic organic chemistry associated with the search for new organic compounds derivatives with desirable properties. Such compounds are widely used in the pharmacetutical industry (Kekare *et al*., 2014) The four-membered cyclic amides commonly known as 2-azetidinones or β-lactams occupy a prominent place in the realm of organic and medicinal chemistry since the structure elucidation of penicillin showed the presence of β -lactam ring in it and the antibacterial activity of Penicillin was attributed to the presence of β -lactam ring. The early investigations in organic chemistry were focused on broadening the spectrum of antibacterial activity. These studies led to development of several novel methodologies for construction of the β -lactam ring and discovery of several β -lactam antibiotics, such as monobactams, cephalosporins, carbapenams, trinems etc. (Singh, 2013). β-lactam antibiotics, since their introduction continue to be chemotherapeutics of incomparable effectiveness, conjugating a broad spectrumof activity with low toxicity (Wright *et al* 1999). It possess pharmacological activities such as anti-viral (Preethi *et al*., 2013)antihyperlipidemic (Piste *et al*., 2014) human leukocyte elastase by



Feledziak *et al* ,2009 antidepressant by Shah *et al*, 2012, anti-parkinsonian (Elumalai *et al*., 2013) anti-tumor (Bricker *et al*., 1992) tubercular (Rao *et al*., 2012) enzyme inhibitory (Parmar *et al*., 2012) and antithrombotic (Kumar, 2011).

Hugo Schiff was the first scientist who described Schiff bases in 1864 (Schiff , 1864) The prepara tion of Schiff bases involves a variety of conditions and is brought about by mixing carbonyl compounds and amines in various proportions and employing a range of solvents. The formation of Schiff bases is generally favored by making use of dehydrating agents. A great care should be taken for the purification of Schiff bases as they are degradable. The acid/base catalysis or heating is employed for the synthesis of Schiff bases as their reactions are mostly reversible (Anis *et al*., 2013).

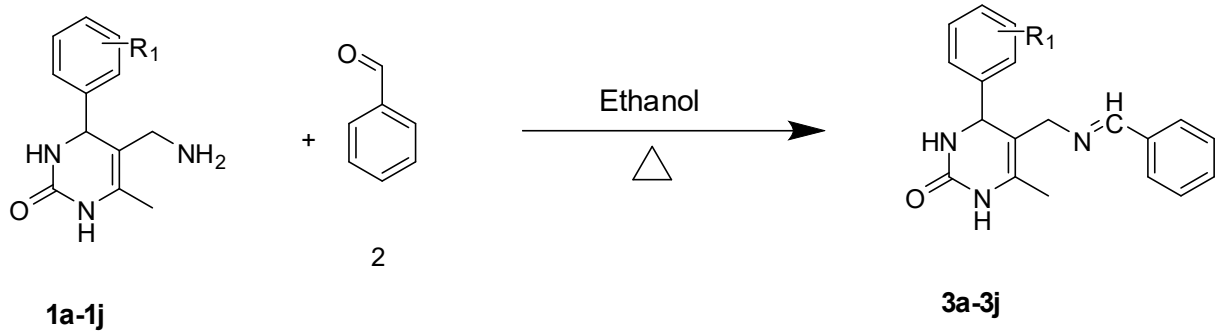
The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R_1, where R and R_1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethine (Ashraf *et al*., 2011) The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centers of cell constituents and interferes in normal cell processes. Schiff bases from an important class of the most widely used organic compounds and have a wide variety of applications in many fields including analytical, biological and inorganic chemistry (Kajal *et al*., 2013).

Schiff bases have been utilized as synthons in the preparation of a number of industrial and biologically active compounds like formazans, 4-thiazolidinies, benzoxazines, 2-azetidinone and so fourth via ring closure, cycloaddition and replacement reactions (Jarrahpour *et al*., 2007). Schiff bases have received more attention mainly because of their wide biological activities including anti-tumor (Pattanail *et al*., 2011) antibacterial (Wadher *et al*., 2009) fungicidal (Kumar *et al*., 2010) anti-inflammatory (Sachdeva *et al*., 2014) antiviral (Kumar *et al*., 2010) herbicidal (Nicolae *et al*., 2013) antipyretic (Kabeer *et al*., 2001) and anticonvulsant (Sen *et al*., , 2013). Schiff bases and their cyclization to produce β -lactam derivatives of biological significance (Kdura *et al*., 2011).

RESULTS AND DISCUSSION

In this communication, the equimolar quantity of 5-(aminomethyl)-4-(substituted phenyl), 6-methyl,3,4-dihydropyrimidin-2(1H)-one (1a-1j) (0.01mol) and benzaldehyde (2) (0.01mol), were taken in Round bottom flask with 5-10ml ethanol as a solvent and the reaction mixture was subjected to reflux for few hours. The product, obtained was poured over crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol to afford pure 5-((benzylidene amino)methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin -2(1H)-ones. (**3a-3j**). And these results are summarized in Table-I.

Reaction:



1a-1j **3a-3j**

Scheme 1:
5-((benzylideneamino)methyl),-4-(substituted phenyl),6-methyl, 3,4-dihydropyrimidin-2(1H)-one
Where R_1 = a) –H , b) 4-OCH_3 , c) 4-NO_2 , d) 4-Br , e) 4-Cl f) 4-OC_2H_5 , g) 3-Cl , h) 2-Cl , i) 2-F , j) 2-OC_2H_5 .

Table I : Analytical data of synthesized 5-((benzylidene amino)methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin-2(1H)-ones.

Sr. No.	Compound	R_1	Reaction time (hrs)	M.F.	M.W.	Yield	M.Pt (^{0}C)
1	3a	H	1.5	$C_{19}H_{19}ON_3$	305	80%	240^{0}C
2	3b	p-OCH_3	1	$C_{20}H_{21}O_2N_3$	335	75%	232 ^{0}C
3	3c	p-NO_2	0.50	$C_{19}H_{18}O_3N_4$	350	82%	215 ^{0}C
4	3d	p-Br	1.20	$C_{19}H_{18}ON_3Br$	383.9	80%	239 ^{0}C
5	3e	p-Cl	0.50	$C_{19}H_{18}ON_3Cl$	339.5	85%	245 ^{0}C
6	3f	p-OC_2H_5	1.45	$C_{21}H_{23}O_2N_3$	349	90%	222 ^{0}C
7	3g	m-Cl	1.30	$C_{19}H_{18}ON_3Cl$	339.5	88%	233 ^{0}C
8	3h	o-Cl	1.30	$C_{19}H_{18}ON_3Cl$	339.5	81%	248 ^{0}C
9	3i	o-F	1.20	$C_{19}H_{18}ON_3F$	323	76%	240 ^{0}C
10	3j	o-OC_2H_5	1	$C_{21}H_{23}O_2N_3$	349	79%	242 ^{0}C

Experimental Section: The melting points of all synthesized compounds were recorded using open capillaries and are uncorrected. The IR spectra were recorded on a PERKIN ELMER Spectrophotometer in the frequency range 4000-400 cm^{-1} in Nujol mull and as KBr pellets. 1H NMR spectra were recorded on BRUKER ADVANCE II 400 spectrometer with TMS as internal standard using DMSO as solvents. All the compounds are synthesized in R. B. Flask by using water condenser and refluxed for several times. Purity of the compounds were checked on pre- coated silica-G plates by TLC.

Chemicals (Reagents) used in the synthesis of 5-((benzylidene amino)methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin -2(1H)-ones, were of AR gread.

General Procedure : In this case, the equimolar quantity of 5-(aminomethyl)-4-(substituted phenyl), 6-methyl,3,4-dihydropyrimidin-2(1H)-one (1a-1j) (0.01mol) and benzaldehyde (2) (0.01mol), were taken in Round bottom flask with 5-10ml ethanol as a solvent and the reaction mixture was subjected to reflux for few hours. The product, obtained was poured over crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol to afford pure 5-((benzylidene amino)methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin -2(1H)-ones. (3a-3j).

Spectroscopic data of Representative 5-((benzylidene amino) methyl) - 4-(substituted phenyl) - 6-methyl - 3,4-dihydropyrimidin-2(1H)-ones.

1. 5-((benzylidene amino) methyl)-4-(phenyl), 6-methyl, 3,4-dihydropyrimidin-2(1H)-one. (entry-1): m.p 240^{0}C, IR(KBr) :[cm $^{-1}$] 3416, 2929, 1678, 1436, 1388, 934, 713 ; PMR (DMSO-d6):7.7(1H,s,CH),7.9(4H,m,ArH),6.9 (1H,bs,NH),2.3(3H,s,CH_3),10.0(1H,s,NH).

2. 5-((benzylidene amino) methyl)-4-(4-methoxy-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one.(entry-2): m.p 232^{0}C ,IR(KBr) :[cm $^{-1}$] 3414, 2929, 1678, 1386, 1029, 752 ; PMR (DMSO-d6) :6.9(1H,s,NH),7.4(4H,m,ArH),6.9 (1H,bs,NH),2.5(3H,s,CH_3),7.2(1H,s,CH), MS(m/z,%) 335(M^+), 331[M-$C_6H_5(OCH_3)$,Imine ring]$^+$

3. 5-((benzylidene amino) methyl)-4-(4-nitro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-3): m.p 215^{0}C, IR(KBr):[cm $^{-1}$] 3624, 3227, 1663, 1341, 1075, 727 ; PMR (DMSO-d6) :7.0(1H,bs,NH),7.6(4H,m,ArH),8.7 (1H,bs,NH),2.0(3H,s,CH_3),5.3(1H,s,CH).

4. 5-((benzylidene amino) methyl)-4-(4-bromo-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-4): m.p 239^{0}C, IR(KBr): [cm $^{-1}$] 3487, 3118, 1760, 1442, 1039, 752 ; PMR (DMSO-d6) :8.7(1H,bs,NH),8.2(4H,m,ArH), 6.9(1H,bs,NH),2.0(3H,s,CH_3),6.9(1H,s,NH).

5. 5-((benzylidene amino) methyl)-4-(4-chloro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-5): m.p 245^{0}C, IR(KBr):[cm $^{-1}$] 3435, 3235, 1710, 1402, 1087, 706 ; PMR (DMSO-d6) :7.0(1H,bs,NH),7.6(4H,m,ArH),8.6 (1H,bs,NH),2.1(3H,s,CH_3),5.28(1H,s,CH).
6. 5-((benzylidene amino)methyl)-4-(4-ethoxy-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one.(entry-6): m.p 222^{0}C ,IR(KBr) :[cm $^{-1}$] 3622, 3187, 1791, 1702, 1499, 1046, 706 ; PMR (DMSO-d6) :8.5(1H,bs,NH),6.8(4H,m,ArH), 5.1(1H,bs,NH),2.5(3H,s,CH_3),9.9(1H,s,CH), MS(m/z,%) 349(M^+), 331.28[M-$C_6H_5(OC_2H_5)$,Imine ring, CO]$^+$

7. 5-((benzylidene amino) methyl)-4-(3-chloro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-7): m.p 233^{0}C, IR(KBr): [cm $^{-1}$] 3327, 2929, 1607, 1436, 996, 717; PMR (DMSO-d6) :7.2(1H,bs,NH),7.3(4H,m,ArH), 7.5(1H,bs,NH),2.0(3H,s,CH_3),8.6(1H,s,CH).

8. 5-((benzylidene amino)methyl)-4-(2-chloro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one.(entry-8): m.p 248^{0}C ,IR(KBr) :[cm $^{-1}$] 3741, 3018, 1698, 1438, 993, 782 ; PMR (DMSO-d6) :6.9(1H,bs,NH),7.3(4H,m,ArH),7.4 (1H,bs,NH),2.0(3H,s,CH_3),5.6(1H,s,CH), MS(m/z,%) 338.5(M^+), 301.14[M-C_6H_5,Imine ring]$^+$

9. 5-((benzylidene amino) methyl)-4-(-fluoro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-9): m.p 240^{0}C, IR(KBr):[cm $^{-1}$] 3741, 3616, 1557, 1433, 991, 710 ; PMR (DMSO-d6) :6.9(1H,bs,NH),7.2(4H,m,ArH), 7.3(1H,bs,NH),2.0(3H,s,CH_3),8.6(1H,s,CH).

10. 5-((benzylidene amino) methyl)-4-(2-chloro-phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one. (entry-10): m.p 242^{0}C, IR(KBr): [cm $^{-1}$] 3622, 3088, 1674, 1450, 1038, 741 ; PMR (DMSO-d6) :7.2(1H,bs,NH),7.1(4H,m,ArH),6.9 (1H,bs,NH),2.0(3H,s,CH_3),8.5(1H,s,CH).

CONCLUSION

we have developed a simple quick and efficient method or the synthesis of 5-((benzylidene amino)methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin -2(1H)-ones by using benzaldehyde and ethanol. Apart from its Simplicity, the important advantage of the present protocol is the ability to tolerate variations in all the three components of the reaction. To the best of our knowledge, this is one of the quickest, economical and simple alternatives towards the synthesis of the 5-((benzylidene amino) methyl)-4-(substituted phenyl), 6-methyl, 3,4-dihydropyrimidin -2(1H)-ones. This introduces another important use of Benzaldehyde in the synthetic Organic Chemistry.

Acknowledgement:
The Authors are very thankful to the Principal of G. S. Science, Art's & Commerce College, Khamgaon, for providing the necessary facilities in the Laboratory and also to, CDRI, Lucknow, SAIF, Chandigarh , for providing the Spectral analysis.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:63-67
UGC Approved Journal No 48951

Original Article Open Access

Microwave Assisted Synthesis and Biological Evaluation of Transition Metal Complexes of p-methyl isonitrosophenyl Acetate

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Hiware NN , Raut RD , Choudhary MD and Wasim Khan (2018) Microwave Assisted Synthesis and Biological Evaluation of Transition Metal Complexes of p-methyl isonitroso-phenyl Acetate, *Int. J. of. Life Sciences*, Special Issue, A12: 63-67.

ABSTRACT

The shorter reaction times offered by microwave assisted deals with the synthesis of p-methyl isonitrosophenyl acetate using p-cresol , acetic anhydride and n-amyl nitrite. The Fe(II), and Fe(III) complexes of Schiff base derived from p-methyl isonitrosophenyl acetate have been synthesized. The complexes of the type ML2 have been synthesized and characterized on the basis of elemental analysis, conductivity, magnetic measurement, IR and electronic spectral studies. The conductivity data of the complexes suggests their non electrolytic nature. The biological activity of Schiff base and their metal complexes are studied against gram positive and gram negative bacteria by disc diffusion technique which shows that complex exhibit promising antibacterial activity than that of Schiff base against tested bacteria.

Keywords: p-methyl isonitrosophenyl acetate, complexes, spectroscopy, antimicrobial activity

INTRODUCTION

Chemist are looking for cleaner , more environmentally benign ways to make targeted synthesis In industry the shorter reaction times offered by microwave assisted synthesis are suited to explore the wide range of applications. Schiff bases are considered to be the important class of chelating agents especially when -OH functional group also present with azomethine group. In recants years, researcher shows much interest in the synthesis and characterization of Schiff bases metal complexes due to their importance as catalyst in many reactions (Huges, 1984; Chatterjee *et al*, 2000; Ali *et al*., 2002). Transition metals are essential for normal functioning of living organism and are, therefore, of great interest as potential drugs [Malhotra *et al*., 2006]. Coordination compounds derived from numerous Isonitroso ketones have been reported because of their anti-tuberculosis, antimicrobial and corrosion inhibitors [Fouda *et al*., 2008; Ali *et al*., 1988; Ferrari *et al*., 1999]. Isonitroso ketones are of great

interest since it has the ability to chelate metal ion through nitrogen and or oxygen donor centers. The interaction of metal ion with ligand containing oxygen and nitrogen as donor atom were undertaken by many chemist. It was also established that the biological activity of Schiff bases is altered many folds on coordination with metal ions [Malik *et al*., 2010]. Keeping the above fact in our mind and in continuation of work on transition metal complexes with Schiff bases [Saraf *et al*., 2012(a)] the ligand p-methyl isonitrosophenyl acetate (L) has been synthesized. and its metal complexes with Fe(II) , and Fe(III) were synthesized. The structure of ligand and metal complexes had been characterized by FTIR, H NMR and UV spectroscopy. The biological activity also studied against gram positive and gram-negative bacteria for ligand and metal complexes.

MATERIALS AND METHODS

All chemical used were of analytical grade and of highest purity available and used without further purification. P-cresol and n-amyl alcohol were obtained from M/S Merck chemicals. Metal (II) chlorides and acetate salts were also obtained from Merck. Solvents used were distilled and purified before used.

Synthesis of p- Methyl isonitroso phenyl acetate

p-cresol (100ml) and acetic anhydride(120ml) was taken in round bottom flask. Fused sodium acetate (4g) was added to it and reflux for 1hour. The reaction mixture was cooled and poured into ice cold water. Liquid layer was separated by separating funnel and distilled to obtain pure p-methyl phenyl acetate at 210^{o}C-212^{o}C.

Dissolved 12g of sodium in 250ml of absolute alcohol and to this solution, added in small portion, and with cooling, first 60ml n-amyl nitrite and then 70ml of p-methyl phenyl acetate. This mixture was allowed to stand for 2 days in well stopper bottle in a refrigerator. At the end of this time, the brown sodium salt was filtered and dried in air. The dried sodium salt was dissolved in a minimum quantity of ice cold water and treated with a calculated quantity of glacial acetic acid. Precipitate p-methyl Isonitroso phenyl acetate was then filtered through suction, and dried in vacuum. The crude product was recrystallised from benzene.

Synthesis of Metal Complexes:

1. Preparation of Fe(P-MINPA) $_2$:

00.358g of P-MINPA was dissolved in minimum quantity of alcohol was added. Similarly 0.392g of ferrous ammonium sulphate was dissolved in water. The FAS solution was added to reagent solution drop by drop with constant stirring. The pH was adjusted to 5.5-6.0 with buffer tablets. This solution was refluxed on sand bath at 100^{o}C for 2hrs. and then kept in vaccum desicator for overnight. A blue coloured complex was formed, filtered and recrystalised from chloroform.

2. Preparation of Fe(P-MINPA) $_3$:

Aqueous solution of Ferric Chloride and P-MINPA was mixed in the molar ratio of 1:3 and pH of solution was maintained 5-6 by HCl/NH_4OH. On putting in microwave oven for 4 to 5 hour yellow colour complex was formed, filtered and recrystalised from chloroform.

RESULTS AND DISCUSSION

Elemental analyses were carried out on a model 240 Perkin elemental analyzer. Metal contents were determined gravimetrically. The infrared spectra were measured on a Nicolet 400 D FT- IR spectrophotometer using KBr pellets from. The electronic spectra of the metal complexes in DMF were recorded on JASCO 7800 Elico SL-159 and Shimadzu UV-160A UV-VIS spectrophotometer. Magnetic susceptibility measurements of the complexes in the solid state were determined by Gouy balance using $CuSO_4$ as the calibrant at room tempera-ture. Molar conductance measurements were made in anhydrous DMF on a Systronic model 305 conductivity bridge.

Above synthesized compounds and ligands (Schiff base) were screened against bacteria Escherichia coli by the filter paper disc method [Dubey amd Maheswari, 2002] at various concentrations using nutrient agar as medium. Sterilized filter paper of 5 mm diameter was soaked in solutions of different concentrations of test samples and introduced on nutrient agar plates. These plates were incubated for 48 hours at 35^{0}C.

On the basis of physicochemical characteristics, it has been found that the complexes are non- hygroscopic, stable at room temperature, insoluble in water but fairly soluble in DMSO. The magnetic moment data indicates that the Fe(II), Ni(II) and Co(II) complexes are paramagnetic in nature. The molar conductance values for all the complexes in 10^{-3}M DMSO are in the range of 9.5-14 W^{-1} cm^2 mol^{-1} suggesting their non-electrolytic nature[Kumar *et al*., 1994] and that no anion are present outside the coordination sphere. Elemental analysis data and molar conductance value for ligand and metal complexes given in Table 1.

Table 1: Elemental analysis data and molar conductance value for ligand and metal complexes

Sr. no.	Ligand/ Complexes	Elemental analysis (%)Found/ (Calculated)				Colour	Yield (%)	μ_{eff}
		C	H	N	M			
1	P-MINPA	60.24 (60.34)	5.33 (5.03)	7.66 (7.82)	……	Colourless	98%	…..
2	Fe(P-MINPA) $_2$	52.17 (52.19)	4.25 (4.34)	6.65 (6.71)	13.40 (13.48)	Blue	87%	5.18
3	Fe (P-MINPA) $_3$	51.22 (51.81)	4.24 (4.31)	6.66 (6.71)	14.22 (14.12)	Yellow	94%	4.63

Infrared Spectroscopy:

The infrared spectral data of Schiff base ligand and its metal complexes are listed in Table 3.

- The IR spectra of the complexes indicate that the ligand behaves as bidentate and coordinates with metals via azomethine nitrogen and C=O group. The IR spectra of Schiff base ligand P-MINPA shows sharp band observed for ligand at 1640 cm−1, is due to azomethine >C=N linkage which is shifted to lower frequency (1590-1560 cm^{-1}) on going from ligand to its metal complexes due to coordination of azometine nitrogen with metal ion [Sece *et al*., 2000]. It is expected that coordination of nitrogen to the metal atom would reduce the electron density in the azomethine link and thus lower -HC=N absorption.

- In the spectra of P-MINPA show pick at 1680 cm−1 which may be attributed to the ν C=O, The disappearance of this band in all the metal complexes indicating the involvement of this group in complex formation [Saraf *et al*., 2011(b)].

- Metal-ligand vibration is observed in far-IR region usually give information regarding the bonding of ligand with metal ion. The new band is appear in the region of 510 cm−1 due the >M-O suggest the coordination of oxygen with metal ion.

- The presence of sharp band in the region 575 cm−1 in all the complexes due to the >M-N coordination of azomethine nitrogen [Ghosh *et al*.,2012; Saraf *et al*.,2012(c)]. The appearance of νM-N and νM-O vibration support the involvement of N and O atoms in complexation with metal ions under investigation [Thomas *et al*., 1995]

The IR sperctra of ligand and its complexes, the band at 1210-1220cm^{-1} can be attributed to C-O bond [Ibrahim Dermir, *et al*., 2008].

The N ⟶ O stretching vibration due to N — O of =NOH Which found near 930-960 cm^{-1} [Palm and Werbin 1954] metal complexes have coordination through the oxime oxygen or nitrogen atom only.

Electronic Spectra and magnetic measurements

The electronic spectral measurements were used for assigning the stereochemistry of the metal ions in the complexes based on position and number of d-d transition peaks. Electronic spectra of ligand and its metal complexes were displayed in DMF (Dimethylformamide) solution. Electronic spectra of ligand shows absorption in UV/ visible region two high intensity base bands at273 nm (40421 cm^{-1}) and 270 nm (37037 cm^{-1}) which indicate Π and Π^* transition of azomethine group in the ligand [Boghaei *et al*., 2000]. The electronic spectra of Fe(II) complex exhibits two bands. The bands at 11350cm-1may be assigned to $^5T_2g \rightarrow {}^5Eg$ (G) transition [Dwivedi and Dhakarey, 2003]and the other at 19700cm-1 to charge transfer. Similar types of transitions are reported for octahedral Fe(II) complexes [Aswar *et al*., 2006]. The magnetic moment value of Fe(II) complex is 5.40 B.M. indicating an octahedral geometry [Patel, *et al*., 2000]of this complex.

The electronic spectra of theFe(III) complex exhibits two bands at10242 cm^{-1} and 19945 cm-1 assigned to [$^3A_{2g} \rightarrow {}^3T_1g(P)$] and [$^3A_2g \rightarrow {}^3T_1g(F)$] transitions respectively, expected for octahedral geometry. The observed magnetic moment value of Fe (III) complex (4.63 B.M.) is in good agreement with this geometry [Aryane *et al*., 2009].

Table 2: IR spectra of Schiff base ligand and its metal complexes in cm-1

Sr. No	Compound	C=N	C=O	C-O	M-N	M-O	N--O
1	P-MINPA	1640	1680	1220	-	-	-
2	Fe(P-MINPA) $_2$	1560	-	1220	575	420	955
3	Fe (P-MINPA) $_3$	1590	-	1210	560	280	965

Table 3: Electronic spectral data of complexes

Ligand/Complexes	Geometry	Band Assignments	λ_{max}
P-MINPA	-	$\Pi \rightarrow \Pi^*$ $n \rightarrow n^*$	40421 cm $^{-1}$ 37037 cm $^{-1}$
Fe(P-MINPA) $_2$	Octahedral	$^5T_{2g}$ ^5Eg (g) ligand →metal charge transfer	11350 cm $^{-1}$ 19700 cm $^{-1}$
Fe (P-MINPA) $_3$	Octahedral	$^5T_{2g}$ ^5Eg (g) ligand →metal charge transfer	10242cm $^{-1}$ 19945 cm $^{-1}$

Table-4: NMR Spectra of P-MINPA and complexes

Sr.No	Compound	=NOH	-CH=N-	CH_3	Aomatic ring
1	P-MINPA	8.60δ	8.40δ	2.2δ	7.60δ
2	Fe(P-MINPA) $_2$	-	8.20δ	1.6δ	7.10δ
3	Fe(P-MINPA) $_3$	-	8.20δ	1.6δ	7.20δ

Table 5: Antibacterial activity of ligand and its complexes

Ligand/Complexes	Gram + ve		Gram -ve	
	S.aureus	*S.Pneumoniae*	*E. coli*	*P.aeruginosa*
P-MINPA	+	+	+	+
Fe(P-MINPA) $_2$	+ + +	+ + +	+ + +	+ + +
Fe (P-MINPA) $_3$	+ + +	+ + +	+ + +	+ + + +
Amoxicillin	+ +	+ +	+ +	+ +

H NMR Spectra:

H NMR Spectra of Schiff base (P-MINPA) and their complexes were recorded in DMSO (Dimethyl sulfoxide) solution and TMS (Tetramethylsilane) used as internal standard. The azomethine proton (-CH=N-) appears at 8.4δ, it has been shifted to down field in metal complexes and appear at ~8.2 which confirm coordination of ligand with metal by azomethine nitrogen [Vashi, *et al*., 2013]. NMR spectrum of (P-MINPA) show a peak around 8.60 δ due to the=NOH group. Two groups of band corresponding to $-CH_3$ and the aromatic proton in (P-MINPA) appears at 2.2 δ and7.60 δ respectively. It may be mentioned that etyl-ά-isonitrosoacetoacetate(HEINA), Isonitrosoacetylacetone [Thakkar and Deshmukh, 1994] (HINAA), Isonitrosoacetophenone [Pathak and Haldar, 1994] (HINAP)and p-chloroisonitrosoacetophenone [Raut *et al*., 2011] (HP-CIINAP), show =NOH proton resonance at -9.27 δ, 8.65 δ, 8.6 δ and-8.64 δ respectively. NMR spectra of Fe(P-MINPA)$_2$, Fe (P-MINPA)$_3$,in DMSO solution exhibit peak due to methyl, azometine proton (-CH=N-) and aromatic ring proton and do not show any proton signal due to the =NOH group. This suggests that their complexes have been formed by the replacement of the proton of the =NOH group by the metal ion. It is interesting to note that the peak due to methyl proton Fe(P-MINPA)$_2$ exhibit at lower value compared to that of methyl proton in the reagent (P-MINPA). Further peak due to aromatic ring proton in these complexes occur at higher field side with respect to that of aromatic ring proton signal in (P-MINPA).

Antimicrobial Activity:

The antibacterial activity data is presented in Table 4. The antibacterial activity of ligand and their metal complexes were screened on gram positive bacteria: Staphylococcus aureus and Streptococcus pneumoniae and gram negative bacteria: Escherichia coli and Pseudomonas aeruginosa by disc diffusion technique. The diameter of susceptibility zones measured in mm

[Rehman *et al*., 2001]. Filter paper disc of diameter 6 mm were used for the incresults were recorded. The antibacterial activity of ligandubation period of 60 hours at 25-30°C and and their complexes were tested by measuring inhibition zone observed around material. Ligand showed significant range of activity on growth of all selected bacterial stain. The results suggest that complexes increase the antibacterial activity [Valarathy and Subbalakshami, 2013].

Acknowledgements

Authors thanks Department of Chemistry, B. D. College of Engineering and Technology, Sewagram, Dist-Wardha for Support and Facilities.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:68-72
UGC Approved Journal No 48951

Original Article Open Access

Synthesis and Biological Evaluation of Some Novel 1,3,4-Oxadiazoles Bearing Coumarine Moiety

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Siddiqui Naqui J, Mohammad Idrees and Manisha Rohankar (2018) Synthesis and Biological Evaluation of Some Novel 1,3,4-Oxadiazoles Bearing Coumarine Moiety, *Int. J. of Life Sciences*, Special Issue, A12: 68-72.

ABSTRACT

A series of 1,3,4-Oxadiazoles bearing Coumarine moiety (5a-c) were synthesized by refluxing Schiff's bases (4a-c) with acetic anhydride. 4a-c needed for the synthesis was obtained by **r**efluxing ethyl-2-oxo-2*H*-chromone-3-carbohydrazides (3a-c) with benzyloxy benzaldehyde. Similarly, 3a-c were prepared by reacting hydrazine hydrate and ethyl-2-oxo-2*H*-chromene-3-carboxylates (2a-c), which in turn were synthesized by treatment of substituted 2-hydroxy benzaldehydes (1a-c) with diethyl malonoate. The structures of the newly synthesized 1,3,4-Oxadiazoles have been established on the basis of chemical transformations, elemental analysis, IR, ^{1}H NMR, and Mass spectral studies. The title compounds were screened *in-vitro* for antibacterial activity against two Gram positive and two Gram negative bacterial strains such as *E. coli, S. aureus, B. thurengienesis* and *E. aerogenes*. The zone of inhibition measured in mm revealed that the title compounds exhibited moderate to good antibacterial activity against Chloramphenicol as standard.

Keywords, 1,3,4-Oxadiazole, Coumarine, Schiff's base, Carbohydrazides.

INTRODUCTION

1,3,4-Oxadiazole is an important isomer among the class of oxadiazoles and has become an important structural theme for the development of new drugs because of its various biological activities. Review available in the literature (Patel *et al*., 2014) have suggested different methods for the synthesis of 1,3,4-Oxadiazoles. The most commonly used pathway for synthesis 1,3,4-Oxadiazole backbone includes reactions of properly substituted acid hydrazides with either acid chlorides/carboxylic acids or by direct cyclization of diacylhydrazines using a variety of dehydrating agents (Bentiss and Lagrenee, 1999; Liras *et al*., 2000; Gomes *et al*., 2001; Kadi *et al*., 2007; Mickevicius *et al*., 2009; Souldozi and Ramazani, 2007). Similarly, 1,3,4-Oxadiazole is a highly privileged structure, the derivatives of which have been found to possess broad spectrum antimicrobial activity and exhibit a wide range of biological activities (Sahu *et al*., 2011) including antibacterial (Barbucenu *et al*., 2011), antitubercular (Kumar et

al. 2010), vasodialatory (Shirote and Bhatia, 2010), antifungal (Parkash *et al*., 2010) cytotoxic (Padmavathi *et al*., 2009), anti-inflammatory and analgesic (Idrees *et al*., 2009) hypolipidemic (Jayashankar *et al*., 2009) anticancer (Kumar *et al*., 2009) and ulcerogenic (Shashikan *et al*., 2008) activities. Hence, in view of the importance and inspections of the research work on these heterocycles and continuation of our previous work (Siddiqui and Mohammad, 2008) on hydrazides, it was found to be fascinating to synthesize and subsequently treat carbohydrazide derivatives bearing coumarine moiety with benzyloxy benzaldehyde followed by acetic anhydride for the synthesis of few novel 1,3,4-Oxadiazoles and view for spectral characterization and study their biological importance.

MATERIALS AND METHODS

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, v max in cm-1). ^{1}H NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as solvent. Chemical Shifts are given in parts per million (ppm). Positive-ion electrospray ionisation (ESI) mass spectra were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer. Elemental analysis (CHN) was done using Elemental analyzer, Vario EL III. All the chemicals used for the synthesis were of AR grade of Merck, S.D. Fine and Aldrich. The compounds were analyzed for carbon, hydrogen, nitrogen and sulphur and the results were in good conformity with the calculated values.

Experimental

Synthesis of starting materials substituted ethyl-2-oxo-2*H*-chromene-3-carboxylates **(2a-c)** and substituted 2-Oxo-2*H*-chromene-3-carbohydrazides **(3a-c)** was done according to the reported procedure (Siddiqui and Mohammad, 2017) (Scheme **1**).

2-Oxo-2*H*-chromene-3-carbohydrazide (3a),

Colourless needle like crystal; mp, 136-138 °C yield, 90.0%; M. F. $C_{10}H_8O_3N_2$; Recrystallizing solvent, Ethanol.

6-Chloro-2-oxo-2*H*-chromene-3-carbohydrazide(3b)

Colourless needle like crystal; mp, 158-160˚C yield, 80.0%; M. F. $C_{10}H_7O_3N_2Cl$; Recrystallizing solvent, Ethanol.

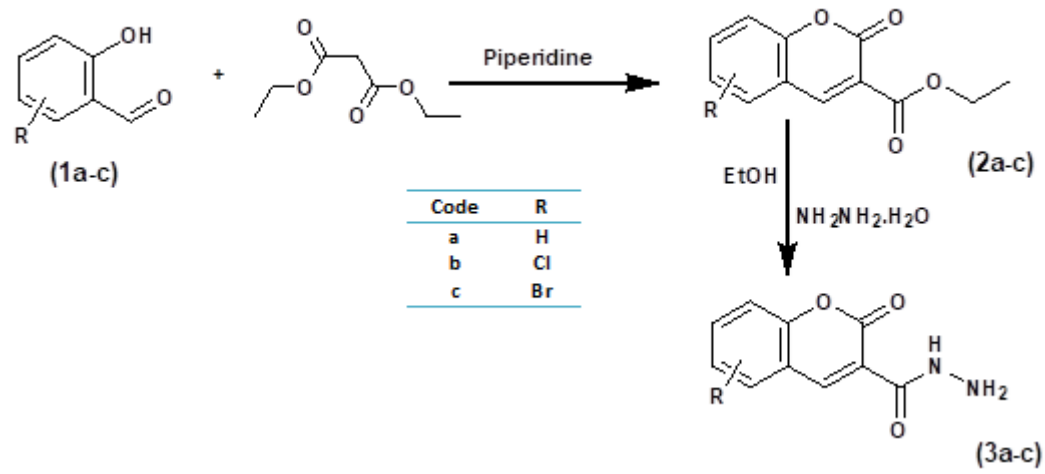
6-Bromo-2-oxo-2*H*-chromene-3-carbohydrazide(3c)

Colourless needle like crystal; mp, 174-175˚C yield, 85.0%; M. F. $C_{10}H_7O_3N_2Br$; Recrystallizing solvent, Ethanol.

Procedure for the synthesis of N'-(4-(benzyloxy) benzylidene)-substituted 2-oxo-2*H*-chromene-3-carbohydrazide (4a-c):

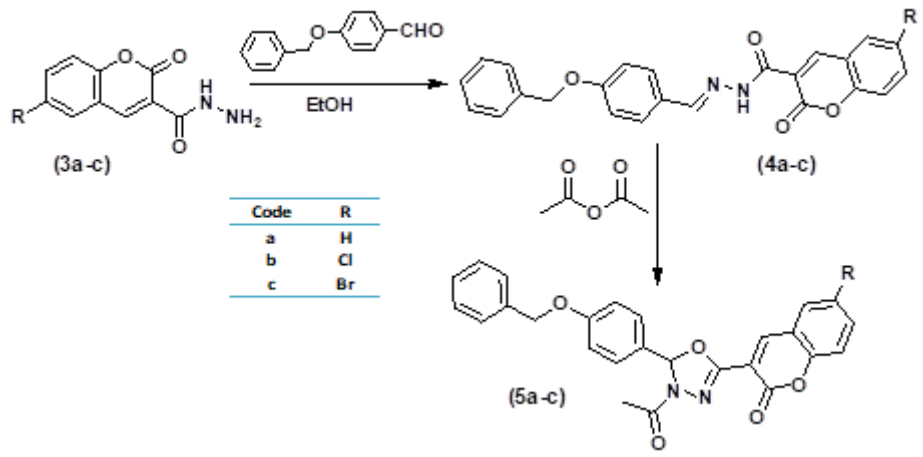
Ethyl-2-oxo-2*H*-chromene-3-carbohydrazide **3a** (10 mmol) and 4-benzyloxy benzaldehyde (10 mmol) in ethanol (90 mL) containing 2-3 drops of concentrated acetic acid was refluxed for 2h to get **4a**. The reaction mixture was cooled, filtered, washed, dried and recrystallized from 1,4-dioxane (Scheme **2**). Similarly, **4b-c** were synthesised from **3b-c** by extending the same procedure followed for **4a.**

Reaction Scheme : 1



Code	R
a	H
b	Cl
c	Br

Reaction Scheme : 2



Code	R
a	H
b	Cl
c	Br

N'-(4-(benzyloxy)benzylidene)-2-oxo-2*H*-chromene-3-carbohydrazide(4a): Yellow amorphous; mp, 192-194 °C yield, 90.0%; M. F. $C_{24}H_{18}O_4N_2$

N'-(4-(benzyloxy)benzylidene)-6-chloro-2-oxo-2*H*-chromene-3-carbohydrazide (4b): Yellow amorphous solid; mp, 258-260˚C, yield, 89.0%; M. F. $C_{24}H_{17}O_4N_2Cl$

N'-(4-(benzyloxy)benzylidene)-6-bromo-2-oxo-2*H*-chromene-3-carbohydrazide (4c): Yellow amorphous solid; mp, 278-280˚C, yield, 91.0%; M. F. $C_{24}H_{17}O_4N_2Br$

Procedure for the synthesis of 3-(4-acetyl-5-(4-(benzyloxy)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)-substituted-2*H*-chromen-2-one (5a-c), A mixture of N'-{[4-(benzyloxy) phenyl]methylidene}-2-oxo-2*H*-chromene-3-carbohydrazide **5a** (2 mmol) and acetic anhydride (10 mL) was refluxed for 1h. The excess acetic anhydride was distilled off at reduced pressure and residue was poured into ice cold water. The solid produced was filtered, dried and recrystallized from 1,4-dioxane (Scheme **2**).

3-(4-acetyl-5-(4-(benzyloxy)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (5a), Yellow amorphous solid; mp, 208-210˚C, yield, 89.0%; M. F. $C_{26}H_{20}O_5N_2$, IR, 1767 (C=O, ester), 3015 (ArH), 2927, 2861 (CH_3, CH_2), 1508 (C=C),1604,1619 (C=N), 1244 (C-O, ester); ^{1}H NMR, 7.25-8.67 (m, 15H, ArH), 5.20 (s, 2H, -CH_2), 2.37(s, 3H, -CH_3), MS , 440 $[M]^+$, 473$[M+Na]^+$; Calculated , C, 70.91%; H, 4.55%; N, 6.36%; Found, C, 69.99%; H, 4.64%; N, 6.01%.

3-(4-acetyl-5-(4-(benzyloxy)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)-6-chloro-2*H*-chromen-2-one (5b), Yellow amorphous solid; mp, 219-221˚C, yield, 86.0%; M. F. $C_{26}H_{19}O_5N_2Cl$; IR, 1775 (C=O, ester), 3028 (ArH), 2918, 2845 (CH_3, CH_2), 1525 (C=C),1624,1621 (C=N), 1241 (C-O, ester); ^{1}H NMR, 7.37-8.91 (m, 14H, ArH), 5.24 (s, 2H, -CH_2), 2.41 (s, 3H, -CH_3), MS, 476 $[M+1]^+$, 498 $[M+Na]^+$; Calculated , C, 65.76; H, 4.03; N, 5.90; Found, C, 65.71%; H, 4.00%; N, 5.87%.

3-(4-acetyl-5-(4-(benzyloxy)phenyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)-6-bromo-2*H*-chromen-2-one (5c), Yellow amorphous solid; mp, 225-227 ˚C, yield, 88.0%; M. F. $C_{26}H_{19}O_5N_2Br$; IR, 1772 (C=O, ester), 3019 (ArH), 2917, 2864 (CH_3, CH_2), 1561 (C=C),1622 (C=N), 1240 (C-O, ester); ^{1}H NMR, 7.33-8.88 (m, 14H, ArH), 5.23 (s, 2H, -CH_2), 2.39 (s, 3H, -CH_3), MS, 519 $[M]^+$; Calculated , C, 60.13; H, 3.69; N, 5.39; Found, C, 60.09%; H, 4.00%; N, 5.27%.

Antimicrobial activity

The novel synthesized heterocyclic compounds such as were screened for their *in vitro* antimicrobial activity using cup plate method against two gram positive bacterial strains, *B. thurengienesis* and *S. aureus* and two gram negative strains, *E. coli* and *E. aerugenes* using Chloramphenicol as the standard drug.

General Procedure for the Determination of Zone of Inhibition by Cup Plate method: Test solutions were prepared with known weight of compound in DMSO and half diluted to give the resultant concentration of 31-500µg/mL. Whatmann no. 1 sterile filter paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar obtained from Himedia Ltd., Mumbai. Petri plates were prepared by pouring 10mL of agar for bacteria containing microbial culture and were allowed to solidify. The discs

were then applied and the plates were incubated at 37˚C for 24h (bacteria), then inhibition zone were measured in mm. The results were compared using Chloramphenicol as standard. The zone of inhibition of the compounds is given in the Table 1.

RESULT AND DISCUSSIONS

The synthesis of the novel compounds (5a-c) is described in reaction scheme 2. The identities of the newly synthesized compounds have been established on the basis of their elemental analysis and spectral data[19] such as IR, ^{1}H NMR and Mass spectral studies. Substituted 2-hydroxy benzaldehydes (1a-c) and diethyl malonate were reacted in the presence of piperidine in ethanol to form ethyl-2-oxo-2*H*-chromene-3-carboxylate (2a-c); which on treatment with hydrazine hydrate resulted in 2-oxo-2*H*-chromonene-3-carbohydrazide (3a-c), which was further reacted with different aldehydes to form Schiff bases (4a-c). Schiff bases on refluxing with acetic anhydride was found to cyclize to 1,3,4-Oxadiazoles (5a-c) which was confirmed from their elemental and spectral analysis.

IR spectra of 5a-c showed absorption bands in the range of 1767-1775 cm^{-1} for C=O, 1240-1244 cm^{-1} for C-O-C stretching and bands at 2927-2861 cm^{-1} for -CH_3, -CH_2 aliphatic stretch. The ^{1}H NMR spectra showed a multiplet of fifteen, fourteen and fourteen aromatic protons in the range of δ 7.25-8.67, 7.37-8.91 and 7.33-8.88 ppm for 5a, 5b and 5c respectively. Similarly, singlet in the range of δ 5.20-5.24 in 5a-c also confirms the presence of two methylene protons -CH_2. Mass spectra also confirms the formation of 5a-c, as molecular ion peaks are obtained at 440 $[M]^+$, 476$[M+1]^+$ and 519$[M]^+$, having the molecular formula $C_{26}H_{20}N_2O_5$, $C_{26}H_{19}O_5N_2Cl$ and $C_{26}H_{19}O_5N_2Br$ respectively (Scheme 2).

Antimicrobial activity, Synthesized title compounds (5a-c) were screened for antimicrobial activity. Table no. 1, shows the inhibition zone calculated in mm at different concentrations from 31-500 µg/mL using Chloramphenicol as the standard drug. The zone of inhibition revealed that the title compounds exhibited moderate to good antibacterial activity against the standard. 1,3,4-Oxadiazoles bearing chloro and bromo substitution i.e. 5b and 5c were found to have good antibacterial activity when compared with the

Table 1: Antibacterial Activity

Sr. No.	Compd. Code	Concentration (µg/mL)	Zone of Inhibition (mm)			
			Antibacterial Activity			
			S. aureus	*E. coli*	*B. thurengienesis*	*E. areogenes*
1.	Chloramphenicol	500	28	19	19	15
		250	28	18	20	17
		125	20	16	16	15
		62.5	18	15	14	14
		31	20	20	15	15
2.	**5a**	500	20	13	12	10
		250	19	12	14	11
		125	14	10	11	9
		62.5	12	10	8	9
		31	13	10	10	7
3.	**5b**	500	24	15	14	12
		250	21	14	14	13
		125	17	12	13	12
		62.5	15	15	11	10
		31	17	16	14	11
4.	**5c**	500	23	14	15	11
		250	21	13	15	12
		125	15	12	14	10
		62.5	16	14	11	10
		31	15	16	12	9

unsubstituted derivative 5a that showed moderate activity against all the four bacterial strain chosen.

CONCLUSION

We have reported here synthesis of some new 1,3,4-Oxadiazole derivatives bearing Coumarine moiety (5a-c) in good yields via cyclization of substituted Schiff's bases (4a-c) in presence of acetic anhydride. Their structures were also confirmed from spectral studies such as IR, ^{1}H NMR, Mass and CHN analysis. Biological screening revealed that the synthesized chloro and bromo substituted oxadiazoles derivatives 5b and 5c exhibited good antibacterial activity as compared to 5a.

Acknowledgments

The authors are thankful to the Director, Institute of Science, Nagpur; Principal, Government Science College, Gadchiroli; Head, Department of Chemistry; research students, Mr. Roshan Nasare and Mr. Satish Kola for their support and cooperation. The authors are also thankful to Dr. Sushama Narkhede former Head, Department of Botany, GSC, Gadchiroli for permitting to use the laboratory for carrying out the microbial activity, similarly the authors are thankful to the Director, SAIF, Punjab University, Chandigarh for providing IR, ^{1}H NMR, Mass spectra and CHN analysis.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:73-74
UGC Approved Journal No 48951

Original Article Open Access

Studies on viscosity of Gum Karaya in Gadchiroli district, Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Rewatkar Suresh B, Kalyankar Sarla N, Dhankar Raksha P and Kadu Ashwini M (2018) Studies on viscosity of Gum Karaya in Gadchiroli district, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A12: 73-74.

ABSTRACT

The present work is related with the variation in viscosity with the concentration of the gum samples. The Gum Karaya is collected from Gadchiroli District of Maharashtra State during Summer-2017. The study of viscosity is carried out at 30^0C. The following results of relative viscosity are obtained. The calculated relative viscosities are 12.26, 13.18, 14.15, 15.29 and 16.58 for the concentration of the gum samples 0.2%, 0.4%, 0.6%, 0.8% and 1.0% respectively.

Keywords : *Viscosity, Gum Karaya, Gadchiroli, Maharashtra*

INTRODUCTION

Gum Karaya, Sterculia gum, is the dried exudates obtained from stem and branches of Sterculia tree, family Sterculiaceae. The gum is collected after tapping or blazing the tree or as natural exudates (Elkhalifa. & Hassan, 2004). The dried Gum Karaya appears as hard lumps.

Major areas producing Gum Karaya in India are Tropical Himalayas, West and Central India, Deccan Plateau and throughout the Eastern and Western Ghats (Chopra et al., 1956). The fully mature tree attains a height of more than 30 feet in forest areas with a significant smooth greenish-grey bark or white bark peeling off (Krishnamurthy, 1993).

According to WHO, the medicinal plants would be the best source to obtained variety of drugs (Dewick, 1996). About 80% of individuals from developed countries use traditional medicine. Natural Gums are hydrophilic carbohydrate polymer of high molecular weight, generally composed of monosaccharide units joined by glucosidal bonds. Gum Karaya in the dry state is not soluble in water but only forms viscous suspensions. The gum enormously swells in water and forms thick suspensions (Rao & Gayatri, 2016). They are generally insoluble in oil and

organic solvents such as ether, hydrocarbons, alcohols (Evans et al., 1989).

The present study is focused on viscosity studies of Gum Karaya at different concentrations.

MATERIALS AND METHODS

All the Gum Karaya samples were collected from the Gadchiroli District of Maharashtra during Summer-2017. Gadchiroli District has 78.4% reserve forest which consists of high dense forest and rich biodiversity.

Gum samples were dried at room temperature and cleaned by hand to remove foreign particles. The samples were further ground by using a mortar and pestle, sieved through sieve No. 4 and kept in air tied glass containers. The viscosity measurements were carried out by using Ostwald's Viscometer by taking the gum samples with concentrations 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, at 30^0C.

RESULTS AND DISCUSSION

The relative viscosity for Gum Karaya varies from 12.26 to 16.58 for the concentration 0.2% to 1.0% sample. These values are comparable with the literature values of Gum Karaya. The viscosity and swelling ability of the gum decides the quality of gum in industrial applications.

Table 1: Relative Viscosity variation with concentration of gum samples.

Sr. No.	Concentration of Gum Sample (%)	Relative Viscosity
1	0.2	12.26
2	0.4	13.18
3	0.6	14.15
4	0.8	15.29
5	1.0	16.58

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:75-77
UGC Approved Journal No 48951

Original Article Open Access

Green route of Synthesis of 3,5-Diaryl-4-Benzoyl-1-Pyridoyl-Δ^2- Pyrazolines

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
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ABSTRACT

Some new of 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines have been synthesis by the action of isoniazid on 3-aroyl flavanones in pyridine medium. In this synthesis p-cresol and m-cresol are used as starting material. Isoniazid is use as anti tuberculosis drug. Structures of newly synthesized compound are identified by spectral and elemental analysis.

Keywords : Synthesis, Pyrazoline, Isoniazids

INTRODUCTION

Heterocyclic compounds are well known for their wide range of biological applications out of which pyrazolines occupy unique position due to dominant applications. Pyrazolines are known to possess antimicrobial, ant tubercular, antiviral, anti-HIV, molluscicidal and cerebroprotective properties. Pyrazolines are and important nitrogen containing five-member ring heterocyclic compounds. Pyrazoline derivatives have been found to possess a broad spectrum of biological activities such as tranquillizing, muscle relaxant, psychoanaleptic, anticonvulsant, antihypertensive, and antidepressant activities (Turan-Zitouni *et al*., 2000; Rajendra *et al*., 2005) Pyrazolines derivatives are also used as Anesthetics (Rao and Subbaraju, 1995), Analgesic (Banoglu *et al*., 2007), Antitubercular (Babu *et al*., 2004), Antitumor (Taylor *et al*., 1992), Immunosuppre-ssive (Joseph and Otterness, 1981), Antidepressant (Ruhoglu *et al*., 2005; Prasad *et al*., 2005), Cerebroproc-tive (Ohto and Shigo, 1995), Antidiabetic (Soliman *et al*., 1987; Cottineau *et al*., 2002), Anticancer (Abdolhamid *et al*., 2008) Antiviral (Sechi *et al*., 2005), anticonvulsant (Srivastava *et al*., 2002), molluscicidal (Flora, *et al*., 2006), Insecticides (Kristopher, S.S. and David , 2005; Li *et al*., 2006), Fungicides (Shinde *et al*., 2006), Antiinflammatory (Mohammad, *et al*., 2008; Reddy *et al*., 2008), Herbicides (Suratkumar and Rastogi, 1987), Antiimplanatory (Jamode *et al*., 2004), Antimicrobial and antibacterial (Singh *et al*., 2004; Shinde *et al*., 2006).

The literature survey clearly indicates that 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines are not yet synthesized. It was, therefore thought of interest to synthesize 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines from 3-aroyl flavanones.

Thus the present work deals with synthesis of 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines from 3-aroyl flavanones (scheme) and Isoniazid in pyridine medium . Structures of this compound have been established by spectral and elemental analysis.

MATERIALS AND METHODS

Melting points are uncorrected. IR spectra in KBr were recorded on PE-983/PE-781IR spectrophotometer. NMR in DMSO on Varian EM 390-cw NMR spectrophotometer and UV on Varian Cary 239 OUV spectrophotometer.

(1) Preparation of 1,3-diaroyl-1,3 propadione (1a – 1f)

2-benzoyloxy acetophenone was dissolved in dry pyridine. The solution was warmed up to 60^{o}C and pulverized KOH was added slowly with constant stirring. After about 4 hours the reaction mixture was acidified by adding ice cold HCl. The brownish yellow product obtained was filtered; wash with sodium bicarbonate solution and sufficient water. The product obtain was crystallized from ethanol-acetic acid mixture.

(2) Preparation of 3-aroyl flavanones (2a-2f)

1,3-diaroyl-1,3 propadione (1a – 1f) and aromatic aldehydes (p -iodo benzaldehyde, *m*-iodo benzaldehyde, *o*-iodo benzaldehyde) where reflux for about 2 hours in ethanol containing a few drops of piperidine. The resulting mixture was cool and the product separated was crystallized from ethanol-acetic acid mixture. The structure of this compound where confirm by spectral analysis.

Spectral interpretation of 3a:

IR (νmax):1630 cm^{-1} ν(C=O); 1605 cm^{-1} ν(C=O);1590-1610 cm^{-1} ν(C=C);1190 cm^{-1} ν(C-O-C).

^{1}H NMR: $^\delta$2.50(S, 3H, Ar-CH_3); 4.80(S,3H Ar-O-CH_3);7-8.5(m,11HAr-H)

UV(λ max):340 nm.

(3) Preparation of 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines (4a-4f)

3-aroyl flavanones where reflux with isoniazid for 8 to 10 hours in pyridine solvent. The reaction mixture was decomposed by acidified water, filtered and wash with sufficient water. The product obtain was crystallized from ethanol-acetic acid mixture. To obtain a crystalline solid. Yield 60 – 80%.

Spectral interpretation of 4a:

IR (νmax) :1625cm^{-1} ν(C=O);3350 cm^{-1} ν(OH);1620 cm^{-1} ν(C=N);1500 cm^{-1} ν(C=C);1390 cm^{-1} ν(C-N);1035 cm^{-1} ν(C-O)(Phenol)

^{1}H NMR: $^\delta$1.9(S, 3H,-CH_3); 7.2-7.6(m,17H,-Ar-H); 12(S,1H,-OH).

UV(λ max):256nm.

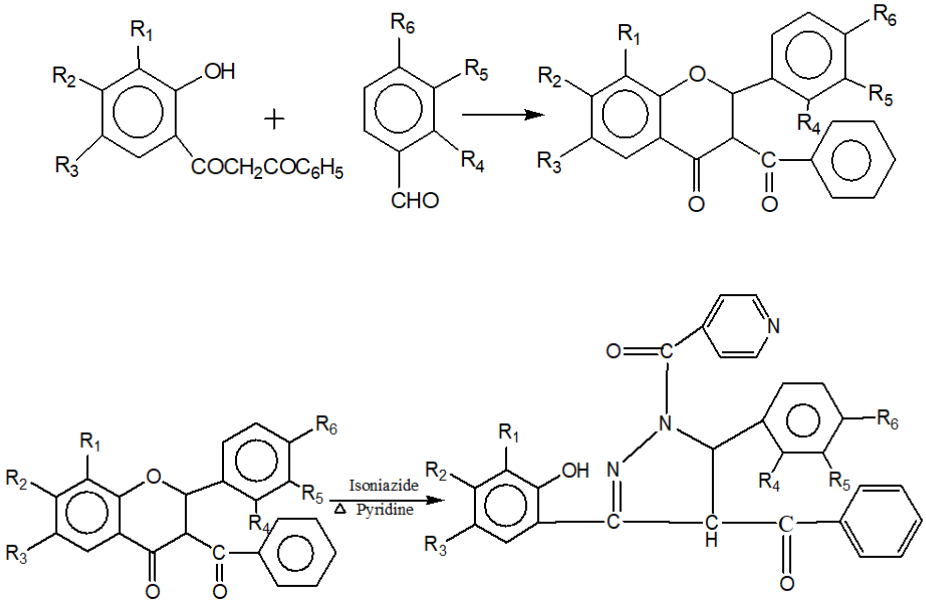
Table 1: Physical Characterization data of Synthesized Compound 3-Aroyl flavanones (3a-3f)

Compound	R_1	R_2	R_3	R_4	R_5	R_6	Yield (%)	M.P.(^{o}C)	Molecular Formula
3a	H	H	CH_3	H	H	I	85	140	$C_{23}H_{17}O_3I$
3b	H	H	CH_3	I	H	H	85	155	$C_{23}H_{17}O_3I$
3c	H	H	CH_3	H	I	H	85	148	$C_{23}H_{17}O_3I$
3d	H	CH_3	H	H	H	I	75	150	$C_{23}H_{17}O_3I$
3e	H	CH_3	H	I	H	H	90	145	$C_{23}H_{17}O_3I$
3f	H	CH_3	H	H	I	H	85	158	$C_{23}H_{17}O_3I$

Table2: Physical Characterization data of Synthesized Compound 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2- pyrazolines (4a-4f)

Compound	R_1	R_2	R_3	R_4	R_5	R_6	Yield (%)	M.P.(^{o}C)	Molecular Formula
4a	H	H	CH_3	H	H	I	50	252	$C_{29}H_{21}O_3N_3I$
4b	H	H	CH_3	I	H	H	70	250	$C_{29}H_{21}O_3N_3I$
4c	H	H	CH_3	H	I	H	85	249	$C_{29}H_{21}O_3N_3I$
4d	H	CH_3	H	H	H	I	65	254	$C_{29}H_{21}O_3N_3I$
4e	H	CH_3	H	I	H	H	75	252	$C_{29}H_{21}O_3N_3I$
4f	H	CH_3	H	H	I	H	80	253	$C_{29}H_{21}O_3N_3I$

SCHEME



CONCLUSIONS

In conclusion, we have reported an efficient procedure for the synthesis of 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2-pyrazolines in pyridine medium. The major advantage of this method is that the ease of work-up. This method also offers some other merits such as pure synthesis, high yields of products, and use of various substrates, which make it useful and attractive proceture for the synthesis of 3,5-diaryl-4-benzoyl-1-pyridoyl-Δ^2-pyrazolines.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:75-77
UGC Approved Journal No 48951

Original Article Open Access

Synthesis of 3-(2-hydroxy substituted phenyl)-4-(*o*-methyl benzoyl)-5-(substituted chloro aldehyde) -1-pyridyol pyrazoles

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Tayde Nitin P and Kadu Mangesh V (2018) Synthesis of 3-(2-hydroxy substituted phenyl)-4-(*o*-methyl benzoyl)-5-(substituted chloro aldehyde) -1-pyridyol pyrazoles, *Int. J. of. Life Sciences*, Special Issue, A12: 75-77.

ABSTRACT

Some new 3-(2-hydroxy substituted phenyl)-4-(*o*-methyl benzoyl)-5-(substituted chloro aldehyde) -1-pyridyol- pyrazoles (3) have been synthesized by the action of isoniazid on 3-aroyl flavones (2) in pyridine medium. Structures of these compounds have been established by spectral (IR, NMR and UV) and elemental analysis. The compounds were tested for their antimicrobial activities and because of electronegative fluorine atom the compounds showed enhanced antimicrobial activities. Heterocyclic compounds are well known for their wide range of biological applications out of which pyrazoles occupy unique position due to dominant applications. Pyrazoles are important nitrogen-containing five-member ring heterocyclic compounds. In the same way substituted pyrazoles constitute in the field of agricultural and medicinal chemistry because of their broad spectrum biological activities. They are widely used as fungicide, insecticide, herbicide, and antitumor agent. Pyrazoles are anti-diabetic, anti-inflammatory, anti-parasitic, anti-oxidant, anti-cancer agents, anti-microbial, anti-depressant and anti-protozoa.

Keywords : *o*-methyl benzoyl, 1-pyridyol- pyrazoles, anti-diabetic, anti-inflammatory, anti-parasitic, anti-cancer agents.

INTRODUCTION

Pyrazoles have been synthesized by different workers, 3-substituted 1-phenyl-1*H*-pyrazole-4-carbaldehydes and the corresponding ethanones by Pd-catalyzed cross-coupling reactions were synthesized (Egle et al., 2011). Abdel et al. (2012) had synthesized Pyrazole as corrosion inhibitor pyrazole derivatives for C- Steel in hydrochloric acid medium. Rao et al. (2012) had reported pyrazolo [3,4-c] pyrazole derivatives bearing indole moiety showing antimicrobial activity. Anticancer and antimicrobial activities of some synthesized pyrazole and triazole derivatives had been reported by Eman et al. (2012). Cyanopyridone derivatives pyrazole showing antimicrobial activity had been reported by Shridhar et al.(2012). Chandrakantha et al. (2012) had reported T3P mediated quinoline substituted pyrazole derivatives showing antibacterial.

MATERIALS AND METHODS

(1) Preparation of flavanones (Ia-f):

1,3 diaryl-1,3- propanedione (0.01M) and fluoro substituted aldehyde (*p*-chloro- benzaldehyde, *m*-chloro-benzaldehyde, *o*-chloro-benzaldehyde) (0.01M) were reflux in ethanol (25-30 ml) for about 1 hour containing few drops of piperidine. The reaction mixture was cooled and the product separated was crystallized from ethanol-acetic acid mixture. List of the 3-aroyl flavanones (Ia-f) synthesized is as.

Ia) 2-(4′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavanone.
Ib) 2-(3′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavanone.
Ic) 2-(2′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavanone.
Id) 2-(4′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavanone.
Ie) 2-(3′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavanone.
If) 2-(2′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavanone.

(2) Oxidation of flavanones:

The substituted flavanones (Ia-f) were oxidized by DMSO-I_2 to obtain substituted flavones. Physical characterization and data of synthesized flavones (II a-f) is given in table 1.

IIa) 2-(4′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavone.
IIb) 2-(3′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavone.
IIc) 2-(2′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-7-methyl flavone.
IId) 2-(4′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavone.
IIe) 2-(3′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavone.
IIf) 2-(2′- chloro benzaldehyde)-3-(2′-methyl) benzoyl-6-methyl flavone.

(3) Preparation of 3-(2-hydroxy substituted phenyl)-4-(*o*-methyl benzoyl)-5-(chloro substituted aldehyde) -1-pyridyol-pyrazoles (IIIa-f):

3-aroyl flavone. (IIa-f) (0.1M) were refluxed with isoniazid (0.2M) for 8-10 hours in pyridine solvent. The reaction mixture was decomposed by acidified water, filtered and wash with sufficient water. It was crystallized from ethanol-acetic acid mixture to obtain white crystalline product, yield 60-70%. Physical characterization and data of synthesized 3-(2-hydroxy substituted phenyl)-4-(o-methyl benzoyl chloride)-5-(chloro substituted aldehyde) -1-pyridyol-pyrazoles (IIIa-f) is given in table 2. List of the pyrazoles synthesized is as,

IIIa) 3-(2′-hydroxy-4′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(4′- chloro benzaldehyde) -1-pyridyol-pyrazole.
IIIb) 3-(2′-hydroxy-4′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(3′- chloro benzaldehyde) -1-pyridyol-pyrazole.
IIIc) 3-(2′-hydroxy-4′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(2′- chloro benzaldehyde) -1-pyridyol- pyrazoles.
IIId) 3-(2′-hydroxy-5′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(4′- chloro benzaldehyde) -1-pyridyol-pyrazoles.
IIIe) 3-(2′-hydroxy-5′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(3′- chloro benzaldehyde) -1-pyridyol- pyrazole.
IIIf) 3-(2′-hydroxy-5′-methyl phenyl)-4-(*o*-methyl benzoyl)-5-(2′- chloro benzaldehyde) -1-pyridyol-pyrazole.

Spectral determination of IIIb

IR (V_{max}): 3400 $cm^{-1}\nu$(C-OH); 550 $cm^{-1}\nu$(C-Br); 1550 $cm^{-1}\nu$(C=N); 1200 $cm^{-1}\nu$(C-N);
1150$cm^{-1}\nu$ (C-O);1150 $cm^{-1}\nu$

NMR : δ 2.4(s, 3H, -CH_3); δ 3.6(d, 1H, -CH); δ 6.8(d, 1H, -CH); δ 8 to8.5 (m,15H, Ar-H);
δ 11.8(s, 1H, -OH).

UV (λ_{max}): 280 nm

Table 1: ***Physical Characterization and data of synthesized*** **flavones**

Compound	R_1	R_2	R_3	R_4	R_5	Molecular Formula	Molecular Weight	MP°C	%Yield
IIa	CH_3	H	Cl	H	H	$C_{24}H_{17}O_3Cl$	388.5	173	65
IIb	CH_3	H	H	Cl	H	$C_{24}H_{17}O_3Cl$	388.5	162	65
IIc	CH_3	H	H	H	Cl	$C_{24}H_{17}O_3Cl$	388.5	183	60
IId	H	CH_3	Cl	H	H	$C_{24}H_{17}O_3Cl$	388.5	189	65
IIe	H	CH_3	H	Cl	H	$C_{24}H_{17}O_3Cl$	388.5	160	65
IIf	H	CH_3	H	H	Cl	$C_{24}H_{17}O_3Cl$	388.5	185	60

Table-2: Physical Characterization and data of synthesized 3-(2-hydroxy substituted phenyl)- 4-(o-methyl benzoyl)-5-(chloro substituted benzaldehyde) -1-pyridyol-pyrazoles

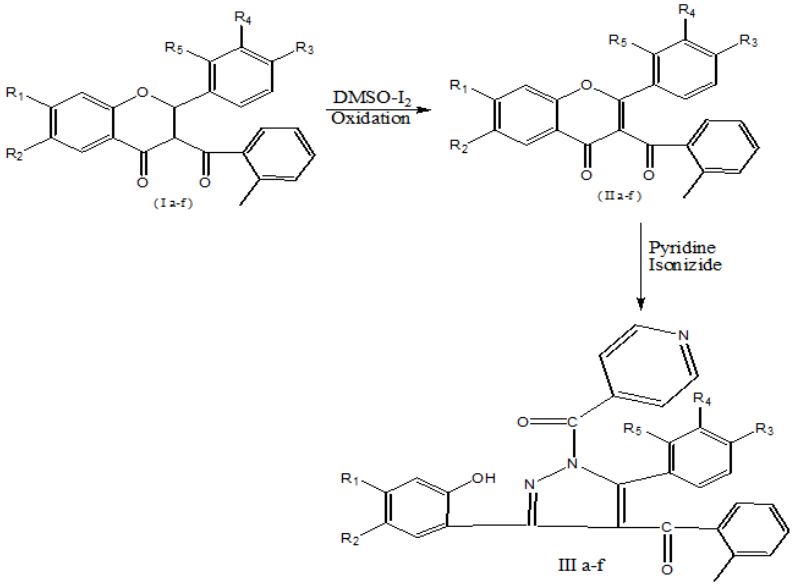
Compound	R_1	R_2	R_3	R_4	R_5	Molecular Formula	Molecular weight	MP°C	%N Cal. (Found)
IIIa	CH_3	H	Cl	H	H	$C_{30}H_{21}O_3N_3Cl$	506.5	360	8.51 (8.50)
IIIb	CH_3	H	H	Cl	H	$C_{30}H_{21}O_3N_3Cl$	506.5	368	8.53 (8.48)
IIIc	CH_3	H	H	H	Cl	$C_{30}H_{21}O_3N_3Cl$	506.5	379	8.50 (8.49)
IIId	H	CH_3	Cl	H	H	$C_{30}H_{21}O_3N_3Cl$	506.5	378	8.57 (8.52)
IIIe	H	CH_3	H	Cl	H	$C_{30}H_{21}O_3N_3Cl$	506.5	362	8.58 (8.52)
IIIf	H	CH_3	H	H	Cl	$C_{30}H_{21}O_3N_3Cl$	506.5	350	8.59 (8.50)

Antimicrobial activities of synthesized pyrazoles

Antimicrobial screening was done by using cup plate method at a concentration of 100µg/ml. The compounds were evaluated for antimicrobial activity against *P. aeruginosa, S. aureus, C. frundii, E. coli, P. mirabilis and S. typhi.* The results of antimicrobial data are summarized in table 1. All compounds show the moderate to good activity. (Zone of inhibitions in mm).

Organisms	IIIa	IIIb	IIIc	IIId	IIIe	IIIf
P. aeruginosa	10	12	14	12	13	10
S. aureus	15	14	10	11	13	14
C. frundii	13	12	10	11	14	13
E. coli	12	12	11	10	15	14
P. mirabilis	13	13	10	10	14	12
S. typhi	14	12	12	12	14	13

Strongly active range; >12 mm, moderately active range: 8-12 mm, weakly active range: < 8 mm. inactive —



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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:81-85
UGC Approved Journal No 48951

Original Article | Open Access

Conventional Energy Source Conservation Practice in Nagpur City, Vidarbha Region, India: A Case Study

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gudadhe Swapnil K (2018) Conventional Energy Source Conservation Practice in Nagpur City, Vidarbha Region, India: A Case Study, *Int. J. of. Life Sciences*, Special Issue, A12: 81-85.

ABSTRACT

Human being uses the earth's bounty in profusion. Energy resources are in verge of extinction due to its over use. Conservation of traditional energy sources and harvesting renewable energy resources like solar energy, wind, biomass and sea-waves can bring out a substantial solution to the problem of energy crisis. To study the same two prime activities undertaken by Nagpur Municipal Corporation were taken into consideration for the study. Many campaigns along with Poornima Diwas Campaign were studied for the project and analysed the amount of energy resources saved through these activities. Along with them, it observed that an aggressive propaganda for harvesting solar energy and marketing several gadgets using solar energy are being systematically done by NMC to bring out a desired effect. The paper ends with the positive note that energy is to be conserved for brighter future.

Keywords: Nagpur, Energy Conservation, Natural Ressources, Poornima Diwas, Solar Energy.

INTRODUCTION

Human being is the most intelligent species on the earth. He uses the earth's bounty in profusion. He seems to be least bothered about the truth that the natural resources that are available on the globe is to be shared by all the living-being on the earth including plants and animal. It is a sorrow state of affair that man is concerned only about his own needs and wants. Our self-centered need has started to take the form of the greed. It is an extremely crucial issue that has to be dealt with in detail, to understand various activities that are undertaken in Nagpur city for conserving non-renewable energy conservation, a project was conducted. The reports that are gained after visits to several NGOs and Nagpur Municipal Corporation (NMC) give a clear picture of the activities conducted at Nagpur level towards conservation of traditional natural resources and the initiatives taken to promote the use of non-conventional energy resources. The present paper aims giving a bird eye view of some

prominent activities done by NMC for conserving non-renewable energy.

As we are aware of the fact that we receive our natural resources from biotic and abiotic material found in the nature. Biotic natural resources include fossil fuels which are formed from organic matter that has decayed. Abiotic resources come from non-living and non-organic material. These resources are further classified into renewable and non-renewable energy resources on the basis of their renewability. Since the resources like coal, petroleum and nuclear energy etc. cannot be replenish once its stock is used up they are known as non-renewable resources, on the contrary the energy sources that are found in abundance in nature and can be revived again and again comes under the category of renewable energy sources. Solar energy, wind, sea-waves and geothermal energy etc. are viewed as renewable energy sources. There is a worldwide debate regarding the allocation of natural resources. Earth minerals, metal ores, fossil fuel (coal, petroleum and natural gas) and ground water are considered to be prominent non-renewable energy sources that are fast depleting due to its over use. The scenario cannot change unless we follow the golden path of reuse, recycle and regeneration of the traditional energy resources and harvesting of the renewable energy resources like solar energy, wind and sea tide etc (Pazare and Raman, 2014).

Nagpur, the second capital of Maharashtra State is uniquely situated in the geographical centre of India. It rich in agriculture and horticulture produce; Nagpur is famous for its oranges and is also known as 'Orange City'. Huge coal and mineral deposits exist in this region. With a population of 2.4 million (2011 Census), Nagpur is estimated to be the 114th largest city and the 143rd largest urban area in the world in 2006 in terms of population. Owing to the availability of rich natural resources in the Nagpur region, mining is a major activity. Several government organisations related to the mining industry are based in Nagpur, which includes Western Coalfields Limited (one of the eight fully owned subsidiaries of Coal India Limited), MOIL (Manganese Ore India Limited) and Indian Bureau of Mines. The Nagpur region has large deposits of coal is a main natural non-renewable source to get electrical energy. The large coal deposits in the region are sufficient to generate 4500 MW of power annually. The planned power generation capacity of Koradi and Khaparkheda will add another 1500 MW of power. But, it is necessary to understand that this natural resource will not last long so it is essential to look forward for renewable energy and energy efficiency model for the natural resources conservation (Madan and Sirse, 2015).

NMC have sensed this threat of energy crisis in time and take some major initiative for creating awareness and culture that will promote a sustainable reinforcement to the use of natural resources. Nagpur was selected under the Local Renewables Model Communities Network as a Model Community in 2006. The Nagpur Renewable Energy and Energy Efficiency Resource Centre (REEERC) was established in November 2006. The Resource Centre has over the years been actively involved in several awareness generating programmes and has developed into a knowledge base for renewable energy and energy efficiency for city officials, citizens, local businesses, etc. In 2007, Nagpur adopted a City Level Renewable Energy and Energy Efficiency Policy, making it the first city to do so in India (along with Bhubaneswar). Under the policy, Nagpur aimed to reduce conventional energy consumption in the city by 3% from 2005 levels by 2012 and municipal conventional energy consumption by 20% from 2005 levels by 2012 (Kale, *et al.* 2013). In addition to the awareness activities, Nagpur has also undertaken several pilot demonstration projects. Some projects and initiatives undertaken by NMC as a part of their action plan to achieve the target for reduction in conventional energy usage for the conservation of natural resources. Some of the projects have been undertaken by NMC for natural conservation are as follows:

Long Term Projects

- Leak Detection, Water Audit & Energy Audit of Water Supply
- Recycling & Reuse of Wastewater in Power Projects
- Use of Culture in existing STP to reduce energy consumption in aeration unit.
- Conversion of Garbage to Carbon Pallets
- Use of Compact Fluorescent Lamps (CFL) Technology in Street Lighting.
- Change of Electric Traffic Signals to Solar Signals.

Short Term Projects

- Installation of 500 LPD Solar Water Heating in NMC's Panchpaoli Maternity Nursing Home.

- Installation of SPV System at Renewable Energy & Energy Efficiency Resource Centre.
- Energy efficient lighting in NMC girls school.
- Energy Audit of NMC main office building.
- Solar Lights installed in Nagpur Municipal Corporation premises.
- Solar Lights installed at High Court premises.
- Installation of Solar Lights in Gardens, parks, etc.
- Installation of Solar based Traffic Signals.
- Installation of SPV System at Renewable Energy & Energy Efficiency Resource Centre
- NMC started offering 10% rebate on Property Tax for SWH installation.

Awareness Generation Activities

- Establishment of Renewable Energy & Energy efficiency Resource Centre for common citizens.
- National & International level workshops
- Celebration of Akshay Urja Diwas.
- Promotion of Solar Water Heater in Residential/Commercial sectors
- Training Programme for School Children.
- National Science Day Celebration
- Participation in Science Express Train Exhibition coupled with renewable energy and energy efficiency exhibitions/stalls.
- Tree Plantation Programme at Schools & in Slum areas.
- Celebration of Earth Day.
- Celebration of World environment day.
- Poornima Diwas (Full Moon Day) campaign

To promote the renewable energy technology at local level NMC passed a resolution on 16th November 2007 to provide 10% rebate in the property tax for the citizens of Nagpur who installs Solar Water Heating System. The tax rebate is applicable for a period of three years upon Solar Water Heating installation. The Local Renewables Model Communities Network is an initiative by ICLEI (International Council for Local Environmental Initiatives – South Asia) Local Governments for Sustainability to enable local governments to anchor the development and promotion of renewable energy resources and energy efficiency in their municipal development strategies and to initiate related activities at city level. The project was supported by German Federal Ministry for Economic Cooperation and Development (BMZ) with the technical support from Gesellschaftfür Technische Zusammenarbeit (GTZ) Germany. Some of the key elements of LR Model Communities project include:

- Citywide Energy Assessments
- Preparation of City Energy Status Report
- Creating Local Policies
- Involving stakeholders
- Establishing competence centres of renewable energy and energy efficiency known as Resource Centres (REEERC)
- Capacity building and Awareness generation activities.

Based on the available data for 5 prime energy sources (Electricity, LPG, Petrol, Diesel and Kerosene), the supply energy scenario for year 2007-08 is developed. The sample survey conducted in the city shows that penetration of SWH in the city is 0.4%. Other renewable have very low penetration and are too small a percentage to be reflected here. The pie-chart mentioned below depicts the usage of energy sources in percentage for supply side energy balance for the 2007-08 (Pazare and Raman, 2014).

From this analysis, it is very clear that the LPG is the major source of energy consumed by the city and the second major source of energy is electricity which needs attention for effective and optimal use through energy efficiency measures. In addition, for the generation of 1 Unit of electricity 0.5 kg of coal and 7.5 litres of water is utilized also it causes 1 kg of greenhouse gasses (GHG) emission. Share solar energy which is one of the best renewable resources in fuels in supply side energy balance for Nagpur city is only 0.4%. Thus, it is necessary to spread the awareness among the citizens about the conservation of non-renewable natural resources and harvest the renewable natural resources (Pazare and Raman, 2014).

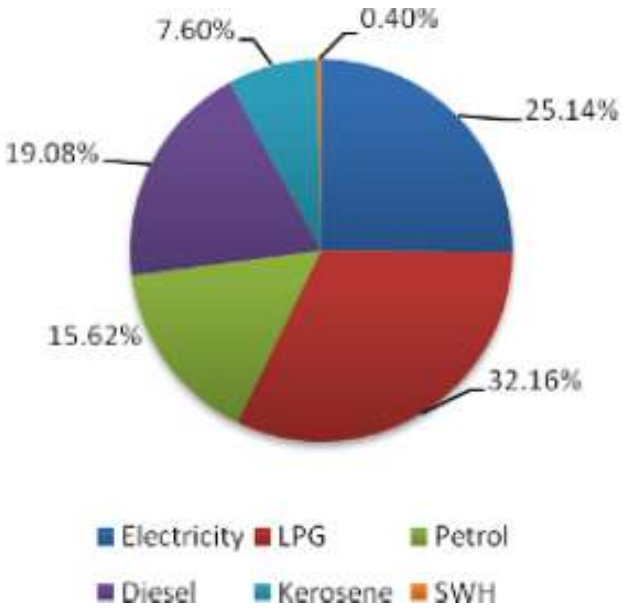

Fig. 1 Shares of Fuels in Supply Side Energy Balance

Solar energy is the largest source of all carbon-neutral energy sources. It is reported that more energy from sunlight strikes the Earth in one hour (4.3 × 1020 J) than all the energy consumed on the planet in a year (4.1 × 1020 J). Solar energy is thus a compelling solution to our needs of energy which is projected to double by 2050 and to more than triple by the end of the century. Solar energy is readily available, abundant source of energy and is secure from geopolitical tension. Nagpur (Latitude 21.1 N, Longitude 79.1 E) receives good amount of solar radiation. Monthly and average solar radiation is 5.09 kWh/m^2/day. for Nagpur is obtained from NASA SSE Satellite data, MNRE Solar Radiation Handbook 2008 and Data from Synergy Enviro Engineers (India) Private Ltd., Hyderabad.

The main aim of NMC is to develop city as model solar city in which the following list provides the present status of works undertaken by NMC:

- Five solar power plant of 25 kW has been installed and producing 3,150 Units of electricity per month.
- The NMC targeted total 2,82,900 Units of electricity will be saved per month by all solar water heaters.
- The work of installation of solar power plant of capacity of 960 kW to producing 1,15,200 Units of electricity per month.
- In addition, NMC has also sanctioned the installation of 200 kW solar power plant for Suresh Bhatt hall of the city, it will be capable of producing 25,200 Units of electricity per month.
- An ambitious project of NMC is to install the solar power plant of capacity of 27 MW at different places for domestic and street lighting to the generation of 34,02,000 Units of electricity per month.
- The Nagpur Metro Rail Corporation Ltd (NMRCL) has decided to installed capacity of 25 MW using the metro railway stations as sites for solar panels.

Poornima Diwas (Full Moon Day) campaign is a new and unique initiative has been taken by NMC to save the electrical energy. In this campaign, the NMC appealed citizens, shopkeepers and residents of particular area to switch off their non- essential electric gadgets and electric appliances for an hour on full moon day during 8:00 PM to 9:00 PM. Due to this campaign total 85639.02 unit's electricity saved in 33 weeks between 15th Jan. 2014 to 14th Nov. 2016.

CONCLUSIONS

In summary, following conclusions are drawn from the present study:

- NMC has taken initiative to develop Nagpur city as Nagpur Model Solar City with Ministry of New and Renewable Energy.
- The presently installed 25 kW solar power plant and 2,189 solar water heaters by the NMC leads to reduce the pressure of 2,86,050 Units of electricity per month from thermal power plants. This saves 1,43,025 kg of coal and 21,45,637 litres of water also it prevent 2,86,050 kg of GHG emission.
- NMC calls the applications from the citizens of Nagpur city to distribute the rest of 1,252 solar water heaters.
- NMC had sanctioned various solar power plants of around 1.16 MW capacity on buildings of NMC and Suresh Bhatt hall.
- A ambitious project of installation of solar power plant of 27 MW by NMC is under study.
- NMRCL has decided to fix solar panels on the roof of Metro stations which could be able to generate 25 MW of electricity which will meet their 40% of the energy requirement including traction power.
- Private firms such as Haldiram have taken initiative to be independent and installed power plant of 1.5 MW.

Social awareness programme Poornima Diwas (Full Moon Day) campaign by NMC successfully saved the electricity of 85639.02 units which leads to the saving of 6,67,084 litres of water and 42,387 kg of coal and reduction of 85,639 kg of GHG emission

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:86-88
UGC Approved Journal No 48951

Original Article Open Access

Assessment of water quality of river and municipal water in urban area

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Thakare Mahendra G (2018) Assessment of water quality of river and municipal water in urban area, *Int. J. of. Life Sciences*, Special Issue, A12: 86-88.

ABSTRACT

Water is essential for living things, including human being. It plays an important role to balance ecosystem by circulation from atmosphere to land and vice versa. Pure, safe and potable water makes its suitability for various uses. Water falls on land surface and drain into water bodies, including river carries heavy sediments which can be categories as physical, chemical and biological. The raw water of the river and its treatment in municipal water treatment plant is the way where quality can be assessed for potability. An attempt has been made to investigate the water quality of the river before and after treatment. Physical and chemical analysis of water shows reduction in water quality parameters viz., conductivity, acidity, total hardness and, sulphate. The result also shows the presence of residual chlorine in household tap water sample

Key words: Municipal, pure, potable, residual chlorine, raw water

INTRODUCTION

About 70% of planet earth is covered with water and rest is land surface (Dara, 2007). Only 2.5–2.75% is considered as fresh water (De, 1994). Industrial and agricultural sectors consume appreciable quantities of water (Sharma, 2000). Polluted water coming out from these sources is other side which account for deterioration of fresh water resources. River water contains impurities so its use before treatment is not so good for human health. Water from the river is pumped through the intake tower and carried by a network of pipelines to municipal water treatment plant for removal of impurities. Raw water passes through various treatment units' viz., screening, equalization, grit removal, coagulation, filtration, disinfection and final distribution through overhead tanks. It is essential to see that water received at tap of each house should comply physical, chemical and biological standards as prescribed by the statutory authority from time to time. In addition to this, the treated water received in houses must contain 0.2 mg/l free residual chlorine to ensure suitability of water for drinking.

METHODOLOGY

Sampling site for the collection of raw river water and municipal water treatment plant sample was selected with consideration of continuous water passes from the river via pipeline to municipal water treatment plant. Domestic houses with a spacing of one kilometer were selected for collection of composite water samples. The analysis methods as described in Standard methods for analysis of water and wastewater (APHA, 2005) were used for container handling and treatment prior to the collection of water sample, water sample preservation and analysis of physical- chemical constituents. Determination of residual chlorine in tap water samples was performed by chloroscope (Device developed by NEERI).

RESULT AND DISCUSSION

pH: pH is a negative logarithm of hydrogen ion activity. It is useful to calculate acidity and alkalinity of water. It can also control the process of coagulation, disinfection and demineralization. At present investigation pH of the river water (7.9) and treated water (7.5) are well within the permissible limit and indicate natural characteristics of water (Table 1). The pH of treated water is less than raw water because in the coagulation process considerable amount of dilute sulphuric acid is produced due to use of alum which reduces the pH of treated water.

Temperature: The values of temperature of water samples (29 °C) correspond to the atmospheric temperature. Temperature is a crucial factor for biota in river water. At elevated temperature solubility of Oxygen and Carbon dioxide is affected.

Electrical Conductivity: Electrical conductivity denotes water capacity to carry an electrical current. Surface water shows less conductivity than ground water. Treated water shows less conductivity (1.21 µs/cm) than raw river water (1.45 µs/cm). In the present investigation, ions are removed in coagulation and filtration process to a lesser extent.

Total acidity: Acidity of water is simply defined as the water's capacity to donate H positive ions. It is caused by free carbon dioxide in water. Carbon dioxide mixes with water and carbonic acid is formed (H_2CO_3). Excess acidity of water may lead to body imbalanced. In present investigation acidity values of treated water (18 mg/l) are slightly higher than river water (10 mg/l), the reason as described in pH section is equally applicable here as well.

Total alkalinity: It is a measure of water capacity to neutralize acids. Alkalinity in water is caused by bicarbonate, carbonate and Hydroxide compounds of calcium, magnesium and potassium According to the World Health Organization, health effects are most pronounced in pH extremes. Drinking water with an elevated pH above 11 can cause skin, eye and mucous membrane irritation. In present investigation alkalinity values are well within limit (Table 1), if, comparison is made between raw river water values (82 mg/l) and treated water (75 mg/l) after coagulation unit operation, it can be said that coagulant addition reduces total alkalinity concentration.

Table 1: Physical-chemical analysis of raw river water and treated water of the municipal treatment plant

Sr. No	Parameters	Raw river water sample	Treated water (MWTP)*	Drinking water standards BIS, IS 10500 : 2012
1	pH	7.9	7.5	6.5-8.5
2	Temperature	29 °C	29 °C	--
3	Electrical Conductivity	1.45 µs/cm	1.21 µs/cm	--
4	Acidity	10	18	--
5	Alkalinity	82	75	200
6	Total Hardness	150	140	200
7	Dissolved oxygen	6.5	7.8	--
8	Sulphate	22	10	200
9	Phosphate	BDL	BDL	--
10	Iron	0.1	BDL	0.3
11	Free Residual chlorine	NIL	0.1	0.2

* Municipal water treatment plant

All parameters are expressed in mg/l except pH, Temperature and Electrical conductivity

Total Hardness: Total hardness in water is caused by divalent cations namely calcium and magnesium (Reva, 1995). Surface water generally shows less total hardness as compare to ground water. Total hardness is removed to some extent in filtration process; its concentration is less in treated water (140 mg/l).

Dissolved oxygen: The solubility of oxygen in water depends on the temperature, pressure of air and the amount of dissolved solids present in the water. Dissolved oxygen of treated water (7.8 mg/l) is higher than raw river water (6.5 mg/l), the reasons being turbulence created during aeration of water in water treatment plant. Aesthetic quality of treated water w.r.t. D.O. concentration is good as compare to raw river water.

Sulphate: Sulphate can cause bitter taste in water if water treatment is not proper. It can create digestion related problems in humans and young livestock. Coagulation process is effective to remove sulphate in water treatment process. Sulphate concentration is considerably low in this study (10 mg/l).

Phosphates: Phosphate in the water is non toxic within permissible range. It can upset digestion if concentration crosses limit. Its concentrations are minimum and don't pose any threat to potability.

Iron: Iron occurs in water in the soluble ferrous and insoluble ferric iron. Dissolved ferrous iron gives water a disagreeable metallic taste, unacceptable taste. Iron is removed by aeration in water treatment plant. Iron concentration below 0.3 mg/l is acceptable, in this study it is within permissible limit (0.1mg/l).

Free Residual chlorine: Chlorine is applied in solid, liquid and gaseous forms in water treatment plant to kill disease causing microorganisms. In the present investigation, liquid chlorine was found used for treatment purpose. Composite samples collected from domestic places and analyzed on the spot by chloscope shows concentration of 0.1 mg/l, are acceptable from a drinking point of view.

CONCLUSION

Raw river water carries heavy load of impurities, such water if used for drinking purpose can pose threats to human life. Physical-chemical analysis of raw river water and water treatment plant sample clearly indicates the need to treat raw river water prior to its use for drinking purpose. Water samples analysed from the taps of houses for free residual chlorine also shows effective water treatment of raw water in municipal water treatment plant in urban areas.

Acknowledgements

I would like to express my sincere thanks to Dr. B. L. Chavan for his guidance. Thanks are also due to Dr. P. R. Chaudhari for his help and encouragement for conducting extensive research.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:89-94
UGC Approved Journal No 48951

Original Article Open Access

Analysis of endosulphan degradation by soil bacterium isolates ED-R1

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Katkar Prafulla and Patankar Sanjeev (2018) Analysis of endosulphan degradation by soil bacterium isolates ED-R1, *Int. J. of Life Sciences*, Special Issue, A12: 89-94.

ABSTRACT

Enzymatic bioremediation of insecticides is receiving considerable attention, particularly since the extensive characterization of enzymes capable of detoxifying a range of organophosphate compounds. The basis of investigations for enzyme capable of detoxifying other classes of insecticides requires a source of enzyme for catalytic detoxification. This study describes the enrichment of a culture of soil bacteria capable of degrading endosulfan. The enrichment was achieved and maintained by providing endosulfan as the only carbon source. Endosulfan is poor biological energy source, as it contains only six potential reducing electrons and previous attempts to enrich for endosulfan degrading microorganism using the insecticide as sulfur source have been not very successful. However, endosulfan has relatively reactive cyclic sulfite diester group70. In this study, microorganisms were selected for their ability to release the carbon group from endosulfan and to use this as source of carbon for the growth. This selection procedure enriches for a culture capable of either the direct hydrolysis of endosulfan or the oxidation of the insecticide followed by its hydrolysis. In particular, enzymatic insecticide bioremediation is the focus of extensive study after the isolation of enzymes capable of detoxifying a range of organophosphate compounds from several bacterial species. An essential step in the investigation of an enzymatic method for endosulfan degradation is the definitive identification of a biological source of endosulfan degrading activity.

Keywords- Endosulfan, Microorganisms, Gas chromatography, Degradati Oxidation

INTRODUCTION

As with the most pesticides, the persistence of and degradation of endosulfan are affected by the environmental conditions in which it is found. Endosulfan does not undergo direct photolysis but is transformed by the chemical hydrolysis under alkaline condition such as in sea water (Armburst 1992).

In soil, endosulfan has been shown to be degraded by a variety of microorganisms (Katayama *et al* 1991). However, degradation rates are usually low and metabolism often results in the formation of endosulfan sulfate, an oxidative metabolite shown to be equally as toxic and persistence as the parent compound, endosulfan. Because of its persistence and toxicity, endosulfan contamination poses a significant environmental concern. There are varieties of soil microorganism that have ability to degrade the endosulfan. The degradation of endosulfan by soil microorganism of family *Pseudomonas Sp*. was studied. In microbial degradation of endosulfan under aerobic condition, soil microorganism degrades the endosulfan and yielded the endosulfan sulphate (30-60%), with some endodiol (2.6%) and endolactone (1.2%). The parenthetical numbers refers to the percentage of the applied endosulfan recovered as a metabolite. Sixteen of 28 fungi, fifteen of 49 soil bacteria and three of 10 actinomycetes metabolized greater than 30% of the applied C-14 endosulfan. Endosulfan sulphate was the major metabolite formed by the fungi and endodiol was the predominant product of the bacteria (Maier-Bode 1968). Only a small amount of C-14 labeled carbon dioxide was detected, indicating minimal mineralization. Microorganisms have increasingly been investigated as a source of xenobiotics-degrading enzymes (Chen *et al* 1998). We are interested in the isolation of endosulfan degrading bacterium for further investigation into enzymatic endosulfan bioremediation. Using endosulfan as the only available carbon source, we can enrich soil inocula for microorganisms capable of releasing the sulfur from the endosulfan, thereby providing a source of carbon for growth (Wegman et al 1978). Since the removal of carbon moiety dramatically decrease the vertebrate toxicity of endosulfan (Stewart *et al* 1974), this results in concurrent detoxification of the insecticide. Results suggest that while both isomers can be degraded by microbial organisms, the degradation materials released counteract the growth of the microorganisms.

In this study, microorganisms were selected for their ability to release the carbon group from endosulfan and to use this as source of carbon for the growth. In this work we have studied the different process optimization parameters to obtain the maximum degradation. We report here on the resultant bacterial culture that, the culture degrades endosulfan to produce a novel metabolite to occur as a result of chemical hydrolysis. These results suggest that the obtained bacterial isolates at optimized growth condition are a potential source of an enzymatic bioremediating agent.

MATERIAL AND METHODS

1. Materials and reagents

Technical grade endosulfan was supplied from Department of microbiology Guru Nanak College of science, Ballarpur (M.S.). Technical grade endosulfan (used commercially) is a mixture of two diastereomers, alpha - endosulfan and beta - endosulfan in a ratio of 7:3, hexane (HPLC grade), and acetone. Standard chemical were used for the preparation of nutrient media. For the chemical and instrumental analysis, spectrophotometric grade chemical were used.

2. Sample collection for isolation studies

The soil sample for the enrichment and the isolation of the microorganisms was collected from the cotton field near Gadchandur (M.S) India at the end of growing seasons. The field had generally received several application of endosulfan in the month of September to October for at least 2-3 times. The soil was fertile gray. The top soil collected from the upper layer (approximately 15 cm) and stored at 4ºC prior to the experimental studies.

3. Nutrient media for the enrichment of microorganisms

The endosulfan enrichment media for the isolation of microorganisms was prepared by the addition of following component (gm/lit). This media is actually a basal medium containing the endosulfan as a carbon source (Katkar *et al* 2015). KH_2PO_4-0.5, K_2HPO_4-0.5, NaCl-0.5, $MgSO_4$ $7H_2O$-0.5, $FeSO_4$ $7H_2O$, $CaCl_2$-0.002, $NaMI_4$-0.001, $CoNO_3$-0.0005, $ZnSO_4$-0.0005, $MnSO_4$-0.0005, Endosulphan-0.001, pH-7.2.

4. Isolation of endosulfan degrading microorganisms

For the isolation of endosulfan degrading microorganisms, soil perfusion apparatus was designed. This work on the air pressure created by the vacuum. The small holes were made at the top and sand pebbles were kept over it for the support and slow perfusion of the soil sample to the medium which is kept at the bottom. The tap water is open to create air pressure; this air pressure is helpful for the aeration to the medium. The soil moistens with the media and perfused to the medium at the bottom. This process recycles

continuously and microorganisms present in the soil enriched into the media. The endosulfan enrichment medium was added to the bottom. The sand pebbles were kept over the holes at the top. The fertile gray soil (approximately 10gm), and then the tap water is open such that the medium rises above the soil and soil sample slowly perfused to the medium. The apparatus were kept run for the 10 days. After the 10 days of incubation, the small alliqout of enriched soil inoculum were plated over the endosulfan enrichment agar. The different population of microorganisms on the endosulfan enrichment agar then achieved.

6. Identification of endosulfan degrading microorganisms

For the identification of single strain of isolates ED-R1 following microscopic, morphological and biochemical studies were been carried out.

6.1. Microscopic Studies

Microscopic details of the isolate ED-R1 have been done. The given isolates are whether Gram positive or Gram negative also been decided.

6.2. Morphological studies

Under the morphological studies, the various colonies characteristics like, shape color and growth pattern have been studied.

6.3. Biochemical studies

Following various biochemical tests have been carried out for each isolates; Indole, Methyl Red, Voges Prausker's and Citrate utilization test, Catalase test, Starch utilization test, Oxidase test, Nitrate reduction test, Urease test.

6.4. Sugar Fermentation Test

For the sugar fermentation test 0.5% NaCl. 0.5% peptone and 0.5% of the sugars were been added and incubated with the given isolate ED-R1. The tubes were observed for the production of acid gas after 24 hours.

7. Analytical Method

7.1 Optimization of bacterial density

Optical densities at λ600 of the endosulfan enrichment media incubated with the given isolates ED-R1 were measured to assess the relationship between growth and metabolic activities of microorganisms, the bacterial growth of the isolate ED-R1 were observed in response to endosulfan supplied as the source of carbon. The optical density of each isolates was measured with the interim of two days by the visible spectrophotometer and respective readings were recorded.

7.2 Optimization of pH of the Medium

The pH of the endosulfan enrichment media was measured in the order to assess the relationship between growth and metabolic activities of the microorganism. The change in the pH of the endosulfan enrichment media with interim of two days were recorded during the 10 days of incubation. The initial pH of the media was adjusted to 7.2.

7.3 Extraction of Endosulfan from the Media

Endosulfan was extracted from the enrichment media for the degradation studies. Approximately 25 ml culture media sample were taken out from the soil perfusion apparatus and equal volume of acetone (i.e. 25 ml) were added. The acetone - sample mixtures were shaken for 1 hr on the magnetic stirrer. 1ml of the mixture were taken out and transferred to 9 ml of hexane. These mixtures were then further shaken for 15 min **(**Siddique *et al* 2003). The sample was dehydrated by the addition of Na_2SO_4. The sample is then store in vials at 4ºC for the further analysis.

7.4 Quantitative Estimation of Endosulfan by Gas Chromatography

The quantitative analysis of endosulfan and its metabolite was done by gas chromatography-chemito model 1000 GC equipped with electron capture detector by using a glass column (8 inches length X 0.25 inch diameter). Nitrogen was used as carrier gas at the flow rate of 1.5 ml / min. The injected volume of sample in GC was 2 µl. The extracted endosulfan sample were been analyzed by Insecticide Residue Testing Laboratory, Nagpur.

RESULTS AND DISCUSSION

1. Microscopic and Morphological characters

The isolate ED-R1 showed Gram Negative rod shaped cells arranged mostly separated. The colonies on the endosulphan enrichment media were red/pink colored, moist, pleomorphic with round shape.

2. Biochemical Test

The results of all biochemical test performed with isolate ED-R1 are given bellow.

Table 1: Biochemical characterization of the isolate ED-R1

Sr. No.	Name of The Test	Inference
1.	Indole Test	Negative
2.	Methyl Red Test	Positive
3.	Voges Proskauer's Test	Negative
4.	Citrate Utilization Test	Positive
5.	Starch Hydrolysis Test	Negative
6.	Oxidase Test	Negative
7.	Catalase Test	Positive
8.	Urease Test	Negative
9.	Nitrate Reduction Test	Positive
10.	Gelatin Hydrolysis Test	Positive

Table 2: Sugar fermentation test of isolate ED-R1

Sr. No.	Sugars	Acid	Gas
1.	Glucose	+	+
2.	Manitol	+	+
3.	Lactose	+	+
4.	Maltose	+	-
5.	Ribose	+	+
6.	Sucrose	+	-
7.	Xylose	+	-
8.	Arabinose	-	-
9.	Mellibiose	+	-
10.	Raffinose	-	-
11.	Tetrahalose	+	-
12.	Cellobiose	+	+

4. Identification of isolated strain of bacteria

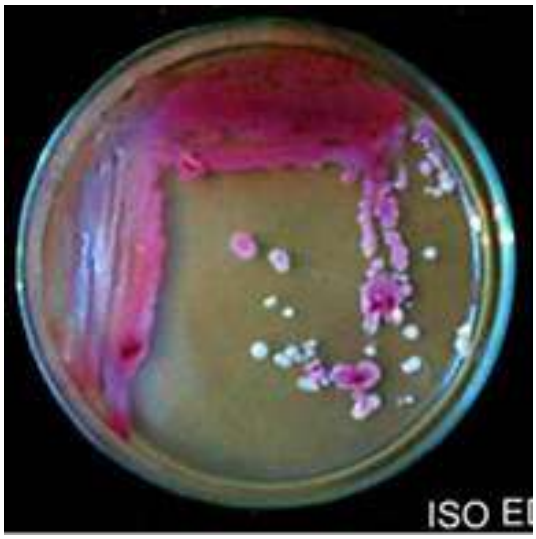

Fig. 1 : Pure culture of bacterial isolate ED-R1 grown on endosulphan enrichment media isolated from mixed culture.

From of the results of microscopic, morphological and biochemical test, the isolate ED-R1 has been identified as *Serratia sp.* The obtained result were studied and compared with standard results of respective bacteria (Hugh *et al* 1973).

5. Measurement of bacterial density

Optical densities (λ600) of the respective isolate are represented in the figure.2. The highest OD_{600} recorded for ED-R1 was 0.47. As per the result, it has been found that the bacterial strain degrading more endosulfan within the culture media showed higher bacterial density.

Siddique *et al* (2003) was observed the same in that bacterial strain that depleted α and β endosulfan as a sulphur source. Sutherland *et al* (2000) and Awasthi *et al* (1997) who observed the substantial disappearance of the endosulfan with the simultaneous increase in the bacterial mass.

Bacterial density obtained with the isolate ED-R1 are quite higher in comparison to Kwon G. S. et al (2005) who were worked with Klebsiella Oxytoca. The utilization of endosulfan was accompanied by the increase optical density (OD595) of the culture media ranging from 0.51 to 0.89 as observed by Hussain et al (2007).

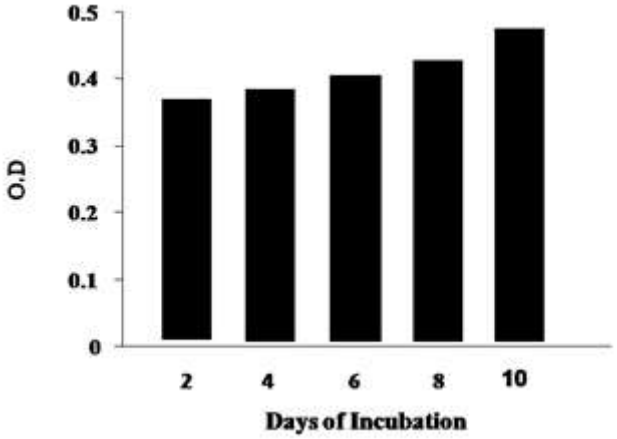

Fig. 2 - Variation in the O.D $_{600}$ of bacterial culture ED-R1 after 10 days of incubation.

6. Measurement of pH of medium

The change in the pH of the endosulfan enrichment media after 10 days of incubation is shown in the figure 3. The culture pH decreased to acidic range due to metabolic activities of the growing organism. The isolate ED-R1 showed the decreased pH of the medium

to 3.5 after the 10 days of incubation. The results are very much similar to the work of Siddique *et al* (2003). It has been observed that the decreased in the pH of the medium was found to be associated with enhanced degradation of the endosulfan. With the interim of two day during each pH reading, pH decreased with the bacterial metabolism. The decrease in the pH may either be due to the dehalogenation of endosulfan resulting in the formation of the organic acid produced by microorganism during their metabolic activities.

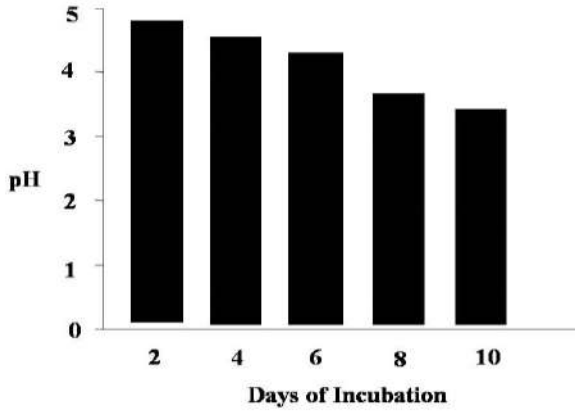

Fig. 3 - Variation in the pH of bacterial culture ED-R1 after 10 days of incubation.

Martens *et al* (1976) were observed that some of the bacteria which showed the pH value of 8.3 and 8.5 at the end of the experiment. This higher pH value was probably due to the chemical hydrolysis but some of the bacteria were having the low pH values which indicate that a large portion of degradation was enzymatic. Endosulfan is susceptible to alkaline hydrolysis occurring with approximately 10 fold increased in hydrolysis with each increased in pH unit. Many previous studies have been unable to differentiate between chemical and biological hydrolysis of endosulfan because microbial growth has led to the increased in alkalinity of the culture media (Guerin *et al* 1992).

7. Degradation of Endosulfan by the Bacterial Isolate ED-R1

The degradation of endosulfan was been confirmed by analyzing the sample by gas chromatography as shown in the figure 4. The degradation was determined by monitoring endosulfan disappearance by GLC-ECD detection. The bacterial isolate ED-R1 degraded 83% (0.171 ppm) endosulfan after the 10 days of incubation. The initial concentration of endosulfan in the culture media was 1 ppm. The isolate ED-R1 degraded 73.4% (0.266 ppm) of α-endosulfan and 85.5% (0.145 ppm) of β-endosulfan. The degradation of β-endosulfan was found to be higher than that of α-endosulfan by ED-R1 isolates. The result of this study suggests that the ED-R1 isolate are a valuable source of potent endosulfan degrading enzymes for use in enzymatic biodegradation. The endosulfan was used separately as a carbon source to identify which microorganism prefers endosulfan as a carbon source and to what extent endosulfan is degraded when used as carbon source. The obtained results are much similar to findings of Siddique *et al* **(**2003) who had worked on *Fusarium ventricosum* which degraded α-endosulfan upto 82.2% and 89.0% of β-endosulfan when endosulfan supplied as carbon source. The bacterium *Pseudomonas Spinosa* and *Pseudomonas aeruginosa* were the most efficient degraders of both α-endosulfan and β-endosulfan as they consumed more than 90% of endosulfan (Hussain *et al* 2007).

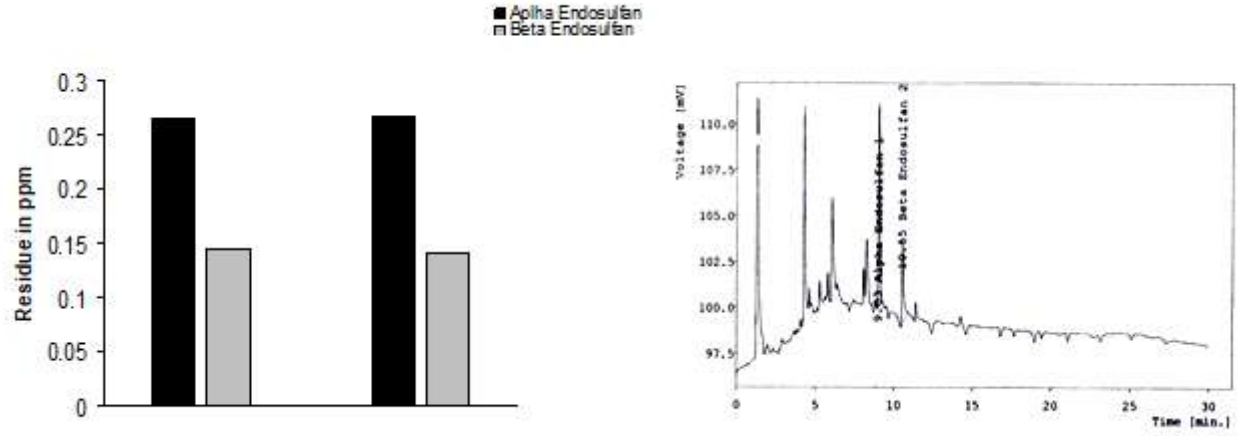

Figure 4- Gas chromatographic analysis of endosulphan degradation by isolate ED-R1.

CONCLUSION

Microorganisms have increasingly been investigated as a source of xenobiotics-degrading enzymes. We are interested in the isolation of endosulfan degrading bacterium for further investigation into enzymatic endosulfan bioremediation. Using endosulfan as the only available carbon source, we can enrich soil inocula for microorganisms capable of releasing the sulfur from the endosulfan, thereby providing a source of carbon for growth. Since the removal of carbon moiety dramatically decrease the vertebrate toxicity of endosulfan, this results in concurrent detoxification of the insecticide. We report here on the resultant bacterial culture that, the culture degrades endosulfan to produce a novel metabolite not reported to occur as a result of chemical hydrolysis. These results suggest that the obtained bacterial isolates are a potential source of an enzymatic bioremediating agent.

We are currently attempting to isolate a pure bacterium from the soil that is capable of detoxifying endosulfan. Such a bacterium would potentially be a valuable source of catalytic enzymes for the development of bioremediating agent to reduce endosulfan residue problems in run-off from irrigation waters..

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:95-100
UGC Approved Journal No 48951

Original Article Open Access

Survival studies of bacterial pathogens and their Immunization effect on fish (*Channa marulias*) in glass aquaria

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Bodhe YG, Wadhai VS, Hajare JW and Atla DG (2018) Survival studies of bacterial pathogens and their Immunization effect on fish (*Channa marulias*) in glass aquaria, *Int. J. of Life Sciences*, Special Issue, A12: 95-100.

ABSTRACT

Present study was carried out to examine for the growth and survival of *Channa marulias* cultivated in glass aquaria. An experiment was conducted in four glass aquaria (size 90 × 30 cm) for a period of 21 days in January 2015. Six fishes of same size (age group) of *Channa marulias*, with mean initial length and weight of 6.5 ± 0.07cm and 5.8 ±0.04 g respectively were assigned to each aquaria. The aim of this work is to determine the concentration of bacterial pathogens to be inoculated in *Channa marulias*, so as to induce bacterial infection but not death during a period of at least two days and, therefore, enable the development of treatment protocols. The clinical exam was done 24 h after inoculation, and the clinical signs suggested bacterial infection in all fishes. In the lowest concentration, fishes demonstrated few clinical signs of disease, and in the highest concentration (4.5 x 10^6 CFU/ml), all fishes died within 24 - 48 hrs of bacterial induction with acute infection. In the intermediate concentration, all fishes presented clinical signs and kept living at the beginning of the time of treatment. Therefore, 2.4 × 10^6 CFU/ml concentrations were defined as viable for the study of experimental infection in different bacterial pathogens.

Key words: *Channa marulias,* Bacterial pathogens, Wainganga river.

INTRODUCTION

Channa marulias is native to South Asia. In South India it is commonly found in reservoirs of eastern Vidarbha region. It is a faster growing fish than most of the other species of the genus. It is a carnivorous species. It is marketed live and fetches high prices in the market. Fishes are well known for their nutritional value. Healthy fishes are prized for their table quality. However, this quality is influenced by several operational environmental factors. Often, they are prone to microbial and parasitic infections. A well known economic loss to the fish industry was the major outbreak of bacterial infection in major carps. The causative agents of the severe acute infectious abdominal dropsy outbreak in Indian major craps.

Cirrhinus mrigala was reported Shome *et al*. (1996). However, the first observation on diseases in Indian major carps was found in descending order of susceptibility on *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* (Gopalakrishnan, 1961). Other well recorded cases have been the severe epidemic due to the diseased condition of Eropean carps (Snieszko 1994).

The studies in the last decade (Kar *et al.,* 1995) showed that species like *Channa striatus, C. punctatus, Clarias batrachus and Anabas testudineus* have been severely affected by bacterial pathogens and the outbreak has been occurring during the period from November to March. Low temperatures appear to influence the severity of infectious lesions. Severe acute infectious abdominal dropsy outbreak in Indian major craps. *Cirrhinus mrigala* was reported Shome *et al* (1996). However, the first observation on diseases in Indian major carps was found in descending order of susceptibility on *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* (Gopalakrishnan 1961). Other well recorded cases have been the severe epidemic due to the diseased condition of European carps (Snieszko 1954). Sabur (2006) isolated and identified five species of *Aeromonas* bacteria in polyculture environment of five carp species namely *Labeo rohita, Cyprinu scarpio, Cirrhinas cirrhosus, Catla catla* and *Hypophthalmich thysmolitrix*. Lately the bacteria *A. hydrophila* was isolated from Thai pangus *Pangasianodon hypophthalmus* (Siddik, 2009) and from climbing perch *Anabas testudineus* (Sayed, 2010). In the present work, experimental infection was done to know the pathogenicity of bacterial pathoens in *Channa marulias*. The virulence of the pathogen was estimated by experimental studies of the LD_{50} (median lethal dose) of *bacterial pathogens* in the glass aquaria.

MATERIAL AND METHODS

2.1 Study Area

This study was conducted on fish species collected for studies of bacterial pathogens and their immunization effect on hematological and biochemical indices in healthy and infected fish from Wainganga river In Gadchiroli district the river flows nearby Armori tehsil. The fish samples were collected from a freshwater during the period October 2015 to February 2016. The numbers of fishes caught were transported on the same day in a container filled with pond water to the laboratory and the analysis was carried out. A total of 10 adult specimens of the species having mean length 20.14±0.40 cm, breadth 3.63±0.08 cm and weight 125.20 ± 4.18 g were utilized in the present investigation.

2.2 Laboratory Analysis

2.2.1 Fish samples

Forty fish samples were collected from Wainganga River between the periods of October 2015 to February 2016. Twenty samples of *Channa marulias* were collected aseptically and immediately from areas separately and transported in a thermal bag to the laboratory and processed within 3hrs of acquisition, and samples were kept in the refrigerator (4–8^{0}C).

2.2.2 Sample preparation

Sample preparation was made using the method described by. About 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples were crushed into small pieces in a sterile mortar with about 10 ml sterile water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This was done for the 40 fish samples (Myiazaki 1972) (Olufemi 1983) (Qureshi *et al.,* 2001) (Refai *et al.,* 1989).

2.3 Sampling

The bacterial counts on the external surfaces, intestines and tissue were estimated as follows:

2.3.1 Skin Surfaces

Sample from different locations of the skin of 40 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of Nutrient broth, MacConkey broth and Selenite F broth which are dispensed in separate tubes. 10 fold serial dilution of the bacterial suspension inoculated in peptone water was prepared induplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by (Slaby *et al.,*1981) and then incubated at 37^{o}Cfor 48 hrs.

2.3.2 Intestines, Gills & Tissues

1g of the fish sample was dissected out, blended and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9 ml of 0.1% sterile peptone water. The bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold serial dilution was carried

out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 hrs as described by (Rodricks 1991). *Coliforms* organisms and gram negative enteric bacteria counts were determined using pour plate method with MacConkey agar, EMB Agar respectively (Chauhan 2013) (Chauhan *et al.,* 2014). Pseudomona isolation Agar for *Pseudomonas spp*. *Salmonella spp*. and *Shigella spp*. were enumerated using Salmonella Shigella Agar (SSA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for pathogenic *Vibrio* spp (Bruno, 1980). The plates were incubated at 37°C for 24hrs. The observed colony growth were counted using Coulter™ Colony counter according to plate count method (Kvenberg 1991) (Laxmareddy, 2013). Identification of the organisms was done using the phenotypic and biochemical characteristics as described by and (Slaby *et al.,* 1981).

2.4 Estimate of mean colony forming unit per gram (CFUg-1)

The mean colony forming unit per gram (CFU g-1) denoted by (x) was calculated as $\Sigma f\chi/\Sigma f$, where Σfx is the sum of the products of number of colonies and the colony forming unit per gram; while Σf is the summation of the number of colonies.

Median lethal dose (LD_{50}) experiment

An amount of 10 mg of fresh culture of the bacteria was carefully scraped and mixed with1 ml PS and desired dilutions were prepared by serial decimal dilution method. In a preliminary test the above stock dilution (10 mg in 1 ml) was calculated to contain around 10^6 CFU/ml. Four serial dilutions having an estimated concentration of 10^7, 10^6, 10^5 and 10^4 CFU/ml were used for the (LD_{50}) experiment. From each of the above 4 dilutions, 0.1 ml bacterial suspension was injected intramuscularly to each of previously stocked and acclimatized 10 fish making a group. The injected fish were observed up to 21 days. No feed was given to the experimental fish and water temperature was recorded twice daily. Immediately after death, each fish was transferred to laboratory; kidney was dissected out, touched with a sterilized loop and streaked onto TSA plates. The plates were incubated at 25°C for 48 hours for *A. hydrophila* colony appearance. From the mortality record, LD_{50} value was worked out according to the following formula:

$$\text{Proportionate distance (PD)} = \frac{\text{50\% mortality - mortality at dilution next below 50\%}}{\text{Mortality at dilution next above 50\% - mortality at dilution next below 50\%}}$$

Dilution factor (DF) = Negative Log of lower dilutions (Next above 50% mortality) …… (i)

PD × DF …… (ii)

Log LD50 titer = (i) + (ii), LD50 titer = 10[(i) + (ii)]

RESULTS AND DISCUSSION

In this study, for all the fish samples ranged between 1.06 x 10^6 and 21.54 x 10^6cfu/ml as shown in Table No.1. Out of the 40 fish samples analyzed, for theskin had the highest number of bacteria with 21.54 x 10^6cfu/ml in *C. marulias*.

Table 1. revealed the isolation of *Pseudomonas sp*. with the skin having the highest number count to be 18.78 x 10^6 cfu/ml. The *S. dysenteriae* isolated had the lowest count to be 1.06 x 10^6cfu/ml from the skin of *C. marulias* as compared with other parts of fish. The intestine is the most colonized part of *E. coli* examined areas in the fish with count to be 12.50 x 10^6 cfu/ml, while the lowest count was examined areas in the fish with count to be *C. marulias* (1.06x 10^6cfu/ml). The gills likewise showed possible colonization but in the lowest count as compared to other parts. Isolation of *Vibrio sp*. on the intestine of fishes. *Coliforms* isolation showed the highest count in *C. marulias* for skin (21.54 x 10^6cfu/ml).

Table 1: Count of bacteria present at different parts of examined sample fish.

Fish	Parts	*Coliforms*	*E. coli*	*S. aureus*	*P. aueruginosa*	*V.cholerae*	*S.typhi*	*S.dysenteriae*
		(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)	(cfu/ml)
		10^6	10^6	10^6	10^6	10^6	10^6	10^6
Channa marulias	Intestine	8.50	12.5	6.10	16.22	8.19	4.17	1.06
	Gill	11.46	14.04	4.82	14.49	2.84	4.18	3.64
	Skin	21.54	11.08	8.46	18.78	3.24	5.24	1.26
	Mouth	16.64	15.26	4.48	13.84	2.48	4.16	4.14

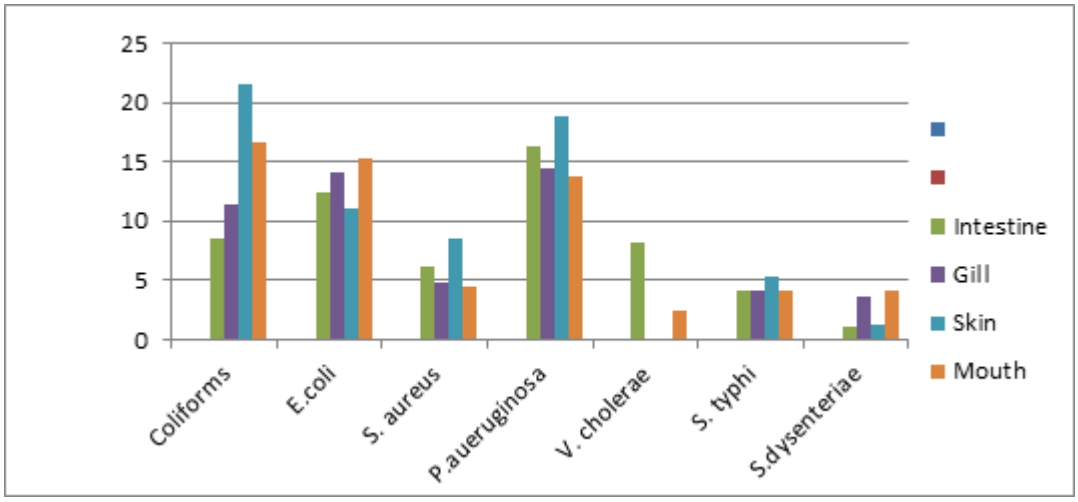

Figure 1.Bacterial count in various parts of fish *Channa morulias*

Table 2: LD_{50} pathogenicity test for fish *Channa marulius.*

Fish	Bacteria	Dose CFU/ml	No. of Fishes	No. of Fishes Died	Mortality	Post Infection Day of Mortality
Channa marulius	*S. aureus*	4.5×10^6	5	4	80%	4-6 days
		4.5×10^5	5	2	40%	4-6 days
	Escherichia coli	4.5×10^6	5	3	60%	2-4 day
		4.5×10^5	5	2	40%	2-4 day
	Streptococcus pneumoniae	4.5×10^6	5	3	60%	2-6 days
		4.5×10^5	5	2	40%	2-6 days
	Pseudomonas aeruginosa	4.5×10^6	5	5	100%	2-4 days
		4.5×10^5	5	3	60%	2-4 days
	V. cholera	4.5×10^6	5	4	80%	1-3 days
		4.5×10^5	5	2	40%	1-3 days
	S. typhi	4.5×10^6	5	5	100%	2-6 days
		4.5×10^5	5	4	80%	2-6 days
	S. dysenteriae	4.5×10^6	5	4	80%	2-4 day
		4.5×10^5	5	2	40%	2-4 day

The mouth and gills were also heavily populated by *E. coli* with the highest exhibited in the gills of *C. marulias. Staphylococcus aureus* had a low isolation rate in all samples analyzed as generally compared with other isolated organisms that had the lowest counts. The human bacterial pathogens that were isolated and identified include *Escherichia coli, Pseudomonas aueriginosa, Shigella dysenteriae, S. aureus, Coliforms, S. typhi and* indicated in the Table 1.

Median lethal dose (LD_{50}) for *Channa marulius*

The pathogenisity tests are shown in Table No. 4.11 of *Channa marulius* was proved to be sensitive to bacterial species as shown by their mortality upto 100%, at a dose of 4.5×10^6 CFU/ml shown by *Pseudomonas aeruginosa* and *Salmonella typhi* compared to 4.5×10^5 CFU/ml contain 60% and 80% respective bacteria. While, maximum mortality of *Staphylococcus aureus, Escherichia coli., Streptococcus pneumoniae, Vibrio cholerae* and *Shigella dysenteriae* shown that 80%, 60%,

80% and 80% respectively in 4.5 × 10^6 CFU/ml dilution factor compared to 4.5× 10^5 CFU/ml contained 40%, 40%, 40% and 40% respected pathogens during 2-6 days of period.

Results of LD_{50} test are presented in Table No. 2 shown that all the fish died with 4.5 × 10^6 CFU /ml within 2 - 5 days, among them 2 fish died at the day of doses, 1 fish died at 2nd day, 1 fish died at 5th day and 1 fishes died at 6th day. With the dose of 4.5 × 10^5 CFU/ml, 4 fishes died out of 5. Among them 1 fish died at the day of doses, 1 fish died at 2nd day, 1 fish died at 4th day and 1 fishes died at 6th day. In case of fishes, streaking and incubation from each dead fish gave rise to the appearance of pure colonies of bacterial pathogens.

DISCUSSION

A high population of bacteria in food indicates the general quality of the food and the degree of spoilage it might have undergone. The occurrence of total bacterial counts of many of the samples investigated having > 5 X 10^6 CFU/g raises concern about the hygienic status of the production and point of sale environment. The results from this study and according to published microbiological guidelines as cited by (Gilbert *et al.* 1996) suggest that the microbiological quality of the fish examined is unacceptable and pose a potential risk to public health. The diversity of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. These opportunistic and pathogenic bacteria were also previously isolated by several other researchers from fish (Mhango *et al.,* 2010).

The fish in this study harbored human disease causing organisms that cause diseases such as food poisoning, diarrhea, typhoid fever and Shigellosis. (Claucas and Ward, 1996). Suggested that when present in food, pathogens such as *S. aureus, Salmonella, Shigella* and *Pseudomonas* are most likely to cause food-borne diseases. The high incidence of *Salmonella* in the fish from the river is a major health concern.

The isolation of *Salmonella, Shigella, and E.coli* indicate faecal and environmental pollution. Coliforms such as *E.coli* are usually present where there has been faecal contamination from warm blooded animals (Chao *et al.,*2003). The organism *E.coli* is recognized as the reliable indicator of faecal contamination in small numbers and in large number sit is an indicator of mishandling. In similar studies, *Escherichia coli, Pseudomonas aueriginosa, Shigella dysenteriae, Staphylococcus aureus and Salmonella typhi were* isolated from the gills, intestines. This was attributed to the heavy load of sewage disposal into the seas which could act as a suitable environment for the growth and survival of the human pathogens.

CONCLUSION

Seven human bacterial pathogens i.e. *Escherichia coli, Pseudomonas aueriginosa, Shigella dysenteriae, Staphylococcus aureus, Vibrio cholerae,* and *Salmonella typhi* were isolated from the two fish species *Channa marulias* collected from Wainganga river of Gadchiroli District.. The presence, in large populations of these bacterial pathogens indicates high levels of faecal contamination in the river. The presence of enteric bacteria may be attributed to faecal contamination due to improper sewage disposal and or water pollution. The fish act as a reservoir of human pathogens and the presence of highly pathogenic agents such as *Salmonella, Shigella* species and of opportunistic pathogens is a potential health risk/hazard to human beings and may cause diseases to susceptible individuals especially the immune-compromised consumers. Moreover the recoveries of various organisms, which are potentially pathogenic to humans, in the fish suggest that if they are improperly handled, undercooked or consumed raw may contribute to the spread of the pathogens in the community. Further examination of fish especially for the presence of pathogens, during handling, storage and up to the very point of consumption is needed for the protection and maintenance of community health by keeping food borne diseases to a minimum.

As the bacteria are species specific parasites, it was found from the present study that bacteria are highly pathogenic to fresh water ornamental fish *C. marulias* causing parasitism. There may be certain toxins present in given species of bacteria which cause pathogenesis in fish lead to change in hematological parameters and varying degree of destruction in the tissue which leads to mortality of fish.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
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Special Issue A 12: March 2018:101-106
ISSN:2320-7817(p) | 2320-964X(o)
UGC Approved Journal No 48951

Original Article Open Access

Methylotrophic activities of *Acenatobactor spp*. from Lonar lake

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Manuscript details:

Received : 05.02.2018
Accepted : 29.04.2018
Published : XX.XX.XXXX

Editor: Dr. Arvind Chavhan

Cite this article as:
Pawar AL (2018) Methylotrophic activities of *Acenatobactor spp*. from Lonar lake, *Int. J. of Life Sciences*, Special Issue A12: 101-106.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Lonar Lake is a saline ecosystem formed entirely on basalt rock. It is a completely closed system a favors the growth of alkalophiles and halophiles. The *Acenatobactor* play a major role for conserving environment by utilizing toxic C1 carbon compound and reduces the pollution produced by methanol, green houses gases etc. In present study, methylotrophic bacterial isolates were isolated from water and sediment of Lonar Lake using minimal salt medium containing 2% (v/v) methanol as sole carbon and energy source. One bacterial strains were isolated and characterized morphologically, biochemically and identified as *Acenatobactor* by 16S rRNA sequencing. These *Acenatobactor* strains were further screened for its ability to utilize methanol by Spectrophotometric method. Results showed that the isolates ALP3 of *Acenatobactor* strains are found to be efficient methanol utilizer and could be effectively use for bioremediation by reducing methanol at polluted sites and also helpful in reducing C1 compound, and global warming gases from environment.

Keywords: Lonar Lake, Methylotrophs, Bioremediation, Methanol and *Acenatobactor*

INTRODUCTION

The Lonar Lake is a saline and hyperalkaline ecosystem formed entirely on basalt rock by meteor impact. The crater is located in India ~ 550 km east of Mumbai and Arabian Sea is 150 m deep and 1830 m across (Fredrikson, 1973) with raised rim up to the 100m in width and 20m to 30 m high and alkalinity of the Lake water is attributed to the high content of sodium carbonate (Thakker and Ranade, 2002). The Lonar Lake is unique in the world for its alkalinity (pH 10) and salinity (NaCl 0.9%) of the water but it was seen that chlorides and salinity of the Lake water is decreasing day by day (Tambekar *et al*., 2010). Microbiological studies using culture-dependent and culture-independent strategies have identified and characterized both bacterial (Kanekar *et al*.,1999, 2002; Nilegaonkar et al., 2002; Wani et al., 2006; Surakasi *et al*., 2007) and archaeal (Thakker and Ranade, 2002;

Surakasi, 2007; Surakasi *et al*., 2007) communities in the Lonar Lake water and sediment.

Methanotrophs are a unique group of methylotrophic bacteria, which utilize one-carbon (C1) compounds such as methane, methanol and methylamine which constitute an important component of microbe-driven food web chains in many ecosystems. Methylotrophic bacteria, are phylogenetically distributed across diverse phyla such as Gammaproteobacteria (type I methanotrophs), Alphaproteobacteria (type II methanotrophs) (Trotsenko and Murrell,2008), filamentous methane oxidizers (Stoecker *et al*., 2006; Vigliotta *et al*., 2007) and Verrucomicrobia (Dunfield *et al*., 2007; Pol *et al*., 2007; Islam *et al*., 2008) and contribute significantly in biogeochemical cycling of carbon by facilitating the incorporation of C1 compound-derived carbon into biomass (Anthony, 1992; Chistoserdova *et al*., 2009). The global cycling of methane and related C1 compounds further affects the important environmental phenomena related to climate change.

The toxicity of methanol has been widely documented and their disastrous effect towards human and environment is greatly concerned. Currently utilization of C1 compound received great attention from many people from industries and researcher due to their toxicity. The present study aimed to isolate and characterize methylotrophic bacterial spp from Lonar Lake which can remediate methanol by which there may reduction in C1 compound pollution in the environment. The phylogenic analysis of these methylotrophic bacterial isolates could be very much helpful in future research, related to microbial diversity and reduction in pollution level of global gases from environment.

MATERIAL AND METHODS

Isolation of Bacterial Strain by Enrichment Method The sediment and water samples were collected from different sites of Lonar Lake (Buldhana District in Maharashtra, India). The medium containing ingredient (g/l): $NaNO_3$ 2.5g, KCl 0.1g, KH_2PO_4 3g, K_2HPO_4 7g, $CaCl_2$ 0.01g, $MgSO_4$ 0.5g, $FeSO_4$ 0.116g, H_3BO_3 0.232g, $CuSO_4$ 0.41g, $MnSO_4$0.008g, $(NH_4)_6$ Mo_7O_{24}, 0.008g, and $ZnSO_4$ 0.174g, 20 ml methanol, is used for isolation of methytrophic bacteria (Haddad *et al*., 2009) The medium was then incubated at 37^0C on a rotary shaking incubator at 100 rpm for 3 days. The subculturing was made for 5 times for enrichment and isolation of pure culture was done on solid nutrient agar plate. Well isolated colonies were selected and stored at 4^0C as stock culture (Tambekar *et al*., 2010).

Identification of Isolates

The isolates were characterized by morphophysiological and cultural characteristics. The biochemical tests were performed by rapid detection kit (Hi-media, KB001 and KB009). The kit contents the tests namely, indol, methyl red, voges prauskar, citrate, lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, glucosamine, dulcitol, inositol, sorbitol, Rhamnose, cellobiose, melizitiose, á-methyl mannose, xylitol, ONPG, esculin, arabinose, citrate, malonate, sorbase, nitrate reduction, urea hydrolysis and starch hydrolysis. The molecular identifications were performed by 16S rRNA sequencing at NCCS, Pune.

Methanol Utilization Studies For estimation of methanol, the bacterial isolates were grown in the nutrient broth and incubated overnight at 37 ^{0}C. This 24 h old culture was inoculated in mineral salt medium containing 2% (v/v) methanol as a sole source of carbon and energy (Tambekar *et al*., 2013). Preliminary studies were carried out bacterial inoculums in medium containing 2% (v/v) methanol. The methanol utilization was determined by analyzing residual methanol at 480 nm in the medium after the each interval 24 h up from 0 h to 96 h by using UV- visible spectrophotometer (Zhan *et al*., 2010).

RESULTS AND DISCUSSION

Lonar Lake represents an extreme environment with high pH and moderate salinity. It is the only known depression in the region and hence may serve as a drain for excess runoff from anthropogenically influenced surrounding areas. However, the contribution of such natural or anthropogenic factors towards elevated phosphate and nitrate levels in the lake sediments warrants further investigation. Lonar Lake water is green throughout the year because of dense cyanobacterial bloom dominated by *Arthrospira* (Surakasi *et al*., 2007). Sediment and water samples were chosen as the source of bacterial isolation and these were enriched in minimal medium using 2% (v/v) methanol as sole source of carbon and energy for one month by repeated subculture after every 96 h.

Those bacterial isolates able to grow on medium containing 2% (v/v) methanol as carbon source were identified as methylotrophs and subsequently isolated in pure culture. The experimental outcome of morphological and biochemical characterization proved that all isolates are gram negative and identified as *Acenatobactor (*Table 1) and represented as ALP3.

These isolated were analysed for 16s rRNA and identified as *Acenatobactor*. The *Acenatobactor* have best potential to utilize methanol. These isolates were able to grow at temperature up to 42^0C.

In this investigation, a new method for the direct determination of methanol using sodium nitroprusside (SNP) is used (Zhan *et al*., 2010). It has been reported that SNP can react with nucleophilic agent such as primary and secondary aliphatic amine however; no studies in the literature to date have been reported on the reaction of SNP and alcohol. This experiment results showed that SNP can react with methanol to form colored product. Absorbance of product is linear with certain extent of the concentration of methanol.

These morpho-biochemically characterized bacteria were identified by 16S rRNA sequencing and phylogenetic tree was constructed. The result of the phylogeny showed that methylotrophic strains isolated from Lonar Lake were related to phylum Proteobacteria. According to 16S rRNA gene sequences, these isolates showed a high level of similarity with the genus *Acenatobactor*.

Table 1: Morphological, Cultural and Biochemical Characteristic of Bacteria Isolated From Lonar Lake

	Character		**Character**
ALP3	Culture Code	ALP3	Culture Code
Acenatactoobr	Bacterial isolates on the basis of 16S rRNA sequencing	*Acenatactoobr*	Bacterial isolates on the basis of 16S rRNA sequencing
-	Dulcitol	S	Source
-	Glycerol	W	Colony Colour
-	Salicin	WSCE	Colony Morphology
-	Glucosamine	-	Gram Reaction
-	Sodium gluconate	CB	Shape
-	Inositol	G	Arrangement
-	Sorbitol	-	Endospore
-	Mannitol	-	Motility
-	Adonitol	-	Catalase
-	Methyl d-glucoside	-	Oxidase
-	Ribose	-	Indol
-	Rhamnose	-	Methyl red
-	Cellibiose	-	Voges Praskaur
-	Melizitose	+	Citrate
-	Methyl d-mannose	-	Lactose
-	Xylitol	-	Xylose
+	ONPG	-	Maltose
-	Esculin	-	Fructose -
-	D-arabinose	+	Dextrose
-	Malonate	-	Galactose
-	Sorbose	-	Raffinose
-	Nitrate Reduction	-	Trehalose
-	Urease	-	Melibiose
14MM	Starch Hydrolysis	-	Sucrose
+	Growth at 6.5% NaCl	-	Larabinose
-	Growth at 4^0C	-	Mannose
+	Growth at 42^0C	-	Inulin
+	Growth at pH10		
Note: Sed-Sediment, W-Water, Gr-Green, Wh-white, I-Irregular, SR-Short rod, S-Single.			

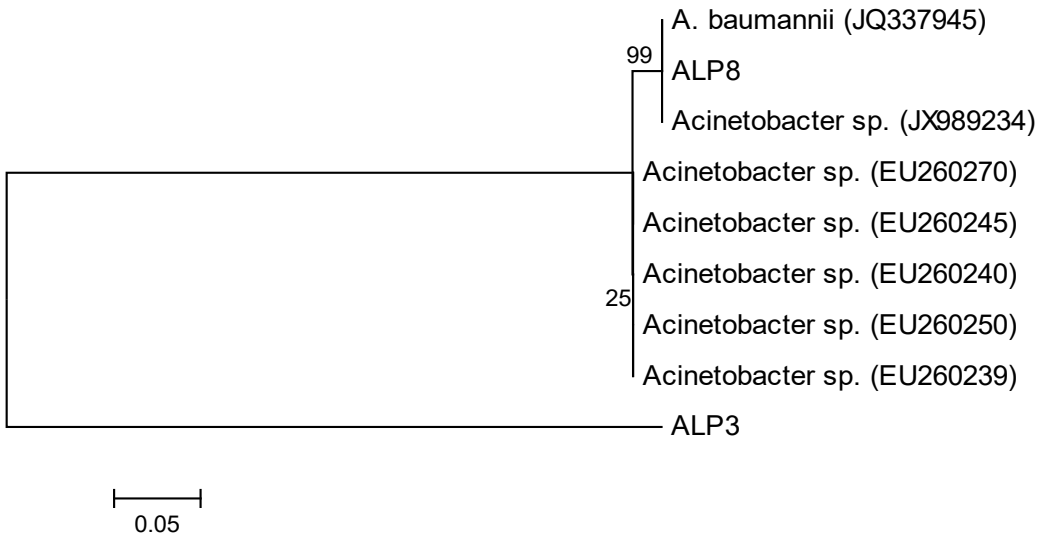

Figure 1: Phylogenic Tree of *Acenatobactor* Species Isolated from Lonar Lake : Phylogenetic tree for two methanotrophic bacteria isolated from Lonar Lake based on 16S rRNA gene comparisons and some of their closest phylogenetic relatives. The tree was constructed for the isolates ALP3 and ALP8. The phylogenic tree was constructed by neighbor-joining method. The number on the tree indicates the percentage of bootstrap sampling derived from 1,000 replications.

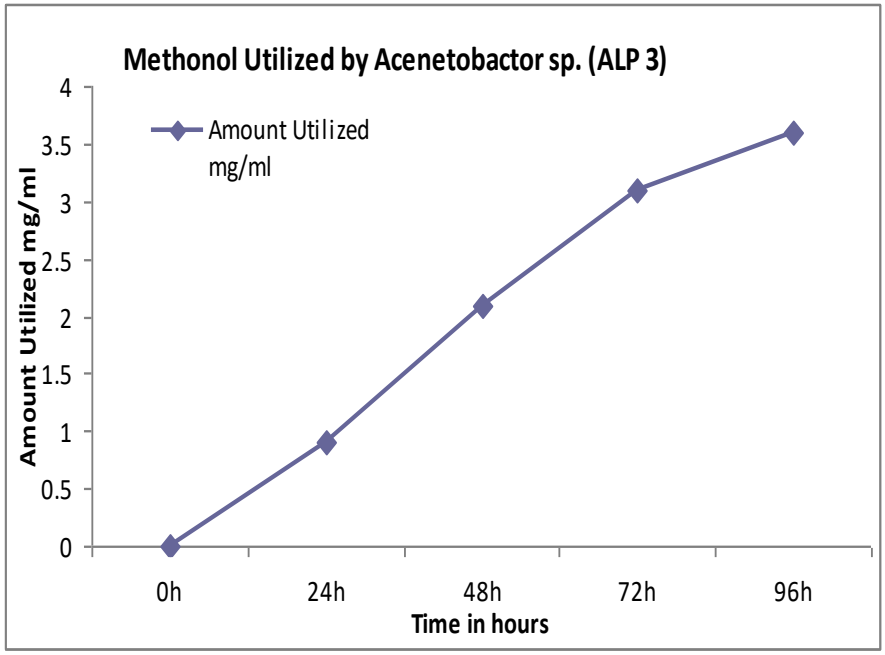

Figure 2: Percent Utilization of Methanol by *Acenatobactor* Species

Sediment is the potential source to isolate *Acenatobactor*. Antony *et al*, (2010) identified *methylmicrobium*, *methylophaga* and *Bacillus spp* as predominant methanotrophs from sediments of Lonar Lake. The utilization performances of selected strain were examined by spectrophotometric method. The experiment designed to find out the percent utilization and rate of utilization after 24h, 48h, 72h and 96h. In these studies, methanol estimated for 96h by spectroscopic method at each 24h time interval. Percent utilization and rate of degradation of methanol for these isolates was found, *Acinetobacter* Sps. indicated 72% methanol degradation at pH 7 and at 37°C temperature and showed 64% methanol degradation at 3% salt concentration. Experiment also showed that *Acenatobactor sp* ALP3 utilize methanol 0.9, 2.1, 3.1

and3.6 mg/mL after 24h time interval and rate of utilization of methanol was about 0.037 for isolates of *Acenatobactor sps* (Figure 2). The rate of utilization of methanol was also almost same for bacterial isolates Tambekar *et al*., (2011) reported that Methylotrophic bacteria not previously recorded bacterial species from Lonar Lake to utilize methanol as carbon source.

The findings of this study provide a window into the diversity of bacterial community members which are methane degrading from the Lonar Lake. These isolated bacterial species may be used to combat industrial pollution of methanol or to control global warming which may found better choice for studies like methane, methanol or toxic chemical degradation to combat Global warming. Till date several works are in progress to isolates efficient microbial strain that have ability to utilize methanol. We report here a new *Acenatobactor* (ALP3) was found to be a potential strain to utilize methanol as sole source of carbon and energy. This work has provided a useful guideline in evaluating potential methanol utilizer isolated from Lonar Lake.

CONCLUSION

The unexplored site of alkaline Lonar Lake contains many methanogenic and methylotrophic genera which might be helpful for the remediation of pollution environment. In present study isolation strategy for methylotrophic bacteria was used and potential methanol utilizing bacterial isolates of *Acenatobactor* were isolated which can be helpful for remediation of site with pollution of C1 compounds and provides a new unexplored site for researcher.

Acknowledgements

The authors would like to thank Commissioner Collegiate Education Telangana, Principal, Govt. Degree College, Jammikunta, for rendering financial assistance and providing necessary facilities.

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Special Issue A 12: March 2018:107-109
UGC Approved Journal No 48951

Original Article Open Access

Inhibition of STX Virulence Factor Biosynthesis in *Staphylococcus aureus* by Thyme EO.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Thool Vaishali U and Khobragade Kavita D (2018) Inhibition of STX Virulence Factor Biosynthesis in *Staphylococcus aureus* by Thyme EO., *Int. J. of. Life Sciences*, Special Issue, A12: 107-109

ABSTRACT

Staphylococcus aureus is a nuisance pathogen and STX is the central eponymous feature of *Staphylococcus aureus*. To counteract with this global problem a more natural approach is required as antibiotic resistance is developing with the passage of time. The aim of this study was to study the antibacterial effect of Thyme EOs (EO) against STX producing *Staphylococcus aureus* isolates from various clinical samples. Thyme EO was found to be effective against all the S.aureus isolates (n=25). Antistaphylococcal activity of thyme oil showed the maximum inhibition zone of 22 mm. More such EOs should be explored for counteracting the increase in growing antibiotic resistance by STX producing staphylococcus isolates.

Key words: Antistaphylococcal activity, Thyme oil, *S. aureus*.

INTRODUCTION

Some strains of *S. aureus* are competent of producing a virulence factor STX (STX) - a carotenoid pigment which gives the pathogen a characteristic golden color. STX are antioxidants in nature and the biosynthetic pathways leading to its synthesis have been known. The carotenoid pigment has the ability to douse the singlet oxygen and helps the microbe to survive killing with reactive singlet oxygen used by the host immune system (*Krinsky,* 1993) the golden pigment is responsible for neutrophil killing and promotes virulence through its antioxidant activity. The antioxidant action of STX helps the microbe evade death by reactive oxygen species produced by the host immune system. (*Clauditz et al.,* 2006). Many researchers are working on drugs to inhibit the bacterium's production of the STX and these drugs may also weaken the activity of STX and renew its susceptibility to antibiotics (*Liu et al., 2005).* Also, a drug developed in the context of cholesterol-lowering therapy has shown to block *S. aureus* pigmentation and disease progression in a mouse infection model. (*Liu et al., 2008). In this context EOs can be explored in different ways as Methicillin Resistant Staphylococcus aureus and Vancomysin Resistant Staphylococcus*

aureus are emerging in India (Thool et al., 2012). EOs are composed of volatile aromatic compounds with strong odor and are produced by plants as secondary metabolites (Bakkali *et al*., 2008). The use of EOs in medicines, perfumes, cosmetics and food preservatives is known from long. Different classes of compounds present in EOs include 1- Terpenes which include: monoterpene and sesquiterpenes, 2-Oxygenated compounds including phenols, alcohols, aldehydes and ketones,esters. Thymus species have been shown to have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities. *Thymus vulgaris* L. (thyme), locally known"zaatar" or "zaitra', a member of the family Lamiaceae, is widely used in Morocco folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants all over the world. *Thymus* species are commonly used as herbal tea, flavoring agents and as medicinal plant. Hence this study was carried out to assess the thyme oil for arresting the STX producing *S. aureus*.

Materials and Methods

Antimicrobial agents and chemicals: The following commercially available compounds were purchased from the indicated manufacturers: Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB) from Hi Media Laboratories, Mumbai, India. All standard chemicals were of analytical grade. For thyme oil, the compound (LR grade) was purchased from Burgyon Urbidges and Co., India.

Isolation of STX producing *S. aureus*: Clinical samples (pus, urine, catheters) were collected from various pathological laboratories of Gadchiroli district and inoculated into Tryptose soya broth. On inoculating the broth on Baird Parker Agar (BPA) and Mannitol Salt Agar (MSA) medium *S. aureus* were isolated and identified using morphological and cultural characteristics. Pigment production was studied by visual inspection of colonies grown on Nutrient Agar after 48 hrs of incubation.

Agar diffusion susceptibility testing : Thyme EO was assessed against all *S.aureus* strains (n=25). Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) plates by the Kirby Bauer disc diffusion method and according to Clinical and Laboratory Standards Institute (CLSI) guidelines. (Bauer *et al.,* 1966; CLSI. 2006).

RESULT AND DISCUSSION

Consequently, the yellow pigment plays the key to the ability of *S. aureus* to survive immune system attacks. It may act as an antioxidant which prevents CCl_4 induced toxicity in liver, kidney and testis in mice (Kurjogi *et al.,* 2010). Production of pigment is influenced by the *rsbUVWsigB* system (Morikawa *et al.,* 2001).

Table 1 Antistaphylococcal activity of Thyme EOs against STX virulent factor producing *S.aureus*

Sr. No.	Sample No.	Zone of inhibition in mm diameter
1	O1	15mm
2	O2	**16mm**
3	O3	08mm
4	O4	10mm
5	O5	12mm
6	O6	08mm
7	O7	10mm
8	O8	13mm
9	O9	12mm
10	W1	12mm
11	W2	15mm
12	W3	13mm
13	W4	12mm
14	W5	14mm
15	C1	09mm
16	C2	**20mm**
17	C3	15mm
18	C4	09mm
19	C5	**16mm**
20	Y1	06mm
21	Y2	07mm
22	Y3	10mm
23	Y4	12mm
24	Y5	10mm
25	Y6	09mm

Note: O – Orange color pigment, W- White color Pigment, C- Cream color Pigment, Y- Yellow Color Pigment

Katzif *et al.,* 2005 reported that pigment production is regulated by *CspA* through a *SigB* dependant mechanism. Lan *et al.,* 2010 indicates an intimate link between purine biosynthesis and oxidative phosphorylation for *in vivo* survival and pathogenesis of *S. aureus* and targeting purine biosynthesis is a promising strategy to develop anti *S. aureus* therapies. In this present study on Nutrient Agar pigment

production was seen after 48 hrs and 09 (36 %) orange colonies, 05 (20%) each of white and cream colonies and 06 (24%) Yellow colonies were noted. In the present study orange and cream pigment producing *S. aureus* isolates were more sensitive to *Thymus vulgaris* EO than other isolates. Zarringhalam *et al.,* 2013 indicated that Thyme EO can play a significant role in inhibition of *Escherichia coli O157: H7* and *Staphylococcus aureus.* Semeniuc *et al.,* 2017 stated that thyme EO exhibited strong antibacterial activity against *E.coli*, moderate against *S. typhimurium* and *B.*cereus, and mild inhibitory effects against *P. aeruginosa* and *S. aureus,* Combinations of lovage/thyme and basil/thyme EOs displayed antagonistic effects against all bacteria, parsley/thyme EOs against *B. cereus*, *S. aureus*, *P. aeruginosa*, and *E. coli*.

CONCLUSION

From this study it is concluded that Thyme oil was effective against STX pigment producing organism. In future drugs like thyme EO in combination with other drugs designed may be used to block the production of the STX and further may increase oxidant sensitivity and decrease whole-blood survival. This study offers a novel therapeutic approach in the treatment of complicated *S. aureus* infections.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:110-112
UGC Approved Journal No 48951

Original Article Open Access

Effect of antistaphylococcal activity of various essential oils against STX pigment producing *Staphylococcus aureus.*

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ABSTRACT

In the background of managing staphylococcal infections, health departments are facing a slow down to curb the antibiotic menace. STX pigment promotes resistance to reactive oxygen species and host neutrophil-based killing and is a hallmark phenotype in virulence and is absent in the mutant or less virulent counterpart. Hence the aim of this study was to study the antibacterial effects of essential oils (EOs) against STX producing Staphylococcus aureus isolates from various clinical samples. Comparative activities of seven different essential oils were recorded. Thyme EOwas found to be most effective against most of the *S.aureus* isolates. All the *S.aureus* isolates (n=25) exhibit sensitivity to all the essential oils, however clove oil and thyme oil showed excellent antibacterial activity. The use of essential oils may prevent the development of infections and will minimize antibiotic use, prevent development of resistance as well as promote healing.

Key words: Antibiotic resistance, Essential oils, STX producing *S.aureus*

INTRODUCTION

Staphylococcus aureus has a virulence factor which produces an array of diseases in humans. Antibiotics are fight against these virulent factor hence it is exact tools to human. The golden color characteristic *S.aureus* strains are producing a virulence factor Staphyloxanthin (STX)-a carotenoid pigment which gives pathogen a golden colour. STX are antioxidants in nature and the biosynthetic pathways leading to its synthesis have been known. The carotenoid pigment has the ability to douse the singlet oxygen and helps the microbe to survive killing with reactive singlet oxygen used by the host immune system (*Krinsky,* 1993) the golden pigment is responsible for neutrophil killing and promotes virulence through its antioxidant activity. The production of pigment increased pathogenesis and impairing neutrophil killing due to associated with harsh environmental conditions. Essential Oil (EO) which excreted from plant extract has been used for therapeutic purposes.

In recent years, much research has been devoted to investigating such plant extracts. EO contains main constituent that is terpenoids which are highly complex. Terpenoids have been found to show antimicrobial activity against *S. aureus* and other bacteria. Increasing in popularity as consumers seek to utilize more "natural" means to stay healthy and treat disease by use of essential oils which promote health and wellness. Consumers are inhaling these oils through the use of in-home diffusers and applying the oils cutaneously to treat a variety of ailments from anxiety to wounds. Several studies have indicated the effectiveness of essential oils against a wide variety of microbial agents.

Plant derived essential oils having numerous studies about discovery of promising novel antimicrobial candidates. In previous studies, the antimicrobial activities of other EOs have also been investigated, and their actions against various pathogens, including clinical Methicillin resistance *S. aureus* (MRSA) isolates, have been demonstrated (Cox *et al.,* 1998). Also, research data indicate that antimicrobial activity was present in many essential oils.

METHODOLOGY

Antimicrobial agents and chemicals:
In the present work numerous commercial chemicals and media were purchases from indicating manufacturer, Media like Nutrient agar, Nutrient broth, Muller Hinton agar, Baired parkar Agar, Manitol Salt Agar from Himedia Company Mumbai, India. Essential oils like Thyme oil, Clove Oil and Lemon Oil (LR grade) from Burgoyn Urbidges & Co, India. Whereas Eucalyptus oil, Sesame oil, Castor oil and Mustard oil purchased from medical shop at Gadchiroli, Maharashtra India.

Tested Bacterial Strain:
Test bacterial strain i.e., *S. aureus* was isolated from various clinical sample collected from hospital & pathological laboratories of Gadchiroli, Maharashtra. These samples inoculated into tryptose soya broth and then culture sample transfer to the Baired parkar agar and Manitol salt agar to get the colonies of *S. aureus.* These colonies transfer over nutrient agar media & incubate for 24 hr it get the golden, orange, cream & white color colonies. 25 (n=25) isolates were taken for antibacterial activity against essential oils. All isolates were identified by standard protocol.

Agar diffusion susceptibility testing:
All *Staphylococcus aureus* isolates (n=25) were individually tested against above 7 essential oils by using Kirby Bauer disc diffusion method and record the values of inhibition zone. Lawn of *S. aureus* culture was swabbed onto Muller Hinton Agar plates. Filter paper discs soaked in different EO was placed on inoculated plates and incubated for 24 hrs. Values of diemeter of inhibition zones were recorded.

RESULT AND DISCUSSION

As evident from table no. 1, all the seven essential oils were effective against the *S. aureus* which was a produces a virulent factor that causes diseases to human beings. Among 7 EO, Thyme & Clove oil was showing the highest zone of inhibition it means that Thyme & Clove oil was highly effective against virulent factor of *Staphylococcal aureus.* Thyme & Clove oil was showing the 20mm inhibitory zone against C2 isolates and 16mm inhibitory zone against C3 isolates respectively. Then Mustard oil and Castrol oil were showing resistance against these isolates and Lemon oil, Eucalyptus oil & sesame oil were showing little effect against these isolates.

In previous reports antimicrobial properties of EOs and their components have been reviewed extensively (Burt 2004). However, only a few studies have reported the mechanism of antibacterial action of EOs in great detail (Cox et al. 1998, Cox et al. 2000, Fisher and Phillips 2006). It is concluded that recent efforts have targeted virulence factors rather than Essential gene functions (Hung *et al.,* 2005). STX of *S.aureus* is a virulence factor for the organism (Song *et al.,* 2009). The golden-colored pigment is a typical secondary metabolite that is not essential for growth an reproduction of the pathogen (Liu *et al.,* 2005) but might aid invasiveness in vivo (Pelz *et al.,* 2005). The STX (VF) producing organism was isolated from clinical sample & treated with various EO for antistaphylococcal purposes.

In present work it was concluded that all EO (Thyme oil, Clove oil, Lemon oil, Eucalyptus oil, Sesame oil, Castor oil & Mustered Oil) which extracted from plant were effective against the STX virulent factor producing *Staphylococcal aureus*. Thyme oil was highly effective against *S.aureus* as compared to clove oil , mustered oil and castrol oil. Sesame oil, eucalyptus & lemon oil were not effective against STX producing *S.aureus.*

Table No.1 Antistaphylococcal activity of various essential oils against STX virulent factor of *S.aureus*

Sr. no.	Sample No.	Clinical Sample	Clove Oil	Thyme Oil	Lemon Oil	Eucalyptus oil	Sesame oil	Mustered Oil	Castrol oil
1	O1	Pus	10mm	15mm	9mm	10mm	4mm	10mm	11mm
2	O2	Pus	12mm	16mm	8mm	9mm	3mm	9mm	10mm
3	O3	Pus	9mm	8mm	7mm	7mm	4mm	7mm	8mm
4	O4	Pus	13mm	10mm	5mm	6mm	4mm	8mm	7mm
5	O5	Pus	11mm	12mm	6mm	9mm	5mm	10mm	10mm
6	O6	Urine	6mm	8mm	4mm	5mm	2mm	6mm	11mm
7	O7	Urine	8mm	10mm	5mm	8mm	3mm	9mm	12mm
8	O8	Urine	10mm	13mm	7mm	10mm	5mm	8mm	6mm
9	O9	Urine	7mm	12mm	5mm	7mm	3mm	10mm	9mm
10	W1	Pus	10mm	12mm	8mm	9mm	4mm	12mm	13mm
11	W2	Pus	9mm	15mm	7mm	10mm	3mm	8mm	10mm
12	W3	Pus	10mm	13mm	6mm	11mm	5mm	9mm	11mm
13	W4	Pus	13mm	12mm	10mm	12mm	6mm	11mm	12mm
14	W5	Pus	11mm	14mm	7mm	10mm	3mm	12mm	8mm
15	C1	Urine	2mm	9mm	6mm	8mm	2mm	6mm	5mm
16	C2	Pus	10mm	20mm	6mm	12mm	3mm	13mm	10mm
17	C3	Pus	16mm	15mm	5mm	13mm	8mm	12mm	11mm
18	C4	Urine	15mm	9mm	8mm	11mm	3mm	10mm	9mm
19	C5	Urine	13mm	16mm	8mm	13mm	5mm	9mm	6mm
20	Y1	Urine	9mm	6mm	4mm	6mm	4mm	5mm	7mm
21	Y2	Urine	10mm	7mm	3mm	5mm	3mm	9mm	12mm
22	Y3	Urine	7mm	10mm	7mm	5mm	2mm	4mm	10mm
23	Y4	Urine	8mm	12mm	6mm	10mm	3mm	9mm	11mm
24	Y5	Urine	9mm	10mm	4mm	7mm	4mm	8mm	6mm
25	Y6	Pus	10mm	9mm	6mm	6mm	3mm	7mm	7mm

Note: O – Orange color pigment, W- White color Pigment, C- Cream color Pigment,Y- Yellow Color Pigment

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:113-122
UGC Approved Journal No 48951

Original Article | Open Access

Induction of Mutation by Gamma Irradiation in *Brassica comprestris L.*

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Awaley Mundeep G (2018) Induction of Mutation by Gamma Irradiation in *Brassica comprestris L*, *Int. J. of. Life Sciences*, Special Issue, A12: 113-122.

ABSTRACT

Pure line seeds of local variety of *Brassica compestris L*. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of these variety were procured from Krishi Viggyan Kendra, Nagpur. This variety well adapted to the agro climatic conditions. The seed of mustard were treated w ith different doses/treatment of physical mutagens. The physical mutagens used were gamma rays. Uniform healthy dry seeds (10% moisture content) of the mustard were exposed to different doses of gamma rays (10GY, 20GY, 30GY, 40GY, 50GY, 60GY, 70GY, 80GY, 90GY and 100GY) with a dose rate of 20Kr/20min. from 60 cobalt source at the Deparment of chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur**.** The mutagenic effect studied on M1 parameters included seed germination, Seedling height, plant survival, and various Quantitative traits. Seed germination, seedling growth, plant survival Increased with an increase in mutagenic treatment. Gamma rays proved to be most effective in causing maximum biological damage. Studies on various quantitative parameters showed the inhibitory effect of Lower treatments and stimulatory effect of Higher or intermediate treatments in M1 generation. The mean values for various quantitative traits Increased at higher treatments, but Inhibitory effects were noticed at some lower treatments. A significant amount of variability was induced in the treated populations as compared to Control. 70 Gy, 90Gy and 100 Gy treatments of gamma irradiation was found to be most effective.

Key words: *Brassica Compenstris L., Gamma rays, Physical mutagen, Quantitative trait.*

INTRODUCTION

The BRASSICACEAE or Cruciferae (also known as Mustered Family) is a large angiosperm (Flowering Plant) dicot family of Plant kingdom which belongs to the order Brassicales and has been divided into 10-19 tribes with a total of 338- 360 genera and 3,709 species.

The Brassicaceae are easily recognized by having unique flowers with four petals, forming a cross or some time reduced or lacking; six stamens, the outer being shorter than the inner four (however sometimes only two or four stamens are present) and capsule (having two valves capsule with a septum dividing it include two chambers). The plant family Brassicaceae includes several plant species of great scientific, economic and agronomic importance including model species (*Arabidopsis* and *Brassica*), developing model generic system (*Boechera, Brassica* and *Cardamine*), as well as many widely cultivated species.

The genus is native in the wild in western Europe, the Mediterranean and temperate regions of Asia and many wild species grow as weeds, especially in North America, South America, and Australia.

A dislike for cabbage or broccoli can result from the fact that these plants contain a compound similar to phenylthiocarbamide (PTC), which is bitter or tasteless to some people depending on their 'taste buds'.

The main objectives of the present study are:

1. To study the effect of different treatments on various biological parameters.
2. To investigate the chromosome behavior of treated populations with respect to controls.
3. To quantify the magnitude of the genetic variability induced in various quantitative traits.
4. To isolate promising mutants based on changes in phenotypic traits.

The present study was conducted in the Experimental Field of Botany Department, of Bhawabhuti Mahavidyalaya, Amgaon. Dry seeds of *Brassica comprestris L*. were irradiated with different doses of gamma rays (00, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Krad) form a ^{60}Co gamma chamber at RTM University Nagpur Chemistry Department. Irradiated seeds along with control were sown in earthen pots of equal size. The pots were maintained in the field in a Complete Randomized Design (CRD), each treatment being replicated four times. Growth parameters such as days to germination, days to completion of germination, germination percentage, survival percentage, shoot length, root length, number of leaves, number of branches were recorded at different periods after sowing was done. Collected data was subjected to Analysis of Variance technique (Fisher, 1985) and LSD test at 5% probability (Steel & Torrie, 1980).

Gamma radiation, also known as gamma rays, and denoted by the Greek letter γ, refers to electromagnetic radiation of an extremely high frequency and are therefore high-energy photons. Gamma rays are ionizing radiation, and are thus biologically hazardous. They are classically produced by the decay of atomic nuclei as they transition from a high energy state to a lower state known as gamma decay, but may also be produced by other processes. Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900, while studying radiation emitted from radium. Villard's radiation was named "gamma rays" by Ernest Rutherford in 1903.

Natural sources of gamma rays on Earth include gamma decay from naturally occurring radioisotopes, and secondary radiation from atmospheric interactions with cosmic ray particles. Rare terrestrial natural sources produce gamma rays that are not of a nuclear origin, such as lightning strikes and terrestrial gamma-ray flashes. Additionally, gamma rays are produced by a number of astronomical processes in which very high-energy electrons are produced, that in turn cause secondary gamma rays. However, a large fraction of such astronomical gamma rays are screened by Earth's atmosphere and can only be detected by spacecraft.

Gamma rays typically have frequencies above 10 exahertz (or $>10^{19}$ Hz), and therefore have energies above 100 keV and wavelengths less than 10 pico meters (10^{-12} meter), which is less than the diameter of an atom. However, this is not a hard and fast definition, but rather only a rule-of-thumb description for natural processes. Electromagnetic radiation from radioactive decay of atomic nuclei is referred to as "gamma rays" no matter its energy, so that there is no lower limit to gamma energy derived from radioactive decay. This radiation commonly has energy of a few hundred keV, and almost always less than 10 MeV. In astronomy, gamma rays are defined by their energy, and no production process needs to be specified. The energies of gamma rays from astronomical sources range to over 10 TeV, an energy far too large to result from radioac-tive decay. A notable example is extremely powerful bursts of high-energy radiation referred to as long duration gamma-ray bursts, of energies higher than can be produced by radioactive decay. These bursts of gamma rays, thought to be due to the collapse of stars called hypernovae, are the most powerful events so far discovered in the cosmos.

Mustard (*Brasicca comprestris L.*) commonly known as Mohari (Rahi) is a well known economic herb of the

family Cruciferae. The young plants serve as vegetable for human consumption seeds as a spice or as herbal medicine (Petropoulos, 2002). Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal aberrations in the biological material in M1 generation (Gaul, 1970). Gamma rays, an energetic form of electromagnetic radiations are known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002). Ethyl methane sulphonate (EMS), a chemical mutagen of the alkylating group has been reported to be the most effective and powerful mutagen and usually causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Van Harten, 1998; Khatri *et al*., 2005). Sodium azide (NaN3) is the least dangerous and the most efficient mutagen and has been reported to be mutagenic in several crop species (Adamu and Aliyu, 2007; Mostafa, 2011). The mutagenicity of sodium azide is arbitrated through the formation of an organic metabolite which enters the nucleus, interacts with DNA and generates point mutations in the genome. According to Nilan *et al* (1977), SA is relatively safe to handle, inexpensive and noncarcinogenic as compared to other mutagens. In mutation breeding studies, it is important to determine a suitable dose/ concentration of mutagen for a crop plant which can be employed for inducing maximum variability through point mutations. Seed germination, seedling growth, pollen sterility and chromosomal aberration are the commonly used criteria for studying radio-sensitivity in plants (Kon *et al*., 2007; Lal *et al*., 2009; Sangle *et al*., 2011; Sheikh *et al*., 2012). The aim of present study was to determine the response of mustard seeds to gamma rays based on germination and survival percentage, root-shoot length, and pollen fertility with the main aim of identifying appropriate dose/conc.

Plant Morphology is considered to be an important tool for isolation of desirable Mutants. Several induced Morphological Mutations have been reported in literature showing alterations in the Morphology of various plant parts. Raw and Jama (1976) Subjected the seed of Black Gram (phaseolus Mungo) to X-rays & EMS treatments with the objective for obtaining some promising mutants. The induced leaf mutants scored comprised of Crinkled leaf and waxy leaf narrow leaf and unifoliate mutants. Crinkled leaf and waxy leaf mutants had normal fertility and vitality and whereas the narrow leaf mutant was partially sterile and the unifoliate and extreme dwarf mutant was also isolated which was completely sterile. Chandra and Tewari (1978) in bean (Phaseolus aureus) var. Pusa Baisakhi observed that increasing doses of gamma rays and neutons caused a gradual reduction in germination of seeds. Irradiation caused the appearance of leaf abnormallities including unifoliate, bifoliate, trifoliate, tetrafoliate and pentafoliate characters. Under the influence of neutrons both tetra and pentafoliate leaves were observed on the same plant of cv. S-8 apparently associated with enhanced luxuriance of plants which resulted in enhanced pod formation. Moh (1972) induced variations in seed coat colour of some black bean (*Phaseolus vulgaris*) varieties of Latin America. Though the varieties under improvement were disease resistant and good yielding yet were considered inferior because of their seed coat colour. The seeds were treated with EMS and gamma rays and a special screening technique was employed in which seed coat colour mutants were correlated with green hypocotyls colour, for isolation of the potential mutants at a very early stage of seeding development. Mutagenesis resulted in inducing some seed coat colour mutants who varied from white, yellow to various degrees of brown and their seed coad colour was associated with a change in hypocotyl colour from red to green. All these mutnts were bearing white flowers instead of red in the parents but their morphology, growth habit and disease resistance wre similar to that of the parents. Further studies revealed that these induced characters were recessive and their inheritance followed a simple Mendalian manner. Kaul and Chaudhary (1972) conducted mutagenic studies in Atropa belladonna after exposing its seeds to different doses of gamma rays. Studies were aimed at assessing the variability in polygenic character released in M1 generations of belladonna. A higher variability was noted in than in M1. After observing a greater variability for tiller number and alkaloid content than that for plant height and leaf length, it was inferred that different characters may respond differently to different mutagenic treatments.

Mouli and Patil (1976) subjected peanuts (*Arachis hypogea*) to gamma irradiation. They isolated a suppressed branched mutant with larger leaves, altered flowering pattern, reduced shelling, smaller kernels and branch length as compared to normal in the autumn and spring s growing seasons respectively. An extremely poor pod shelling was observed in autumn grown plants as compared to spring grown ones. Narsinghani and kumar (1976) in a mutations breeding programme

subjected the seed of Cowpea (*Vigna Sinensis L*.) to EMS and MMS treatments. In M1 generations, reduction in survival percentage, mean pod number, seed yield per plant and average pollen fertility was observed. Gamma ray induced morphological mutationhave also been 27 reported by Morishita (2001) in Buckwheat and by Tah (2006) in Mutabean. Kumar *et al*. (2003) reported several viable mutants induced by gamma rays in Lima bean (*Phaseolus Lunatus L*.) which included earliness, erect plants, profuse flowering and high yielding mutants. Wani (2011) reported a series of morphological mutants in chickpea isolated in separate and combined treatments of gamma rays and EMS. The Various types of mutants reported included plant height, leaf, pod and seed mutants. Combination treatments in general were found more effective and efficient in inducing various types of morphological mutants.

METHODOLOGY

Variety used: Pure line seeds of local variety of *Brassica compestris L*. were used in the present study. The certified, healthy and dry seeds (10% moisture content) of thise variety were procured from Krishi Viggyan Kendra, Nagpur. This variety well adapted to the agro climatic conditions.

Mutagens used: The seed of mustard were treated with different doses/treatment of physical mutagens. The physical mutagens used were gamma rays.

Gamma rays: Uniform healthy dry seeds (10% moisture content) of the mustard were exposed to different doses of gamma rays (10GY, 20GY, 30GY, 40GY, 50GY, 60GY, 70GY, 80GY, 90GY and 100GY) with a dose rate of 20Kr/20min. from 60 cobalt source at the Deparment of chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur**.**

Method of treatment with physical Mutagen: Prior to the mutagenic treatment, the seeds were choose and passes through the gamma rays in different doses of Kr.

Sample size: A set of 150 seeds were chosen for each dose / treatment including the control. Out of these 150 seeds, 10 seeds for each treatment and control were shown in the field for morphological and cytological studies, Whereas the remaining set of seeds was allowed to germinate on a moist cotton in petriplates for measuring Root- shoot length.

Table 1: Details of Mutagenic Treatment given to mustard seeds

Mutagen used	Dose / Concentration	Duration of presoaking (Hrs.)	Duration of Treatment (Hrs)
Control	DDW	12.0	-
Gamma rays (GY)	10	-	-
	20	-	-
	30	-	-
	40	-	-
	50	-	-
	60	-	-
	70	-	-
	80	-	-
	90	-	-
	100	-	-

Sowing of seeds in the field: Nursery beds were prepared for sowing seeds and raising M1 generation. In January, 2015, the treated as well as un treated (control) seeds were shown in three replicates in a complete Randomized Block Design (CRBD) at the Bhawabhuti Mahavidyalaya, Amgaon. The distance between the seeds along a row was kept 5cm whereas row to row distance was maintained at 10cm in each experimental plot in a replication.

Mechanism of action of physical mutagens

a) Physical mutagens: Gamma rays are the most energetic form of electromagnetic radiation, possessing the energy level from 10 kilo electron volts (kev) to several hundred kev, and they aare considered the most penetrating in comparison to other radiation such as alpha and beta rays (Kovacs and Keresztes, 2002).

Gamma rays typically have frequencies above 10 exahe-rtz (or $>10^{19}$ Hz), and therefore have energies above 100 keV and wavelengths less than 10 picometers (10^{-12} meter), which is less than the diameter of an atom. However, this is not a hard and fast definition, but rather only a rule-of-thumb description for natural processes. Electromagnetic radiation from radioactive decay of atomic nuclei is referred to as "gamma rays" no matter its energy, so that there is no lower limit to gamma energy derived from radioactive decay. This radiation commonly has energy of a few hundred keV, and almost always less than 10 MeV. In astronomy, gamma rays are defined by their energy, and no production process needs to be specified. The energies of gamma rays from astronomical sources range to over 10 TeV, an energy far too large to result from radioactive decay. A notable

example is extremely powerful bursts of high-energy radiation referred to as long duration gamma-ray bursts, of energies higher than can be produced by radioactive decay. These bursts of gamma rays, thought to be due to the collapse of stars called hypernovae, are the most powerful events so far discovered in the cosmos.

The first gamma ray source to be discovered historically was the radioactive decay process called gamma decay. In this type of decay, an excited nucleus emits a gamma ray almost immediately upon formation (it is now under -stood that a nuclear isomeric transition, however, can produce inhibited gamma decay with a measurable and much longer half-life). Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900, while studying radiation emitted from radium. Villard knew that his described radiation was more powerful than previously described types of rays from radium, which included beta rays, first noted as "radioactivity" by Henri (1896), and alpha rays, discovered as a less penetrating form of radiation by Rutherford, in 1899. However, Villard did not consider naming them as a different fundamental type. Villard's radiation was recognized as being of a type fundamentally different from previously named rays, by Ernest Rutherford, who in 1903 named Villard's rays "gamma rays" by analogy with the beta and alpha rays that Rutherford had differentiated in 1899. The "rays" emitted by radioactive elements were named in order of their power to penetrate various materials, using the first three letters of the Greek alphabet: alpha rays as the least penetrating, followed by beta rays, followed by gamma rays as the most penetrating. Rutherford also noted that gamma rays were not deflected (or at least, not easily deflected) by a magnetic field, another property making them unlike alpha and beta rays.

Gamma rays were first thought to be particles with mass, like alpha and beta rays. Rutherford initially believed they might be extremely fast beta particles, but their failure to be deflected by a magnetic field indicated they had no charge. In 1914, gamma rays were observed to be reflected from crystal surfaces, proving they were electr-omagnetic radiation. Rutherford and his coworker Edward Andrade measured the wavelengths of gamma rays from radium, and found that they were similar to X-rays but with shorter wavelengths and (thus) higher frequency. This was eventually recognized as giving them also more energy per photon, as soon as the latter term became generally accepted. A gamma decay was then understood to usually emit a single gamma photon.

RESULT

Evaluation of M1 generation

Seed germination: The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Seedling height (cm): Seedling height was estimated on 10 th day of germination by measuring root and shoot length of randomly selected seedling from each treatment as well as control. Seedling injury as measured by the reduction in root and shoot length and calculate in terms of percentage of root and shoot injury.

$$\text{Percent injury} = \frac{\text{Control- Treated}}{\text{Control}} \times 100$$

Plant survival: The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{No. of plants at maturity}}{\text{No. of seeds germinated}} \times 100$$

$$\text{Lethality (\%)} = \frac{\text{Control- Treated}}{\text{Control}} \times 100$$

Quantitative characters of M1 generation: The following morphological parameters were recorded in M1 generation .

1. Plant height (cm): The height of 15 randomly selected plants was measured from the point above the ground to the tip of the main axis of the plant.

2. Number of pods per plant: Total number of pods per plant for a selected number of 15 plants from each concentration including control was recorded.

3. Length of pods per plant (cm): The length of five pods per plant from 15 randomly selected plants in each treatment including control was recorded.

4. Number of seeds per pod: Five pods per plant from 15 randomly selected plants in each treatment were used to calculate the mean seeds per pod.

5. Seed yield per plant: Randomly selected 15plants per treatment were used for calculating the mean seed yield per plant.

Figure 1: Seed germination of Control & Different Doses of Gamma rays

Figure 1 Sowing of Seeds in the Seed Bed

Figure 3 Sowing of Seeds in the Seed Bed

Figure 4 Seed Germination and Seedling Height

Figure 5 Seed Germination and Seedling Height

Control **Treated**

Control **Treated**

STUDIES IN M1 GENERATION:

The mutagenic effects of gamma rays were studied on seed germination, seedling height, plant survival, various quantitative characters in M1 generation of *Brassica compestris L.*

Seed germination:

Germination percentage was found to be significantly reduced in all the mutagenic treatments Except 50 Gy. The maximum inhibition in germination was recorded at 80 Gy treatments. Seed germination was about 100% in control. In gamma rays it ranged from 90% (10Gy) to 60% (80Gy). The percentage of seed germination was found 100% in 50Gy treatment of gamma irradiation.

Plant survival:

Plant survival was higher in control (95%) than in all the ten mutagenic treatments (Table 3). Plant survival tended to decrease with the increase in the dose/concentration of mutagens are follows:

The plant survival was 55.22, 33.33, 87.5, 22.22, 90.00, 44.44, 87.50, 99.00, 88.88, and 85.71 percent at 10Gy, 20 Gy, 30 Gy, 40Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy,90 Gy, and 100 Gy treatment of Gamma irradiation respectively against 95 % in control. The maximum plant survival was found in 50Gy and 80Gy treatment of gamma irradiation.

Table 2: Seed germination

Treatment	No. of seeds sowing	No. of seeds germinate
Control	10	10
Gamma rays		
10	10	09
20	10	09
30	10	08
40	10	09
50	10	10
60	10	09
70	10	08
80	10	06
90	10	09
100	10	07

Table 3: Effect of gamma rays survival and pollen fertility in M1 generation in *Brassica compestris L.*

Treatments	Germination (%)	Plant survival (%)	Lethality (L)
Control	100	95.00	60.00
Gamma rays (Gy)			
10	90	55.55	44.45
20	90	33.33	66.67
30	80	87.5	12.5
40	90	22.22	77.78
50	100	90.00	10
60	90	44.44	55.56
70	80	87.50	12.5
80	60	90.00	10
90	90	88.88	11.12
100	70	85.71	14.29
Mean	**85.45**	**67.96**	**33.17**

Table-4 Effect of various doses (Kr) of gamma irradiation on shoot length (cm), root length(cm), number of branches/plant, number of pods

Doses /Treatment	Shoot length (cm)	Root length (cm)	No. of branches/ plant	No. of pods/ plant
(Control) 00	17	7	2	-
Gamma rays - 10	38	18	3	-
20	17	9	1	-
30	26	10	3	8
40	23	20	2	12
50	75	10	4	10
60	39.5	11	5	14
70	84	17	8	17
80	51.5	13	5	18
90	61.5	18	4	10
100	91	13	6	30

Seedling height (Shoot length): Data recorded on seedling height measured in terms of root + shoot length is presented in Table 4. It is evident from that seedling height Increases with an increase in dose / concentration of mutagens. The shoot length was 38 cm, 17cm, 26cm, 23cm, 75cm, 39.5cm, 84cm, 51.5cm, 61.5cm and 91cm at 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy, and 100 Gy treatment of gamma irradiation respectively against 17cm in control. The maximum Shoot length was found in 100Gy treatment of Gamma irradiation.

Seedling height (root length): The root length was 18 cm, 9cm, 10cm, 20cm, 10cm, 11cm, 17cm, 13cm, 18cm and 13cm at 10Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy, 80 Gy, 90 Gy, and 100 Gy treatment of gamma irradiation respectively against 7cm in control. The maximum root length was found in 40Gy treatment of Gamma irradiation. Mean squares form Table-4 that the differences for various treatment of gamma irradiation were not able to reach the level of significance. Increase in higher germination percentage at higher doses might be due to their stimulating effects on activating RNA synthesis or protein synthesis (Kuzin *et al*., 1975, 1976) or it could be due to the elimination of germinating bacterial populations, their spores and mould fungi (Gruner *et al*., 1992)

Higher exposures of gamma rays may cause injury in seeds (Mehetre *et al*., 1994) and usually shows

inhibitory effects on seeds of angiosperms and gymnosperms (Akhaury and Singh 1993; Thapa, 1999). These results are in line with Sahrif *et al*., (2000), Din *et al*., (2003) and Kon *et al*., (2007).

Shoot and root length: Effect of gamma rays on shoot and root length in this study was adverse and inhibitory as is evident from data in Table-4 Seeds exposed to higher doses produced Tall plants with long roots. This effect of gamma rays on shoot and root length of plants was more pronounced at 70 Kr to 100 Kr (Table-4). Shakoor *et al*., (1978) and Khalil *et al*., (1986) attributed decreased shoot and root lengths at higher doses of gamma rays to reduced mitotic activity in meristematic tissues and reduced moisture contents in seeds respectively. Decrease in shoot and root lengths of a number of crops has been reported by Thimmaiah *et al*., (1998), Muhammad and Afsari, (2001), Al-Salhi *et al*., (2004), Token *et al*., (2005), Kon *et al*., (2007).

Number of Pods and number of branches: Both number of branches and number of pods were highly significantly increased by radiation doses (Table-4). LSD values showed that maximum means were observed 100 Kr (control) and minimum values for number of branches and number of leaves were recorded at control (Table-3).

CONCLUSIONS

The present investigation was conducted to study the mutagenic effect of gamma rays, in the local variety of Mustard. The main objective of the study was to induce the genetic variability in quantitative traits and to isolate the promising mutants associated with increase in yield potential of the crop. The significant findings are summarized as follows:

The mutagenic effect studied on M1 parameters included seed germination, Seedling height, plant survival, and various Quantitative traits.

a) Seed germination, seedling growth, plant survival Increased with an increase in mutagenic treatment.

c) Gamma rays proved to be most effective in causing maximum biological damage.

d) Studies on various quantitative parameters showed the inhibitory effect of Lower treatments and stimulatory effect of Higher or intermediate treatments in M1 generation.

e) The mean values for various quantitative traits Increased at higher treatments, but Inhibitory effects were noticed at some lower treatments.

f) A significant amount of variability was induced in the treated populations as compared to Control.

h) 70 Gy, 90Gy and 100 Gy treatments of gamma irradiation was found to be most effective.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:123-134
UGC Approved Journal No 48951

Original Article Open Access

Pollen diversity in summer honey samples of forest area in Gadchiroli district, India

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Margonwar AS and Kalkar SA (2018) Pollen diversity in summer honey samples of forest area in Gadchiroli district, India, *Int. J. of. Life Sciences*, Special Issue, A12: 123-134.

ABSTRACT

Melisssopalynological analyses are useful in enhancement of bee keeping industries. Pollen analysis of the nectar sources of *Apis dorsata* and *Apis florea* during summer honey samples from forest area of Gadchiroli district was carried out. 50 samples were collected during summer 2005 to 2009. Pollen grains were identified from reference slides, 18 samples were unifloral and 32 were multifloral. A total 72 pollen types were classified and they were representing 29 families. In the study major pollen types were recorded viz. *Ageratum conyzoides, Syzygium cumin, Eucalyptus globulus, Terminalia arjuna, Tridax procumbens, Pongamia pinnata* etc. The data reflect the floral situation of the place where particular honey was produced and identification of geographical origin based on the presence of combination of pollen types of the particular area and this area is suitable for establishing apiary industries.

Key word: pollen, Honey, Geographical origin, Gadchiroli.

INTRODUCTION

Gadchiroli District is located on the North- Eastern side of the Maharastra State. It is situated between 18.43' to 21.50' North latitude, 79.45' to 80.53'East longitude.This essentially indicates the Gadchiroli District is located in the Deccan Plateau. District has State borders of Andhra Pradesh and Chattisgarh and is categorised as tribal and undeveloped district and most of the land is covered by forest and hills. The forest has covered more than 78.40% (11694.059 Km) of geographical area of district. The main profession of the people is farming. There are no large scale industries in entire district except few. District is economically backward. In present work Pollen analysis of honey were carried out to characterize the honeys and provide important information about the pollen composition of regional flora . pollen frequency is often used to verify and label a honey samples as to the major and minor nectar sources (Kalpana 2005, Kalkar 2009, Bhargava *et al* 2009). This information has important commercial value.

Honeybees visit plants for either nectar or pollen or both which serves as an adequate source for survival and growth of bee colonies. Only such areas with a more or less supply of both these raw materials are useful for successful bee keeping. helps to enhance apicultural practices which provides subsidery income to farmer.

MATERIALS AND METHODS

A. Collection of materials

Total 50 samples were collected from various forest tracts of Gadchiroli district during the period 2005-2009. The squeezing of honeycombs was carried out under personal supervision with the help of expert workers. Enough care was taken to see that only the honey storing potion of the comb was subjected to squeezing for the removal of honey.

B. Labeling of honey-samples

The samples collected were labeled by including the first letters of district followed by forest range, village and then type of hive of honeybee (i.e. either *Apis dorsata* or *Apis florea*). For convenience where more than one name begins with same letter then, second and even sometimes third letter was also used for abbreviations. For example , Forest ranges included were Chatgaon and Choudampalli, hence for Chatgaon "Cha" is used as abbrevation and for Choudampalli, "Cho" is used while labeling the samples as both the ranges begin with "Ch", third letter was used. The sample GChoSD means the sample is collected from Gadchiroli district (G), Choudampalli forest range (Cho) and Singanpalli village of *Apis dorsata* (D) hives. Whereas "D" and "F" represents the sample collected from dorsata and florea hives of *Apis*. At the end the numerical number of collected samples is given. For example, GChoSD 2, GGhSD20, GEED 12 and so on.

C. **Field surveys and Preparation of references slides –** Several field trips were undertaken to the Gadchiroli district for the collection of honey. During these visits collection of the existing local flora was carried out. The plant area, their habits, their utility to honey bees as nectar sources were recorded.

Herbarium specimens of the local flora were made and identified with the help of Nagpur University herbarium and standard literature. Polleniferous material of all the identified plants was collected and reference slides were prepared using Erdtman's (1960) acetolysis method. The polleniferous material was fixed in 70% alcohol. It was then crused, filtered and certifuged at 2500 rpm. The pollen sediment was treated with glacial acetic acid and centrifuged. The glacial acetic acid decanted off and pollen sediment was treated with acetolysed mixture (9 part Acetic anhydride and 1 part of concentrated Sulphuric acid was added drop by drop) and heated in water bath till brown colour appeared. After cooling it was centrifuged and supernatant liquid decanted off. The sediment was then treated with glacial acetic acid. After slight warming the pollen was mounted in glycerin jelly and covered by cover slip.

Pollen morphological characters of the local flora were studied with the help of reference slides and standard literature.

D. Analysis of honey samples

I) Qualitative analysis :

Qualitative analysis of honey samples was carried out to recognize the botanical, geographical origin and season of production of honey based upon the pollen types of that particular region.1 ml of the honey sample was dissolve in 10 ml distilled water and centrifuged for 5-7 minutes. This was subjected to acetolysis method (Erdtman, 1960). Three pollen slides were prepared for each sample and studied critically for their pollen contents.The morphological characters of the pollen types recorded were noted and the pollen type was identified with the help of reference slides and relevant literature to genus and species. In some cases however identification was possible only up to family. A few types, which were not be assigned to even a family, were placed in the unknown category.

II) Quantitative Analysis:

Quantitative analysis of honey samples was aimed at estimating the relative numerical frequencies of diverse pollen types recorded in each honey sample to facilitate preparation of pollen spectra and their chief nectar sources.The frequency classes and frequencies (%) of the pollen types of each sample were determined in accordance with Louveaux et al (1978). The pollen grains were counted at random in various microscope fields of all three slides prepared by acetolysis technique. For determining the frequency classes, 300 pollen grains (100 per slide) and for calculating the pollen frequencies 1200 grains (400 per slide) were counted. The counts of pollen grains of non melliferous (nectar less) or anemophilous plants were substracted

from the total number before calculation the frequencies of pollen of melliferous (nectar producing) plants. As recommended by the international commission for Bee Botany for frequency classes were recognized viz.
P – Predominant pollen (45%) of the pollen grains counted.
S – Secondary pollen (16-45%)
I – Important minor Pollen (15-3%)
M – Minor pollen (<3%)

The honeys were then designated as "unifloral" if they contain more than 45% of one pollen taxa, "Bifloral" if they contain two pollen taxa, showed dominance and represented in between 16-45% and "Multifloral" if they contain more than two pollen taxa are showed dominance.

RESULTS AND DISCUSSION

Analysis of 50 summer honey samples has been carried out. Out of which, 36% honey samples were found to be unifloral and 64% were found to be multifloral. Total 72 pollen types (66 melliferous and 6 non melliferous) referable to 29 families were recorded from these honey samples shown below in Table 1.

Table 1: Frequency Classes and Frequencies (%) of pollen types of *Apis dorsata, Apis florea* honeys summer at various forest range in Gadchiroli District

Sr. No.	Honey Samples	Pollen Types		
				%
Choudampali Range				
1	**GChoSF 1**	**P**	Nil	
		S	*Ageratum conyzoides*	28.5%
			Terminalia arjuna	20.5 %
			Schleichera oleosa	18.5 %
		I	*Tridax procumbens*	15%
			Achyranthus aspera	10.5%
			Brassica nigra	3.5%
		M	*Ocimum scantum*	2.5%
			Vernonia indica	1.5%
2	**GChoSD 2**	**P**	Nil	-
		S	Terminalia arjuna	30.5%
			Azadirachta indica	20%
			Ageratum conyzoides	20.85%
			Capparis grandis	15.5%
		I	*Sphaeranthus indicus*	10.2%
			Mimosa hamata	5%
		M	*Nil*	
Etapalli Range				
1	**GEEF 3**	**P**	*Citrus sp*	79.25%
		S	Nil	-
		I	*Woodfordia fruticosa*	10.5%
			Tridax procumbens	9.5 %
		M	*Acacia nilotica*	0.75%
2	**GEED 12**	**P**	Nil	
		S	*Schleichera oleosa*	25%
			Ageratum conyzoids	22%
			Butea onosperma	20%
			Madhuca indica	18%
		I	*Aegle marmelos*	10%
		M	*Evolvulus alsinoides*	2.5%

			Careya arborea	1.9%
			Rungia repens	0.58%
			Acacia nilotica	0.35%
3	**GEEF 95**	***P***	Nil	
		S	*Portulaca oleracea*	38%
			Tridax procumbens	31%
		I	*Woodfordia fruticosa*	15%
			Feronia elephantum	9.8%
			Helictors isora	3%
		M	*Vitex negundo*	2.5%
			Unknown	1.8%
4	**GEGoD 103**	**P**	*Syzygium cumini*	*55%*
		S	**Nil**	
		I	*Terminalia bellirica*	*15%*
			Schleichera oleosa	*13%*
			Albizia lebbeck	9.5%
			Mangifera indica	7.5%
		M	Nil	
5	**GEGoD 104**	P	Nil	
		S	Syzygium cumini	40%
			Mangifera indica	20%
		I	Capparis grandis	7.9%
			Woodfordia fruticosa	5.8%
			Lantana camera	4%
		M	Sphaeranthuis indicus	2.5%
			Citrus sp	*1.8%*
6	**GEED 105**	*P*	*P-Nil*	
		S	*Butea monosperma*	*35%*
			Terminalia bellirica	*28%*
			Mangifera indica	*18%*
		I	*Cappris spp.*	*15%*
			Sphaerathus indicus	*6.5%*
		M	*Mimosa sp*	*2.3%*
			Lantana camara	*1.8%*
7	**GEED 106**	*P*	*Nil*	
		S	*Pongamia pinnata*	*32%*
		I	*Butea monosperma*	*20.5%*
			Mangifera indica	*13%*
			Zizypus mauritiana	*12.18%*
			Schleichera oleosa	*12%*
			Sphaeranthus indicus	*5.3%*
			Mimosa sp	*4.2%*
		M	*Nil*	
Ghot Range				
8	**GGhSD 10**	**P**	*Nil*	
		S	*Terminalia arjuna*	**25%**
			Butea monosperma	23%
			Sapindus emarginatus	*19.30%*
		I	*Careya arborea*	*10.50 %*
			Helianthus annus	*10.5%*
			Syzyzium cumini	*8%*

			Brassica sp	*3.7 %*
		M	*Nil*	
9	**GGhAdF 17**	*P*	*Nil*	
		S	*Tectona grandis*	*30%*
			Capparis grandis	*25%*
			Lantana camara	*20%*
		I	*Ageratum conyzoide*	*10.50%*
			Sphaeranthus indicus	*10%*
			Vernonia cinerea	*5%*
		M	*Nil*	
10	**GGhAdD 18**	*P*	*Nil*	
		S	*Tamirindus indica*	**35%**
			Tectona grandis	**20%**
			Madhuca indica	**19%**
		I	*Ocimum basillicum*	**10%**
			Tridax procumbens	**10%**
			Vitex negundo	**3.5%**
		M	*Xanthium strumarium*	*1.6%*
11	**GGhAdF 19**	*P*	*Rungia repens*	*86%*
		S	*Nil*	
		I	*Vernonia cinerea*	*11.8%*
		M	*Woodfordia fruticosa*	*2.3%*
12	**GGhSD 20**	*P*	*Syzygium cumini*	*92%*
		S	*Nil*	
		I	*Ageratum conyzoides*	*5.5%*
		M	*Sphaeranthus indicus*	*2.5%*
13	**GGhSD 21**	*P*	*Nil*	
		S	*Azadirachta indica*	*30%*
			Ageratum conyzoides	*30%*
			Sonchus spp	*20%*
			Pongamia pinnata	*20%*
		I	*Nil*	
		M	*Nil*	
Bhamragad Range				
1	**GBTD 13**	*P*	*Terminalia arjuna*	*63%*
		S	*Nil*	
		I	*Butea monosperma*	*10%*
			Largerstromia parviflora	*8%*
			Embelica officinalis	*7%*
			Cassia fistula	*7%*
		M	*Cyperus rotundus*	*2.5%*
			Hibiscus hirtus	*2.2 %*
			Bombox ceiba	*1.5%*
2	**GBBD 15**	*P*	*Nil*	
		S	*Pongamia pinnata*	*29%*
			Syzygium cumini	*20%*
		I	*Peltophorum pterocarpus*	*13.5%*
			Madhuca indica	*16%*

			Borassus flabellifers	*10%*
			Woodfordia fruticosa	*5.3%*
		M	*Sphaeranthus indicus*	*2.75%*
			Poaceae type	*0.33%*
Alapalli Range				
1	***GAVF 16***	*P*	*Nil*	
		S	*Schleichera oleosa -*	*32%*
			Prosopis julifera	*28%*
			Tectona grandis	*19.5%*
		I	*Ricinus communis -*	*13%*
			Xanthium strumarium	*5.3%*
		M	*Mimosa pudica -*	*2.1%*
			Borassus flabellifer	**1.3%**
Markhanda Range				
1	**GMaChaF 11**	*P*	*Syzygium cumini*	*50%*
		S	*Nil*	
		I	*Prosopis juliflera*	*10%*
			Coriandrum sativum	*8%*
			Woodfordia fruticosa	*10%*
			Tinospora cordifolia	*8%*
			Carum copticum	*4%*
			Acacia nilotica	*3.5%*
			Bombox ceiba	*3.5%*
		M	**Citrus spp.**	**2.3%**
2	**GMaAsF 14**	*P*	*Eucalyptus globulus*	*78%*
		S	*Nil*	
		I	*Alternanthera sessilis*	*13.7%*
		M	*Cassia fistula*	*2.5%*
			Feronia elephantum	*2.5%*
			Waltheria indica	*2.3%*
			Vernonia cinerea	**1%**
3	**GMaAsF 31**	*P*	*Nil*	
		S	*Carica papaya*	*24%*
			Pongamia pinnata	*22%*
		I	*Schleichera oleosa*	*15%*
			Capparis grandis	*13%*
			Buchanania lanzan	*9%*
			Alternenthera sessilis	*8.5%*
			Sida acuta	*5%*
			Unknown	**3.5%**
4	**GMaAsF 36**	*P*	*Eucalyptus globulus*	*52%*
		S	*Nil*	
		I	*Tectona grandis*	*15%*
			Terminalia arjuna	*15%*
			Sapindus emarginatus	*12%*
			Sida acuta	*2.7%*
		M	**Unknown**	**2.5%**
5	**GMaJaD 37**	*P*	*Nil*	
		S	*Tectona grandis*	*35%*

			Butea monosperma	*25%*
			Alternanthera sessilis	*15%*
			Zizypus maurantina	*13.3%*
			Rungia repens	*10%*
		M	**Cyperus rotundus**	**1.7%**
6	**GMaAdD 38**	*P*	*Lannea coromandelica*	*61.25%*
		S	*Nil*	
		S	*Tridax procumbens*	*25.8%*
		I	*Capparis grandis*	*10.13%*
		M	*Butea monosperma*	*2.8%*
			Bombox ceiba	*1.5%*
			Sphaeranthus indicus	**0.75%**
7	**GMaAdD 39**	*P*	*Nil*	
		S	*Tectona grandis*	*30%*
			Tridax procumbens	*20.3%*
			Lannea coromandelica	*18%*
		I	*Alternanthera sessilis*	*10%*
			Schleichera oleosa	*8.5%*
			Capparis grandis	*7.2%*
			Erythrina indica	**6.5%**
		M	**Nil**	
8	**GMaUD 44**	*P*	*Nil*	
		S	*Tamarindus indica*	*28%*
			Schleichera oleosa	*24%*
			Zizypus xylocarpa	*18%*
		I	*Capparis grandis*	*15%*
			Butea monosperma	*10%*
			Tephrosia purpurea	*3.8%*
		M	**Bombox ceiba**	**2.3%**
9	**GMaKaD 63**	*P*	*Eucalyptus globulus*	*85%*
		I	*Madhuca indica*	*5.6%*
			Rungia repens	*5.1%*
			Hygrophila auriculata	*3.9%*
		M	*Nil*	
10	**GMaKaD 64**	*P*	*Nil*	
		S	*Rungia repens*	*35%*
			Tectona grandis	*30%*
		I	*Lantana camara*	*11%*
			Alternenthra sessilis	*10%*
			Cordiospermum halicacabum	*7.5%*
			Ocimum sanctum	*3.2%*
		M	*Vernonia cinerara*	*2.3%*
			Cassia tora	*2.1%*
11	**GMaAnD 65**	*P*	*Terminalia arjuna*	*80%*
		S	*Nil*	
		I	*Tridax procumbens*	*6.5%*
			Tectona grandis	*5%*
			Butea monosperma	*3.5%*

			Ocimum sanctum	*3.5%*
			Unknown	*3%*
		M	**Nil**	
12	**GMaAnF 66**	*P*	*Nil*	
		S	*Ageratum conyzoid*	*35%*
			Achyrantus aspera	*20%*
			Tridax procumbens	*18*
		I	*Schleichera oleosa*	*12.5%*
			Lannea coromandelica	*10%*
		M	*Xanthium strurmanium*	*2.5%*
			Vernonia cineara	**2%**
13	**GMaAsF 67**	*P*	*Terminalia arjuna*	*89.18%*
		S	*Nil*	
		I	*Xanthium strumarium*	*3%*
		M	*Ocimum basillicum*	*2.5%*
			Bombax ceiba	*2.5%*
			Unknown	**3%**
14	**GMaAsD 87**	*P*	*Nil*	
		S	*Eucalyptus globulus*	*40%*
			Pongamia pinnata	*35%*
		I	*Zizypus xylocarpa*	*12%*
			Tinospora cordifolia	*8%*
			Butea monosperma	*5%*
		M	*Nil*	
15	**GMaUF 88**	**P**	**Pongamia pinnata**	**100%**
16	**GMaUD 89**	*P*	*Nil*	
		S	*Pongamia pinnata*	*28%*
			Butea monosperma	*25.8%*
		I	*Tectona grandis*	*15.25%*
			Madhuca indica	*13.83%*
			Tridax procumben	*9.7%*
			Sphaerantus indicus	*5.8%*
			Capparis grandis	*3.2%*
		M	*Nil*	
17	**GMaKoF 90**	*P*	*Nil*	
		S	*Schleichera oleosa*	*30%*
			Delonix regia	*25%*
			Terminalia tomantosa	*20%*
		I	*Zizypus mourintana*	*10%*
			Waltheria indica	*9%*
			Woodfordia fructicosa	*6%*
		M	*Nil*	
18	**GMaKoF 91**	*P*	*Nil*	
		S	*Eucalyptus globulus*	*30%*
			Tamarindus indica	*23%*
			Pongamia pinnata	*18%*
		I	*Ageratum conyzoides*	*12%*
			Ocimum sanctum	*5.2%*
			Porlulaca oleracea	*4%*

			Vernoria cinerea	*3.5%*
			Cardiospermum halicacabum	*3%*
		M	*Cassia tora*	*2.5%*
19	**GMaKoD 92**	*P*	*Nil*	
		S	*Butea monosperma*	*35%*
			Tridax procumbens	*25%*
		I	*Madhuca indica*	*10%*
			Terminalia arjuna	*9.8%*
			Achyranthus aspera	*7%*
			Ageratum conyzoides	*6.5%*
			Cassia tora	*4%*
		M	*Brassica nigra*	*1.4%*
			Vernonia cinerea	*1.5%*
20	**GMaAsF 93**	*P*	*Nil*	
		S	*Capsicum frutescens*	*30%*
			Capparis grandis	*20%*
		I	*Tridax procumbens*	*15.5%*
			Ricinus communis	*10.5%*
			Zizypus mauritiana	*9.5%*
			Sapindus emarginatus	*9%*
		M	*Achyranthus aspera*	*2.8%*
			Xanthium strumarium	*2.5%*
21	**GMaAsF 94**	*P*	*Nil*	
		S	*Lannea coromandelica*	*30%*
			Schleichera oleosa	*25%*
			Tridax procumbens	*16%*
		I	*Achyranthus aspera*	*12.2%*
			Vernonia cinerea	*7.5%*
			Brassica sp	*5.8%*
			Tinospora cordifolia	*3.5%*
		M	*Nil*	
22	**GMaUF 100**	*P*	*Nil*	
		S	*Eucalyptus globulus*	*38%*
			Schleichera oleosa	*17%*
			Tamarindus indica	*16%*
		I	*Terminalia arjuna*	*9.6%*
			Capparis grandis	*9%*
			Syzygium cumini	*7.3%*
		M	*Citrus sp*	*2.5%*
			Cassia tora	*0.66%*
23	**GMaUF 101**	*P*	*Nil*	
		S	*Terminalia sp*	*28%*
			Lannea coromandelica	*25%*
			Schleichera oleosa	*20%*
		I	*Bombax ceiba*	*12.5%*
			Woodfordia fruticosa	*8.6%*
			Careya arborea	*3.4%*
		M	*Rutaceae*	*1.3%*
			Cassia tora	*1.3%*

24	**GMaKoF 102**	*P*	*Schleichera oleosa*	*51%*
		S	*Lannea coromandelica*	*20%*
			Careya arborea	*16%*
		I	*Citrus sp*	*8%*
			Capparis grandis	*5.2%*
		M	*Poaceae*	*0.75%*
Mulchera Range				
1	**GMuGaF 32**	*P*	*Schleichera oleosa*	*67%*
		S	*Nil*	
		I	*Zizypus xylocarpa*	*11.3%*
			Mangifera indica	*10.5%*
			Sapindus emarginatus	*5.7%*
			Capparis grandis	*3%*
		M	**Borassus flabellifer**	**2.5%**
2	**GMuGaF 33**	*P*	*Syzigium cumini*	*68%*
		S	*Nil*	
		I	*Ageratum conyzoides*	*13%*
			Zizypus xylocarpa	*10%*
			Schleichera oleosa	*4.7%*
			Xanthium starumarium	*3.5%*
		M	*Vernonia cinerea*	*1.5%*
			Poaceae type	**0.33%**
3	**GMuGaD 34**	*P*	*Syzygium cumini*	*75%*
		S	*Nil*	
		I	*Schleichera oleosa*	*8.3%*
			Tectona grandis	*7.3%*
			Terminalia arjuna	*5.8%*
			Borassus flabellifer	*3.1%*
		M	**Nil**	
4	**GMuGaD 35**	*P*	*Nil*	
		S	*Ageratum conyzoides*	*35%*
			Tectona grandis	*20%*
		I	*Feronia elephantum*	*15.5%*
			Butea monosperma	*12%*
			Terminalia arjuna	*9%*
			Clerodendrum inermae	*5%*
			Xanthium strumarium	*3.4%*
		M	*Nil*	
5	**GMuMuF 40**	*P*	*Terminalia arjuna*	*81%*
		S	*Nil*	
		I	*Schleichera oleosa*	*12%*
			Capparis grandis	**7%**
		M	**Nil**	
6	**GMuMu F 41**	*P*	*Terminalia arjuna*	*78%*
		S	*Nil*	
			Woodfordia fruticosa	*10%*
			Justica procumbens	*7.5%*
			Unknown	*4%*
		M	*Syzygium cumini*	*2.3%*

			Vernonia cinerea	*1.5%*
			Poaceae	*0.75%*
Chatgaon Range				
1	**GChaGiD 43**	*P*	*Nil*	
		S	*Ageratum coniyzoides*	*30.50%*
			Mangifera indica	*29%*
			Terminalia tomantosa	*25%*
		I	*Sphaeranthus indicus*	*14%*
		M	*Capparis grandis*	*1%*
			Bombox ceiba	**1%**

Abbreviations:

Adayal – Ad; Allapali – A; Ankhoda – An; Ashti – As; Bhamragad – B; Chatgoan – Cha; Choudampali – Cho; Etapali – E; Gandhinagar – Ga; Ghot – Gh; Goadsur-Go; Gilgaon _ Gi; Jayrampur – Ja; Jimalghata – Ji; Kadoli – Ka; Konsari – Ko; Markhanda – Ma; Mulchera – Mu; Permili – P; Rengewai – R; Saknapur – S; Tadgaon – T; Umari – U; Velgur – V

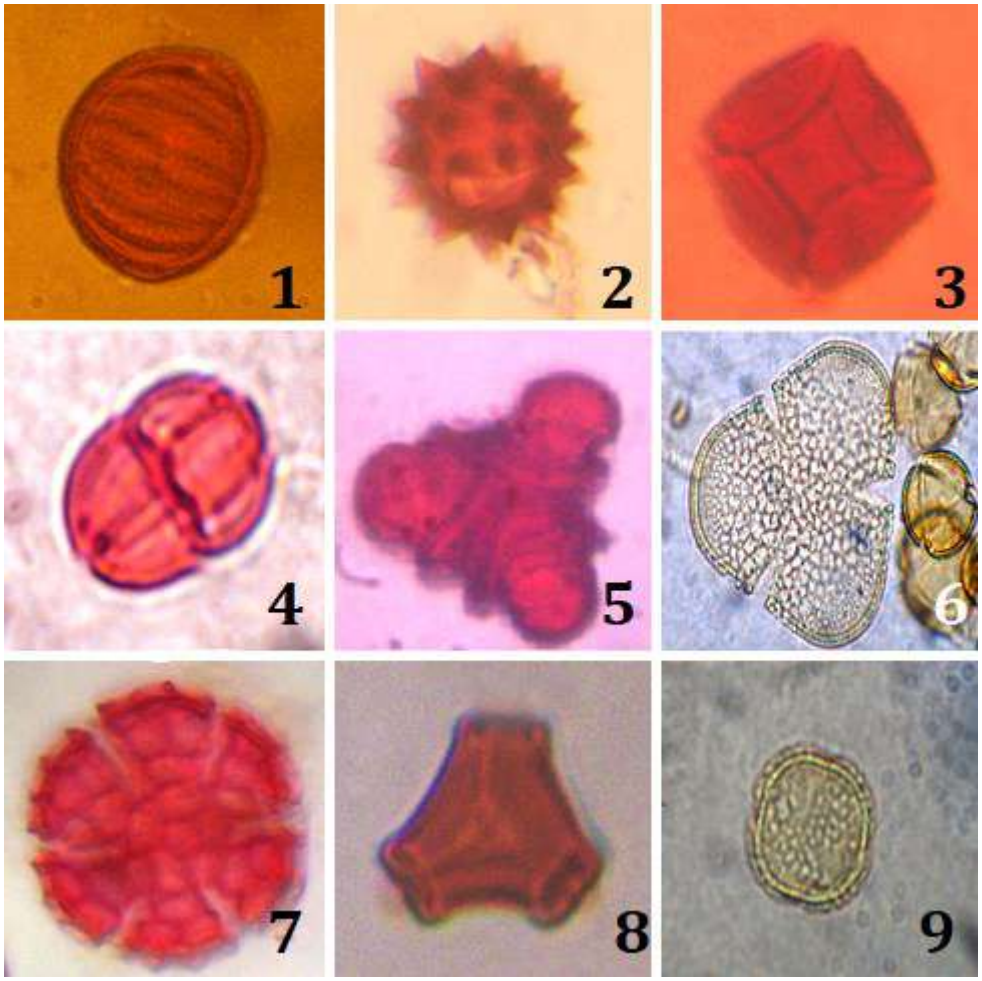

Fig . 1. *Hygrophila auriculata 2. Sphaerantus indicus, 3. Schleichera oleosa, 4.Mimosa hamata 5.Careya arberea 6.Bombox ceiba, 7.Ocimum sanctum8.Eucalyptus globulus, 9.Citrus sp*.

DISCUSSION

The unifloral honeys were assigned to *Syzygium cumini* (5), *Terminalia arjuna* (4), *Eucalyptus globulus* (3), *Schleichera oleosa* (2), *Citrus* sp (1), *Rungia repens (*1), *Pongamia pinnata* (1) and *Lannea coromondelica* (1).

Major Secondary pollen types encountered in summer honeys were- A*geratum conyzoides, Butea monosperma, Eucalyptus globulus, Lannea coromandelica, Mangifera indica, Pongamia pinnata, Portulaca oleracea, Rungia repens, Schleichera oleosa, Sphaeranthus indicus,*

Syzygium cumin,Tamarindus indica, Tectona grandis, Terminalia arjuna, Tridax procumbens.

Common important minor types included pollen types viz. *Sphaeranthus indicus, Justica procumbens, Feronia elephantum, Cleradendrum inermae, Xanthium strumarium, Borassus flabellifer, Zizypus xylocarpa, Sapindus emarginatus, Mangifera indica, Capparis grandis, Woodfordia fruticosa, Careya arborea, Waltheria indica, Vernonia cinerea, Vernonia indica, Achyranthus aspera, Portulaca oleracea, Ocimum sanctum, Tinospora cordifolia, Butea monosperma, Hygrophila auriculata, Tephrosia purpurea, Rungia repens, Alternaethera sessilis, Buchanonia lanzan, Tectona grandis, Terminalia arjuna, Ocimum basillicum, Tridax procumbens* and others. Our observations were supported by Bhusari,et.al, 2005 in *Apis* Honey from Maharashtra.

Sheshagiri (1985), Agashe and Scinthia (1995), Agashe (1997), brought out important findings such as occurrence of unifloral honeys from *Eucalyptus, Coriandrum sativum, Syzygium cumini, Psidium guajava, Pongamia pinnata* and *Phyllanthus* from Dharampuri district, Bangalore and it environs costal Karnataka district of Bangalore and Udupi. Seethalakshmi (1980) on the basis of pollen contents of 12 honey samples from 8 states indicated that the pollen spectra of honey unravel their geographical origin and recorded *Syzygium cumini, Mimosa rubicalis* as a predominant pollen types. Similar finding also reported in our present work

CONCLUSION

The nectar is major raw resource for the bee keeping industry. It transforms into honey after conservation by the bee. Similarly pollen is also needed for the growth of the bees. The outcome of the present studies is that, it explored 64 nectar sources from this region, among these 8 are predominant , 56 are secondary nectar sources and important minor and minor nectar sources. This information of nectar sources will definitely help in promoting bee keeping industry. Such studies will also be helpful in improving economy of the farmer, rural and tribal people as it will add as a subsidiary income

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:135-142
UGC Approved Journal No 48951

Original Article Open Access

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Shende AN, Mohture VM and Korpenwar AN (2018) Traditional medicinal plants used against various diseases in Nagbhid tahsil, Chandrapur (MS) India, *Int. J. of. Life Sciences*, Special Issue, A12: 135-142.

ABSTRACT

Nagbhid is surrounded by abundance of nature and forest. Local people of the area depend on the forest products for earning money as well as aware of the various medicinal properties of the plant. In present study survey of ethnomedicinal plants was carried out during January 2014 to December 2015 from Nagbhid Tahsil. Ninety botanically important medicinal plants belonging to fourty nine families were identified with relevant information and are documented alphabetically with their botanical names followed by local name, family, parts used and modes of preparation of medicine. The local healers in this area use the medicinal plants in cure of various diseases. Documenting the indigenous knowledge is important for the conservation and utilization of biological resources of this area.

Key words: Medicinal plants, Local healers, Nagbhid Tahsil, Indigenous, conservation.

INTRODUCTION

According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000). Researchers have a special interest in the medicinal plants used in Ayurvedas and other traditional system of medicines. Most of the allopathic drugs have been invented but the plant-based medicines have its own unique status as it has no side effects on the human body. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different Indian system of medicines such as Ayurveda, Unani and Siddha. Today there is an increasing desire to unravel the role of ethnobotanical studies in trapping the centuries old traditional folk knowledge as well as in searching new plant resources of food, drug etc. (Jain, 1991). There is an urgent need to document the ethno biological information presently existing among the diverse communities before the traditional knowledge is completely lost. Indian traditional medicine is based on different system such as Ayurveda, Siddha and Unani used by various communities (Gadgil,

1996). The local used of plants as a cure are common particularly in those areas, which have little or no access to modern health services, such as the innumerable tribal villages and hamlets in India indicates that the dependency of traditional societies on the wild collections for subsistence needs (Campbell *et al*., 1997). Nearly 80% of the world population depends upon traditional system of health care (Anonymous, 1998). In India it is reported that traditional healers use 2500 plant species and medicine (Pie, 2001). In recent years, traditional ethno-botanical studies have received much attention due to their wild local acceptability and clues for new or less known medicinal plants (Tripathi, 2000).

People living in the developing countries rely quite effectively on traditional medicine for primary health care (Sullivan and Shealy, 1997; Singh, 2002). Until now, however, there has been little effort to document the volume and impact of national or international trade in India's medicinal plants (Ganesan and Kesavana, 2003). The present paper deals with the listing and documenttation of medicinal plants commonly used on various diseases by the local people, traditional healers and Vaidus in Nagbhid Tahsil of Chandrapur district, Maharashtra.

METHODOLOGY

Study Area: Nagbhid Tahsil is the western most district of the Vidharbha, of the Maharashtra State. The district is situated between $19^0.51$ and $21^0.17$ North latitudes and $75^0.57$ and $76^0.49$ East longitudes. In Nagbhid Tahsil, local Vaidus are natural retainers of traditional knowledge which passed from generation to generation through oral folklore.

Survey and Collection of Information: The field survey was carried out from January 2014 to December 2015 for documentation of medicinal plants used by local people in this area. Information on the use of medicinal plants was obtained through, field tours, interviews and informal conversation with traditional healers, knowledgeable person or medicine men, Vaidus, experienced and aged person, local healers of the villages. They were consulted for recording local name, parts of plant used, methods of drug preparation and recommended doses. Personal interviews and group discussions with local inhabitants revealed some very valuable and specific information about the plants, which were further authenticated by cross checking.

Preparation of Herbarium and Identification: The plants were collected from remote place in vegetative and blooming conditions, simultaneously noting the vernacular names and all the relevant information disclosed by the local practitioners. The plants were brought to the laboratory and processed for herbarium specimen. Plants were identified using relevant scientific literature (Hooker 1872-1877; Cooke 1967 (Rpr.); Sharma *et al*. 1996; Naik 1998; Singh and Karthikeyan, 2000, Singh *et al*. 2001). Subsequent visits were planned to photograph the plants in proper blooming period.

RESULTS AND DISCUSSION:

During present survey 90 medicinal plant species belonging to 49 families were recorded. A brief information including botanical name, family, local name, parts used and their medicinal value by the peoples is given in Table No.1. As the forest area is nearer to Nagbhid, most of the local healers collected the plants from the forest. The medicinal plant parts like leaf, bark, seed, root, tuber, fruit and whole plant were used in raw or cooked forms (Enumeration). The most cited diseases were: jaundice, piles, asthma, skin diseases, fever and rheumatism.

Although this is firsthand knowledge about ethnomedicine in Nagbhid tahsil, thorough pharmacological investigations are recommended since the informants claim the uses with confidence and strong belief. The main aim of this study was to gather the information about the different medicinal plants used to cure different disease in Nagbhid. Most of the local people still dependent and believed on the herbal plants for their remedial properties. There is no written document of such indigenous plant medicine. It spread only by mouth publicity. It is the alarming sign that the knowledge of medicinal plants will disappear in near future. So it is important to preserve this precious knowledge for future generations. These ethnomedicinal plants present in the vicinity of the forest are also a source of income for the local communities. The ethnomedicinal plants are under threat due to deforestation, overgrazing and their over utilization. Due to this many medicinal plants are now come under critically endangered category. There is urgent need of their conservation (Burlakoti and Kunwar, 2008). By taking the active support of local and villagers, and forest persons these plants can be preserved for our future generations.

Table 1: Listing of Medicinal Plants used by local and Traditional peoples of Nagbhid.

SN	Botanical name	Local name	Family	Mode of administration
1.	*Achyranthes aspera* L.	Agadha	Amaranthaceae	The boiled leaves are consumed to relieve internal piles Decoction of plant in the treatment of kidney stone.
2.	*Abutilon indicum* L.	Atibala	Malvaceae	Various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection. The whole plant is uprooted, dried and is powdered. to consume a spoonful of this powder with a spoonful of honey, once in a day, for 6 months until the day of marriage, for safe and quick pregnancy. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men.
3.	*Adhatoda zeylanica* Medik.	Adulsa	Acanthaceae	Gargle with the extract of the leaves with salt to cure tonsillitis. Leaf extract is taken internally to relieve cough and cure asthma.
4.	*Adiantum philippense L.*	Hamsapadi / Lal laajaalu	Pteridaceae	The whole plant is applied externally to burns. The leaves are anti-inflammatory. The plant can be used as anti-poisonous.
5.	*Aegel marmelos* (L.) Correa	Bel	Rutaceae	The bel fruit is used against dysentery and diarrhea. Juice of Bel leaves with black pepper is given orally in jaundice.
6.	*Alangium salvifolium L. F. Wang.*	Ankol	Cornaceae	The roots and the fruits are used for the treatment of rheumatism and haemorrhoid. Externally, it is used for the treatment of bites by rabbits, rats, and dogs. The root-bark is also used in traditional medicine skin problems and as an antidote for snake bite.
7.	*Aloe vera* (L) Burm	Korphad	Liliaceae	Pulp juice of leaf is used to cure piles, jaundice and stomach ache and apply locally to recover the burnt skin and for wound healing.
8.	*Andrographis paniculata* Burm.	Bhui-neem	Acanthaceae	One teaspoon of fresh plant juice is taken twice a day for seven days to treat snake-bite and scorpion-bite
9.	*Argemone Mexicana* L.	Piwla Dhotura	Papaveraceae	The paste of seeds with salt and mustard oil is used as tooth paste by those suffering from pyorrhea. The Bhils apply fresh leaves or their juice on eyes in conjunctivitis.
10.	*Aristolochia bravteata* Retz.	Badakvel	Aristolochiaceae	Root powder is combined with honey and given internally in case of gonorrhea, boils, ulcers and other skin disease.
11.	*Asparagus racemosus Willd.*	Satavari	Asparagaceae	The prevention and treatment of gastric ulcers and dyspepsia, and also been used for nervous disorders. The roots are used in regimen of processing and drying. Roots used as a uterine tonic.
12.	*Bacopa monnieri* L.	Jadpala	Scrophulariaceae	Plant extract is used in snake bite, scorpion sting and in asthma.
13.	*Baliospermum montanum* Willd.	Jamalgota	Euphorbiaceae	Seed paste applied externally on swellings and seed oil applied locally in rheumatic pains. Root decoction is given in asthma and seeds are used as purgative.
14.	*Bauhinia vahlii* Wight and Arn.	Chamul / Mahul	Fabaceae	The Fruits are light, dry and have binding properties to cure diseases of pitta and the whole plant is healer and coagulant. It purifies blood and checks body weights.
15.	*Boerhaavia diffusa* L.	Khaparkhuti	Nyctaginaceae	Decoction of roots as an expectorant to cure asthma and jaundice.
16.	*Boswellia serrata* Roxb.	Dinkya	Burseraceae	The leaf-juice is used to cure eye infection and bark decoction is taken orally to cure chronic cough and cold.
17.	*Butea monosperma* Lamk.	Palash	Fabaceae	Seed powder with goat milk is given as an aphrodisiac. Seed powder is taken orally as contraceptive. Shoot paste is applied twice a day for one week piles.

18.	*Calotropis gigantea* (Linn.) R.Br.	Akawa	Asclepiadaceae	Root decoction is given for lactation. Flowers (2-3) consumed to cure cough and asthma.
19.	*Capparis decidua* Forssk dgew	Karira	Capparaceae	It is used as vegetable for diabetic patients and the root bark is used to cure swollen joints.
20.	*Caralluma adscendens* Grav. & Mayur.	Dagadkakdi	Asclepiadaceae	Stems are eaten raw for a week to cure bleeding piles. Stem is crushed with ginger and taken internally to cure cough.
21.	*Careya arborea* Roxb.	Kumbi	Lecythidaceae	Fruit decoction is prescribed orally for snake- bite. Decoction of root bark is taken in piles
22.	*Cassia tora* L.	Tarota	Caesalpiniaceae	Powder of seeds used on Vata. Pregnanat women prepared coffee from powder against cold.
23.	*Cassis fistula* L.	Amaltash	Caesalpiniaceae	Fruit pulp is advised for constipation. Leaf poultices are applied externally for paralysis and rheumatism.
24.	*Cayratia trifolia* L.	Wajwel	Vitaceae	Leaf Powder is taken orally with milk for the early recovery for fractured bone.
25.	*Celastrus paniculatus* Willd.	Malkangi	Celastraceae	Seed oil is applied externally in the treatment of knee-pains and paralysis and dropped in eyes for better eyesight.
26.	*Celosia argentea* L.	Rankurdu	Amaranthaceae	Plant powder with a cup of milk is given to the ladies twice a day for a week to cure white discharge. The root decoction is effective in the treatment of kidney stone.
27.	*Chlorohytum borivilianum* Roxb.	Safed moosli	Liliaceae	1 gram powder of tuberous root is mixed with water and given to male as a tonic. Small amount of tuber is given to female to check leuchorroea.
28.	*Cissampelos pareira* L.	Patha	Rananculaceae	Leaf extract is used as Antimalarial as well as its antiviral properties, especially against Dengue virus.
29.	*Clerodendrum serratum* L.	Bharungi	Verbenaceae	Decoction of root is taken in malarial fever and ophthalmic complaints. The paste of leaves is applied externally to ripen the wounds. Decoction of root powder is prescribed as blood purifier.
30.	*Cocculus hirsutus* L.	Vasan	Menispermaceae	Leaf extract is taken in peptic ulcers. The leaf extract taken internally along with milk for treatment of supermatorrhoea. The extract of root is taken internally in paralysis.
31.	*Convolvulus pluricaulis* Choisy	Shankapushpi	Gentianaceae	With cumin and milk leaves are used in fever, nervous debility and loss of memory.
32.	*Corallocarpus epigaeus* Rottl. Et. Willd. Hook.	Akasgaddah	Cucurbitaceae	The tuber is used for skin disease, cough and it also used for eye disease.
33.	*Costus speciosus* Koen.	Jangli-adrak	Costaceae	Spoonful rhizome powder with a glass of water in empty stomach is taken as aphrodisiac. Juice of rhizome is taken to cure urinary tract infections.
34.	*Cucumis callosum* L.	Indrava	Cucurbitaceae	The paste of tuber is applied on swelling areas on neck and in earache.
35.	*Curculigo orchioides* Gaertn.	Kala kand	Hypoxidaceae	Tuber powder is taken orally as an aphrodisiac and to cure gonorrehea. One teaspoon powder with milk is taken orally by to cure leucorrhoea.
36.	*Curcuma pseudomontana* J. Graham	Jangali Halad	Zingiberaceae	Roots are boiled and eaten against dysentery and cardiac diseases
37.	*Datura Stramonium* L	Pandhara Dhotra	Solanaceae	Datura is used as herbal medicine in case of Ayurveda for asthma. the oil extract from it is used for growth of hair.
38.	*Desmodium gangeticum* L. Dc.	Ranganjya	Fabaceae	The roots are used for treating the diseases like chronic fever ,cough ,diarrhea ,vomiting ,piles
39.	*Dioscorea bulbifera L*	Kadu Kanda / Ratalu	Dioscoreaceae	Bulb used for treatment of diabetes.
40.	*Dioscorea hispida*	Bhul-kand	Dioscoreaceae	Boiled tubers are taken twice a day for a week to cure piles.

	Dennst. Schl.			The tuber is eaten as vegetable after keeping it overnight in water or after boiling.
41.	*Diospyros melanoxylon* Roxb.	Tembhru	Ebenaceae	Decoction of flower is effective in night-blindness and in diarrhea. Leaf paste is applied in scabies and timorous glands. Paste of fruit is applied in bone fracture.
42.	*Dolichandron falcate* Seem.	Medshingi	Bignoniaceae	The mixture of leaf extract 50 ml and 50 gm curd is taken twice a day for a week to cure bleeding piles. Leaf powder with water is given in diabetes.
43.	*Echinops echinatus* Roxb.	Ulati	Asteraceae	Paste prepared from powder of the root bark is applied on male genitals externally for sexual vigour. Root decoction is an effective remedy for hernia.
44.	*Enicostema axillare* lam. Raynal	Kadu Nai	Gentianaceae	The plant is used to treat diseases like diabetes, hernia ,swelling , itching and insect poisoning.
45.	*Ensete superbum* Roxb	Ran keli	Musaceae	Stem extract is used in treatment of Leucorrhea & debility.
46.	*Eulophia ochreata* (Lindl.)	Amarkand	Orchidaceae	Tuber powder with one cup milk is used against cancer diseases
47.	*Ficus benghalensis* L.	Wad	Moraceae	The milk extract of plant with 1 teas full sugar is used against ulcers, vomiting, vaginal complaints, fever, inflammations, leprosy etc.
48.	*Ficus religiosa* L.	Pimpal	Moraceae	The juice of its leaves used as the ear drop. Its power bark used to heal the wounds. The bark of the tree is useful in inflammations and glandular swelling of the neck. The roots are even chewed to prevent gum diseases. Its fruit is laxative which promotes digestion and checks vomiting. The powered fruit is taken for Asthma. Its seeds are used in urinary troubles.
49.	*Gardenia gummifera* L.	Dikemali	Rubiaceae	Bark is used in headache, juice of leaves is given in body pain. Root powder is used in impotency.
50.	*Geodorum densiflorum* L.	Harghati	Orchidaceae	Fresh root paste mixed with 2 drops of ghee and 5 ml of honey and taken orally to regularized menstrual problems.
51.	*Gloriosa superba* L.	Kal-lavi	Liliaceae	About 10 mg tuber powder is taken orally by the tribal ladies only once to regularize menstrual disorder. Tribals crush tubers of the plant in water and apply on head to kill the lice.
52.	*Glossocarda bosvallea* L.	Patthar suva	Asteraceae	Paste of leaves is applied on healing and on wounds.
53.	*Grangeama deraspatana* L.	Mustaru / Mashipatri	Asteraceae	The leaf sap is used to treat ear ache.
54.	*Helicteres isora* L.	Marophali	Sterculiaceae	Fruit paste with honey internally is good remedy for diarrhea, stomachache, chronic dysentery in children, a general practice in tribals.
55.	*Hemidesmus indicus* (L) R.Br.	Kawdi / Anantmul	Apocynaceae	Root is powdered and given with honey in jaundice. Latex is applied in the form of paste of sores and wounds. Root decoction is taken once a day for blood purification
56.	*Holarrhena antidysenterica* Wall.	Kuda	Apocynaceae	Seeds are dip in water and in powdered form given for dysentery and in worm infections.
57.	*Holarrhena pubescens* Buch.	Indrajao	Apocynaceae	Leaves are used for treatment of skin diseases such as scabies, ringworm ,itching and other infections.
58.	*Leea crispa* Van.	Wanchalita	Vitaceae	The root tuber is used as a treatment against guinea worms.
59.	*Leeam acrophylla* Roxb.	Hathikana	Vitaceae	The roots are used for treatment of guineaworm and ringworm.
60.	*Leucas aspera* Willd.	Kombda	Lamiaceae	Leaf juice (2-3 drops) dropped into nostrils to get relief from heavy cold. The leaves decoction is very useful in chronic rheumatism.

61.	*Limonia acidissima* L.	Kawath	Rutaceae	Leaf juice with onion juice and camphor is taken orally in cholera.
62.	*Momordica dioica* Roxb.	Jangli Karla	Cucurbitaceae	Roasted root is used to stop bleeding from piles. A piece of tuber is recommended internally for liquor addiction.
63.	*Mucuna pruriens* (L.) DC.	Khaj-kuiri	Fabaceae	One spoonful seed powder with a glass of milk is given to increase sexual vigor and as a health tonic. Seeds are given for improving retention of semen and night dreams. Roots are effective in dysentery.
64.	*Ophiglossum reticulatum* L.	Ran Palak	Ophioglossaceae	The plant is used as an anti inflammatory medicine and .the leaves are applied to wounds.
65.	*Ophioglossum costatum* R. Br.	Sapa- Jeebh	Ophioglossaceae	The leaves are eaten as salad or cooked it is good for heart.
66.	*Phyla nodiflora* L.	Panmundi	Verbenaceae	Juice obtained from the plant is given against blood dysentery and pneumonia. The leaves are chewed to cure toothache.
67.	*Phyllanthus amarus* Schum.	Kadu-awla	Euphorbiaceae	Young leaves are good for dysentery. About 10g paste of hole plant is given thrice daily for one week for both plant in hepatitis and chronic liver problems.
68.	*Plumbago zeylanica* L.	Chitramula	Plumbaginaceae	Juice of 5-10 leaves is taken orally as an antidote in snake-bite. Tribals apply the paste of roots on the piles. Root paste along with milk applied externally in leprosy and other skin diseases.
69.	*Psoralea corylifolia* L.	Bawchi	Fabaceae	Seed powder one spoonful with a glass of milk is prescribed twice a day for a month in the treatment of impotency, premature ejaculation and to improve vitality. Seed oil of applied externally in psoriasis, leprosy and leucoderma.
70.	*Pterocarpus marsupium* Roxb.	Bijasal	Fabaceae	Water is kept overnight in a glass made out of the stem and taken in the morning to treat diabetes. Leaf decoction is taken in active stomach pain and dysentery.
71.	*Pueraria tuberose* Roxb.	Bhuikohla	Fabaceae	Tubers are crushed and applied o joints to treat rheumatism. Tuber decoction is prescribed for lactation after childbirth. In painful urination.
72.	*Sida cordifolia* L.	Chikana	Malvaceae	Decoction of seed against dysentery and stomach pain. Crushed fresh leaves applied on cut surface.
73.	*Solanum virginianum* L.	Kateringani	Solanaceae	The seeds are expectorant. They are used in the treatment of asthma and catarrh.
74.	*Sopubia delphinifolia* G. Don Gen. Syst.	Dudhali	Scrophulariaceae	The stem is given orally after pregnancy for milk secretion.
75.	*Spilanthes calva Dc.*	Akkal-kadha	Asteraceae	The flower heads are chewed to relieve the toothache and other mouth related troubles.
76.	*Sterculi aurens* Roxb.	Karu	Sterculiaceae	Seed powder one teaspoonful is taken orally with milk as an aphrodisiac. Bark powder is taken orally with water in tuberculosis and rheumatism.
77.	*Stereospermum chelonoides* Dc.	Kalagori / kalgari	Bignoniaceae	The juice of bark is used to treat indigestion.
78.	*Tamarix ericoides* Rottl.	Kadsherni	Tamaricaceae	Leaves are used for treatment of liver disorder.
79.	*Tephrosia purpurea* Linn.	Diwali	Fabaceae	Decoction of root against diarrhea, rheumatism, asthma and urinary disorder.
80.	*Terminalia arjuna* (Roxb.)	Aanjan	Combretaceae	Bark powder is used for heart diseases.
81.	*Tinospora cordifolia* (Thunb.) Meirs	Gulvel	Menispermaceae	Leaf juice is used for diabetes, upset stomach, lymphoma and other cancers, rheumatoid arthritis and high shivering.

82.	*Tridax procumbance* L	Kambarmodi	Asteraceae	Leaf juice is used for wound healing and skin diseases.
83.	*Triumfettar homboidea* Jacq.	Chirchiri	Tiliaceae	Leaf paste is applied on the affected areas of scabies and eczema. Leaf juice is taken internally in jaundice and urinary complaints. Leaf paste applied externally in bleeding piles.
84.	*Tylophora indica* Burm	Anant-mool	Asclepiadaceae	The plant root is used by common people for the treatment of various diseases including asthma,cancer,fever etc.
85.	*Uraria picta* Desv.	Pitvan	Fabaceae	Leaves are used for snake bite by Tribal people. Decoction of root is given against coughs, chills and fever.
86.	*Vanda tesselata* (Roxb.) Hook.	Rashna	Orchidaceae	Leaf is given orally with betel leaf to women having irregular menstruation.
87.	*Viscum nepalense* L.	Harjor	Loranthaceae	Paste of shade dried powder of the plant with water is applied on the chest to cure swellings and fractured bone and dislocation.
88.	*Vitex negundo* L.	Nirgudi	Verbenaceae	Leaf extract is dropped in the eyes to cure conjunctivitis. Fruit powder decoction (50ml) is taken orally in the treatment of kidney stone.
89.	*Woodfordia fruticosa* L.	Van mehandi / Dhayati	Lythraceae	Flower extract is used for treatment of thirst, blood disorders and also improve heart health it is applied on wounds and ulcers for quick healing.
90.	*Ziziphus mauritiana* Lamk.	Kate-Bor	Rhamnaceae	Decoction of the root bark is used in the treatment of diarrhea and dysentery. The twigs are used as tooth-brush in bleeding gums.

TABLE: NO.2 Total families and number of plant species listed during study:

Families	**Number of plant species**
Rhamnaceae, Loranthaceae, Tiliaceae, Combretaceae, Plumbaginaceae, Lamiaceae, Rubiaceae, Zingiberaceae, Hypoxidaceae, Costaceae, Lecythidaceae, Rananculaceae, Musaceae, Celastraceae, Burseraceae, Nyctaginaceae, Aristolochiaceae, Papaveraceae, Pteridaceae, Cornaceae , Asparagaceae, Capparaceae, Ebenaceae, Tamaricaceae, Lythraceae	**1 Each**
Menispermaceae, Sterculiaceae, Scrophulariaceae, Malvaceae, Moraceae, Bignoniaceae, Dioscoreaceae, Gentianaceae, Amaranthaceae, Euphorbiaceae, Caesalpiniaceae, Rutaceae, Acanthaceae, Solanaceae, Ophioglossaceae	**2 Each**
Verbenaceae, Orchidaceae, Cucurbitaceae, Apocynaceae, Liliaceae, Vitaceae , Asclepiadaceae	**3 Each**
Asteraceae	**5 Plants**
Fabaceae	**9 Plants**

CONCLUSION

The result of the present study provides evidence that medicinal plants continue to play an important role in the healthcare system. The people of Nagbhid tahsil are still depend on indigenous knowledge for their health care, providing a cheaper and accessible alternative to the high cost pharmaceutical remedies. In spite of the overwhelming influence and our dependence on modern medicine and tremendous advance in synthetic drugs, many people still rely on herbal drugs the reason is that, if the herbal medicines are used properly they don't have any side effects.

The possible benefit of plant-derived medications constitutes a rewarding area of research, particularly in countries such India which have a rich biodiversity of plant resources coupled with a high prevalence and variety of infectious diseases where sustainable utilization of the biodiversity can be carried out. Therefore, documentation of these plants is the only way to preserve the traditional knowledge of the plant resources endemic to this area.

Acknowledgment
The authors express sincere thanks to traditional healers, Vaidus and local people who co-operated in sharing their knowledge at the time of study.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :143-147
UGC Approved Journal No 48951

Original Article Open Access

In-vitro micropropagation of *Cassia absus* (l.) An Indian medicinal plant

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Mungole Arvind, Kharwade Priyanka and Wadekar Mohan (2018) *In-vitro* micropropagation of *cassia absus* (l.) An Indian medicinal plant, *Int. J. of. Life Sciences*, Special Issue, A12: 143-147.

ABSTRACT

In the present study the protocol for callus induction and regeneration in *Cassia absus* was standardized. Young apical leaves, cotyledonary leaves, epicotyle and hypocotyle were used as explants for callus induction on Ms Medium containing IBA and kinetin in different concentrations also used BAP and kinetin in different concentrations. The maximum percentage of callusing was observed on the medium supplemented with 0.8mg/L IBA and 0.1mg/L Kinetin was found to be 100% for cotyledonary leaf & 90% for epicotyle explants. The calli in most of the cultures were brown and soft in nature. Initiation of shooting of *Cassia absus* established from cotyledonary leaf explants on MS medium supplemented with combination of hormones BAP 0.2 mg/L & Kinetin 0.6 mg/L. This study was aimed to develop standard protocol for callus induction, protocol for organogenesis & standardization of media and growth hormonal concentrations which may helps in conservation and cultivation of this species.

Key words: In-vitro Micropropagation, Regeneration, *Cassia absus*.

INTRODUCTION

In-vitro micropropagation is an important tool from rapid multiplication of medicinal plants (Atal Kapur 1982 a&b). *In-vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. The capability to regenerate and propagate plants from cultures cells and tissues is one of the most exciting and useful aspects of In-vitro cell and tissue culture. Increasing demand of those plants, which are specially use for the food and medicine, is one of the cause of their rapid depletion from the natural habitats. In-vitro micropropagation offer a great potential for conservation and large-scale multiplication of such useful species and subsequent exploitation as well as for the extraction of active ingredient. Thus, the exploration of tissue culture technique in medicinal plant is indeed desirable. Therefore, the whole world is diverting towards the multiplication of these plants.

Besides preventing from depletion of stocks of wild plants, the contamination of plant material may lead to inferior quality of product. Tissue culture is one way by which plant material can be supplied in a pure from and continuously throughout the year (Datta, 1993). *Cassia absus* is medicinally important plant, belongs to the family Fabaceae a small herbaceous plant grown as weed in the natural habitat it is widely distributed throughout the worlds tropics and subtropics. It has a long history of use by indigenous and tribal people in Ayurvedic natural herbal medicines. The leaves and fruits of *Cassia absus* is very useful source of drugs most commonly used for medicinal purposes, though the roots have also been studies. The leaves and fruit of *Cassia absus* are widely used to treat wound, ringworm, sores abscesses, ulcers and inflammation (Van der Maesen, 2008). In many parts of Africa and Asia, seeds are used to treat diabetes, conjunctivitis, ulcers and cataract (Ghani et al, 1997, Van der Maesen, 2008; Hussain et al., 2008). The seeds are also considered to be astringent and hypertensive (Aftab et al., 1996) and may help to reduce swelling and prevent hemorrhaging. The seeds and leaves of the plant are most commonly used for medicinal purposes though the roots have also been studied. The leaves are bitter, acrid and have been used traditionally for a cough, diseases of the nose and as an astringent to the bowel. It is regarded as useful enriching the blood as tonic, a bitter astringeunt for the bowels, applied locally to heal ulcers. (Ghani *et al.,*1997). Chloroform, petroleum ether and acetone extract of *Cassia absus* have anti-bacterial (Manjusha et al., 2009), hypertensive and anti-spasmodic effect (Aftab *et al*., 1996) seeds, leaves and roots contain two alkaloids, Chaksine and isochaksine (Aftab *et al.,* 1996).

The seeds also contain oils, fatty acids, sterols and flovonoids. The antroquinones, Chrysophenol and emoclin, isolated from Chaksu roots have laxative effect (Rao et al., 1979) & phytochemical studies involving the extracts of seed have shown antibacterial, antimalerial and blood pressure lowering effect (Aftab *et al*., 1996) A recent Study has also evaluated the antiinfommatory and the anti-histuninic activity of an eye drop formulation containing the seeds of the plant. A lot of work has been done antibacterial, antimalerial activity of this plant (Aftab *et al.,* 1996). A large number of publications on the Chemistry, Pharmacology and several other aspects have been made, but here have been a few reports on in-vitro regeneration of *Cassia absus*. Therefore it has attracted the attention of Botanists, Chemists, Pharmacologists because of its medicinal importance in Ayurvedic mixture. In nature, seed production in this plant is irregular, with a low germination percentage due to the impermeability of the integument. It is highly demanded by the different Pharmaceutical companies. Little work done on *in-vitro* regeneration of *Cassia absus*. Keeping entire importance of this taxa in mind decided to do in-vitro Micropropagation of it. The present study was undertaken to examine the potential of different explants with different concentrations of hormones in combination, to rapid initiation of callus and regeneration.

METHODOLOGY

Cassia absus plant used in the present study was collected from the wild population of campus of N. H. College, Bramhapuri, Dist. Chandrapur. Different explants were used for establishing callus including apical leaf, cotyledonary leaf, epicotyls and hypocotyls. They were washed thoroughly under running tap water for 10 min. subsequently sterilization was carried out in laminar air flow cabinet under asceptic conditions. Then explants were surface sterilized with 0.1% (W/v) mercuric chloride for 2-3 min. followed by 70% ethyl alcohol 2-3 min. then washed 2-3 time sterile double-distilled water and inoculated on agar solidified MS (Murashige & Skoog, 1962) medium Supplemented with different concentration of IBA, Kinetin & BAP in combination. All media contained 3% sucrose & 1% agar with pH 5.8 adjusts before sterilization. For shooting cultured on freshly prepared shooting medium containing MS medium with BAP 0.2 mg/L of kinetin - 0.6 mg/L hormone concentration cultures were maintained at 27 ^{0}C with 10 hr. photoperiod.

RESULT & DISCUSSION

The MS medium supplemented with various concentration of BAP and kinetin, IAA and IBA, IBA and Kinetin inducing callusing. The MS medium supplemented with all this combination showed brown and soft callus induction. The maximum percentage of callusing was observed at the medium supplemented with 0.8mg/L IBA and 0.1mg/L kinetin was found to be 100% for cotyledonary leaf & 90% for epicotyle explants. Callus induction were followed by the hormonal combination supplemented with 0.4mg/L BAP + 0.8mg/L Kinetin i.e. 90% for cotyledonary leaf & 60%

for epicotyle explants with 0.9mg/L IBA + 0.2mg/L Kinetin (Table 1). The MS medium supplemented with hormonal concentration 0.2mg/L BAP + 0.6mg/L Kinetin on which Hypocotyle explant was found to be more responsive and induced callus ie. 90% with brown and soft nature. It is followed by the combination 0.3mg/L IAA + 0.6mg/L IBA ie. 60% callus induction was reported (Table 1).

Table No.1: Induction of callus on MS media supplemented with different concentration of hormones.

Hormone concentrations	Explants used	Percentage of callus induction	Duration of induction of callus in days	Colour and nature of the callus
0.2mg/L BAP + 0.6mg/L Kinetin	Epicotyle	50	20	Brown and soft
	Hypocotyle	100	20	Brown and soft
0.4mg/L BAP + 0.8mg/L Kinetin	Cotyledonary leaf	90	15	Brown and soft
0.3mg/L IAA + 0.6mg/L IBA	Cotyledonary leaf	50	25	Brown and soft
	Hypocotyle	60	25	Brown and soft
0.7mg/L IBA + 0.1mg/L Kinetin	Hypocotyle	50	20	Brown and soft
0.8mg/L IBA + 0.1mg/L Kinetin	Cotyledonary leaf	100	20	Brown and soft
	Epicotyle	90	20	Brown and soft
0.9mg/L IBA + 0.2mg/L Kinetin	Epicotyle	60	25	Brown and soft
	Hypocotyle	50	20	Brown and soft
	Cotyledonary leaf	60	25	Brown and soft

Table 2: Effect of different concentration of hormones on shoot regeneration of *Cassia absus/*

Hormonal concentration	No of shoot per treatment	Shoot length in cm.	Shoot morphology
BAP 0.2mg /L Kinetin – 0.6 mg /L	3	2	Green & long
	2	1.5	Thin Short
	2	1.2	Thin Short
	1	1	Thin Short
	2	1	Thin Short

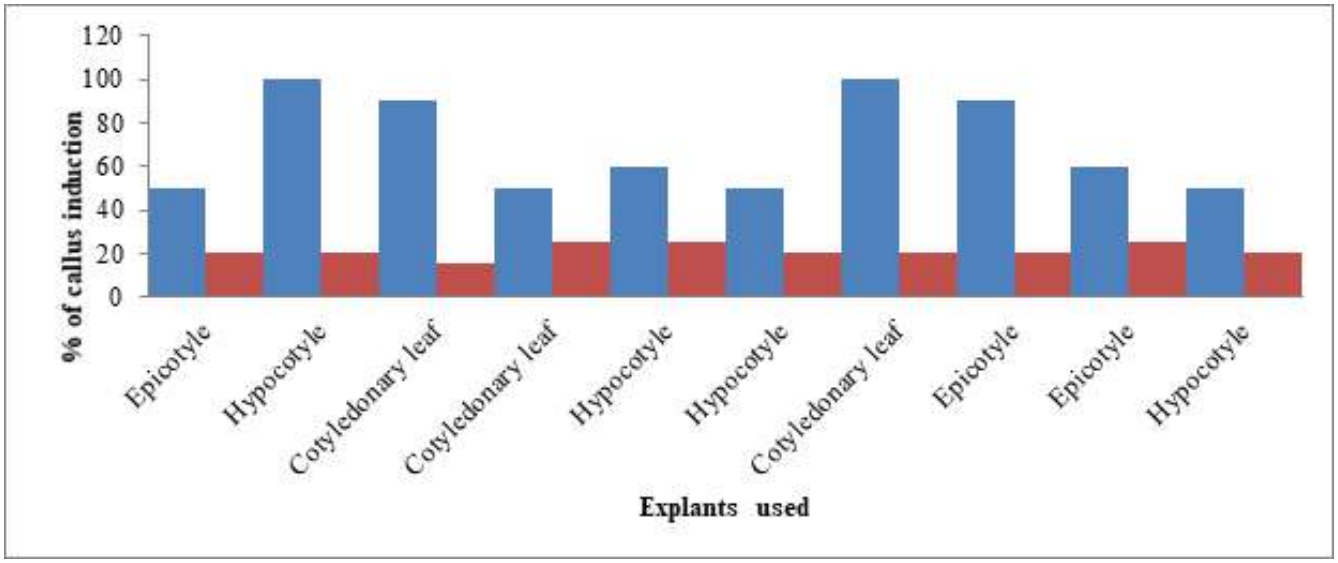
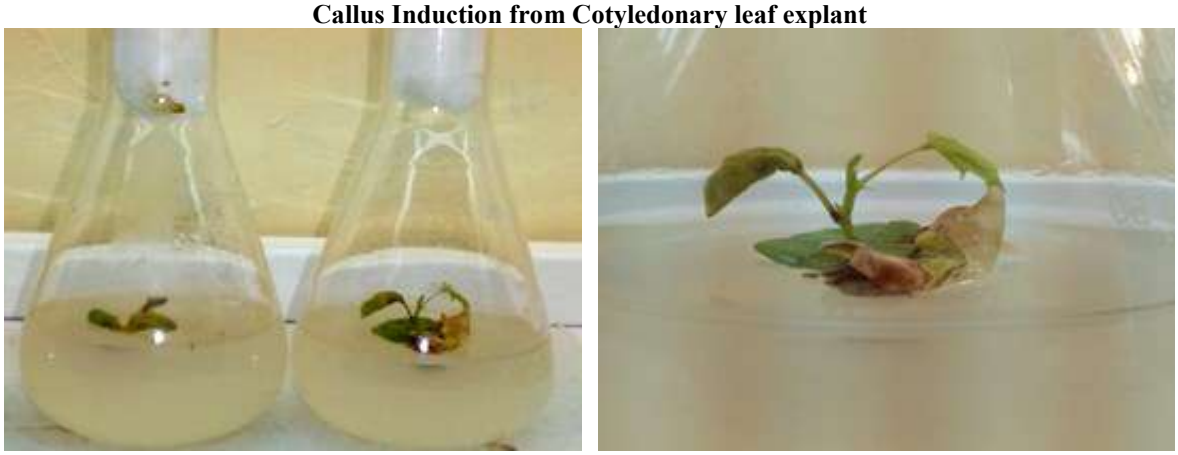


Fig. 1: Response of different explants to callus induction and duration of time

Habit and habitat of the *Cassia absus*

Callus Induction from Cotyledonary leaf explant



Direct regeneration and shooting of *Cassia absus*

Photo plate 1: Showing habit, habitat and different stages of in-vitro regeneration of *Cassia absus*

The cotylodanry leaf found to be more responsive explants on IBA and kinetin. Toker. *et. al.,* (2003) studied the formation of callus using different type of explants like stem, root, leaf and seed of *Ecbollium elaterium* where seed and root explants did not yield callus at all while, stem node and leaf explants formed the callus to a lesser extent. Thus the differential response of various explants can be attributed to differences in cultural requirements of explants and also the variation in endogenous hormone level (Ghosh and Sen, 1994). Further studies were carried out for shoot regeneration capacity by using cotylodanry leaf explants. Shoot were initiated from Cotyledonary leaf explants by showed indirect organogenesis. The best result of shooting (2 cm) was observed with MS medium supplemented with the combination of 0.2mg/L BAP and 0.6mg/L kinetin after. 10th day with good and long morphology in which 3 shoot per treatment wear

recorded. Followed by shoot length (2 cm) was recorded (Table 2). Tissue culture provides the best approach for preservation and multiplication of medicinal herbs. Bera and Roy (2000), proposed the plant tissue culture as a tool for rapid multiplication of plants. Advantages of *in vitro* culture method lie in its ability to produce huge number of true type individuals in a short time and limited space. Tissue cultural techniques a means for conserving and multiplying medicinal plants have been reported by Le (1994), Nin*, et. al.,* (1994) and Wawrosch *et. al.,* (2001).

CONCLUSION

Plant having medicinal importance where collected from wild condition. This lead to gradual depletion due to in discriminate collection. This wild population depletion can be prevented by cultivating such plant for commercial use through In-vitro micropropagation. From the all types of explants collected from *Cassia absus* that is apical leaf, cotyledonary leaf, epicotyls and hypocotyls, cotyledonary leaf was found to be better for callusing on IBA and Kinetin supplemented in MS medium. The best responsive combination of medium is one which supplemented with 0.8mg/L IBA and 0.1mg/L kinetin. Shoot were induced at concentration 0.2mg/L BAP and 0.6mg/L kinetin from cotyledonary leaf explants.

Acknowledgements:
Author is thankful to the Principal N. H. College, Bramhapuri for providing the Tissue culture Laboratory Facility & valuable guidance.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:148-152
UGC Approved Journal No 48951

Original Article Open Access

Report of a Triserial Capsular Fruit from the Deccan intertrappean series of Paladaun, M.P., India

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Patil SP and Kapgate DK (2018) Report of a Triserial Capsular Fruit from the Deccan intertrappean series of Paladaun, M.P., India, *Int. J. of. Life Sciences*, Special Issue, A12: 148-152.

ABSTRACT

The present paper deals with the report of a new trilocular, triserial, capsular, monocot fruit from the Deccan Intertrappean Beds of Paladaun (Lat. 22^0 3' 25.78''N; Long. 78^0 56' 17.53'' E), Chhindwara district, Madhya Pradesh, India. The specimen is tricolular, elongated multiseeded, capsular, dehiscent fruit. The fruit measures about 8 mm in length and 3 mm in cross section tapering at both ends. The anatomy of fruit consists of outer pericarp, Pericarp is thick and differ into epicarp, mesocarp and endocarp. Each locule contains 12 seeds in two rows of 6 seeds each. Central axis is thick. Locules are separated by thin septa. Seeds are small and total 36 in all. Embryo is monocot type. Placentation is axile. Vasculature is seen along the central axis. Dehiscence is loculisidal type. The fossil fruit is named as *Liliaceocarpon paladaunensis* gen.et sp.nov.

Keywords: Deccan, Intertrappean, fossil, capsular, trilocular, fruit

INTRODUCTION

The present paper deals with the report of a new trilocular, triserial, capsular, monocot fruit from the Deccan Intertrappean Beds of Paladaun (Lat. 22^0 3' 25.78''N; Long. 78^0 56' 17.53'' E), Chhindwara district, Madhya Pradesh, India. The number of capsular fruits is reported from the Deccan Intertrappean beds of India, but very few are monoct fruits. The capsular fruits reported are- *Tricoccitesspp* (Sahni, Rode 1937 and Chitaley 1956), *Enigmocarpon parijai* (Sahni 1943), *Indocarpa intertrappea* (Jain 1964), *Harrisocarpon sahnii* (Chitaley and Nambudri 1973), *Sahniocarpon harrissi* (Chitaley and Patil 1972), *Daberocarpon gerhardli*(Chitaley and Sheikh 1971), *Deccanocarpon arnoldi* (Paradkar 1975) etc. Some drupaceous fruits described from the same locality include *Biloculocarpon mohgaoense* (Yawale 1975) and *Grewia mohgaonse* (Paradkar and Dixit 1980). There are also record of Leguminous fruits from the Deccan traps described as *Leguminocarpon eocenum* (Yawale 1973) and *Lomentocarpon deccanii* (Yawale 1982). The baccate fruits are *Kremocarpon aquatica* (Chitaley and Kate 1975) *Mohgaocarpon eyedi*

(Yawale, 1977), *Kremocarponindicum* (upadhye 1979), *Centerospermocarpon chitaleyi* (Sheikh and Khubalkar 1979), *Ramanujamocarpon indicum* (Kolhe 1980), *Tillliaceocarpon interterrappeae* (Dixit 1984), *Juglandiocarpon agashii* (Adhgo 1986), *Erythroxylocarpon intertrappea* (Khubalkar 1982), *Chitaleyocarpon deccani* (Kumar 1984), achenical fruits *Ceratocarponspinosa* (Adhao 1986), winged seeded unilocular fruits are also reported. *Wingmospermocarpon mohgaoense* (Sheikh and Kapgate 1984) and *Wingospermocarpon arilis* (Sheikh and Kapgate 2000.), *Schizocarpon aliformii* a shizocarpic fruit by (Bhowal 1998).

This report will add some more information in the reports of the flora of Deccan Intertrappean beds of India.

MATERIAL AND METHOD

The material was collected from Paladaun locality of Chhindwara district in Madhya Pradesh during the field visit in the form of black chert. While breaking the chert the present fruit specimen was exposed in longitudinal plane. After etching with Hydroflouric (HF) acid serial peels of the material are taken along its longitudinal plane with cellulose acetate peel technique. The peels were mounted in DPX mountant and observed under micro-scope. Micro photographs are taken and camera lucida sketches are also drawn for detailed study. The x-ray photographs are also carried out in the laboratory of Flourida University, USA for detailed study.

Description :

The fruit is exposed in longitudinal plane shows elongated shape in appearance. The fruit measures about 8 mm in length and 3 mm in cross section tapering at both ends. The anatomy of fruit consists of outer pericarp, three locules with two series of 6 seeds in each locule showing axile placentation, well preserved central axis with vasculature, seeds are well preserved with prominent monocot type embryo. The fruit shows dehiscence zone at its upper end. Stalk is not found.

Pericarp- The pericarp (fruit wall) well preserved and is about 334 μm in in thickness divided into three zones i.e. outer Epicarp, middle Mesocarp and inner Endocarp. The upper portion of pericarp shows dehiscence zone.

Epicarp- it is the outrmost layer of pericarp measuring about 98 μm in thickness made up of 4-5 layers of thick walled compact parenchymatous cells.

Mesocarp-it is the middle layer of the pericarp. It is thicker than epicarp and endocarp and measuring about 173 μm in thickness made up of 8-9 layers of thin walled loosely arranged parenchyma giving it a soft nature.

Endocarp- it is innermost layer of the pericarp measuring 63 μm in thickness made up of 3-4 layers of elongated compact parenchyma.

Central Axis – Central axis is well preserved and can be clearly seen along the longitudinal and transverse plane. It measures about 0.6 to 0.7 mm in its cross section. It is made up of multiple layers of circular to oval parenchyma. The vasculature can be seen along the central axis.

Locules – The longitudinal section shows only two locules but the cress section it is clear that the fruit is trilocular. Each locule is 2.45 mm X1.05 mm in size separated by thin parenchymatous septa. Each locule contains two series of 6 seed i.e. each locule contains 12 seeds in two rows of 6 seeds each.

Seed- The fruit contains 3 locules each containing two rows of 6 seeds i.e. the fruit contains 36 seeds in all. The larger seed measures 1.25 X 0.96 mm in size while the smaller seed measures 0.90 X 0.75 mm in size. the seeds show axile placentation. The seed coat is thin and undifferentiated. The seeds are smaller in size and endosperm tissue is not clear. Embryo is ill preserved but it seems to be monocot type.

Placentation – the seeds shows their attachment to the central axis indicating the axile placentation.

Vasculature –Along the central axis the vascular tissue can be clearly seen to supply nutrition to the developing embryos.

Dehiscence – At the upper portion of the pericarp breakage is clearly observed indicating its dehiscence zone. This indicated that the fruit is mature and about to dehisce. The dehiscence is clearly loculisidal type.

DISCUSSION AND IDENTIFICATION

From the above description the present specimen fruit shows following characters-

- The specimen is tricolular, elongated multiseeded, capsular, dehiscent fruit.
- Pericarp is thick and differ into epicarp, mesocarp and endocarp.
- Each locule contains 12 seeds in two rows of 6 seeds each.
- Central axis is thick.
- Locules are separated by thin septa.
- Seeds are small and total 36 in all.

- Embryo is monocot type.
- Placentation is axile.
- Vasculature is seen along the central axis.
- Dehiscence is loculisidal type.

From the above discussion the present fruit specimen is compared with living genus of the modern families and with the reported fossil capsular fruits for its identification.

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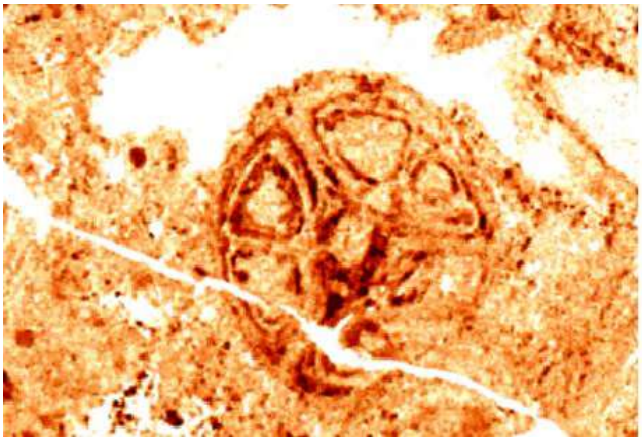

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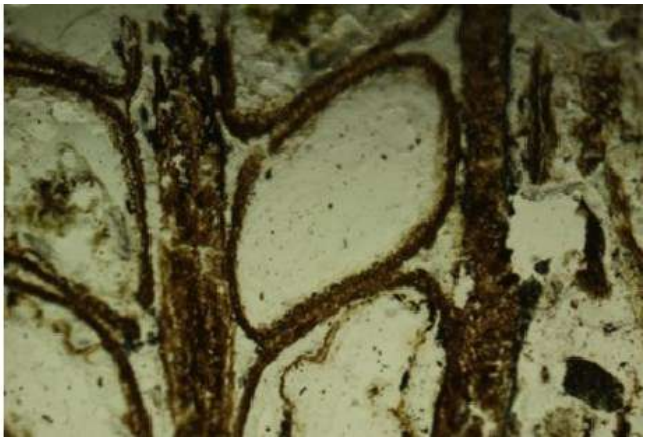

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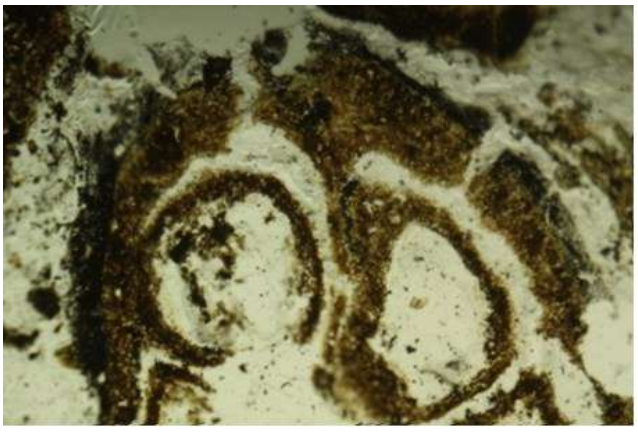

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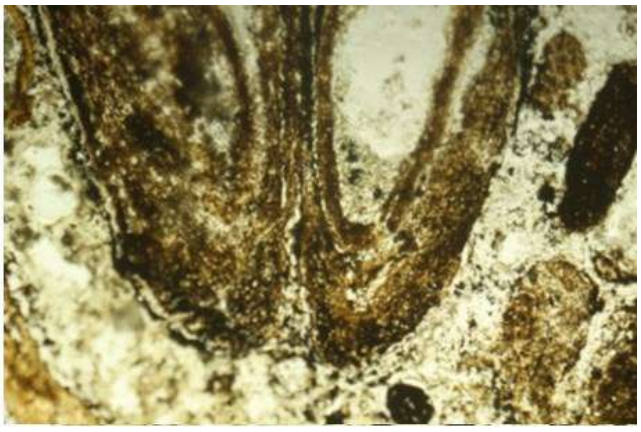

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Figure 1 & 2 : Complete view of fruit in L.S. **Fig. 3:** Complete view of the fruit in T.S.**, Fig. 4 :** Showing Pericarp, central Axis & Seeds, **Fig. 5:** A part enlarged showing dehiscence zone**, Fig. 6 :** A part enlarged showing base and vasculature.

Comparison with Modern families

The present fruit is compared with the living genera of the Monocot families showing similar characters. The present fruit is compared with the capsular fruits of modern living monocot families like. Marantacea, Liliaceae, Xyridacea, Junacacea, Ericaulacea, Burmanniaceae (Cook, 1967; Corner, 1976; Mathew, 1983). In the following families fruit are dry capsular dehiscent but differs from the present in following respect. In family Marantaceae fruits are loculicidal capsules having three locules but differ in having one seed in each locule. Liliaceae shows much resemblance with present fruits in having loculicidalcapsules, generally they have 3 locules with two series of many seed in each locule and loculicidal dehiscence (e.g. *Lilium*sp.) but differ in having numerous seeds and wedged pericarp while present specimen is circular in nature. Juncaceae show resemblence in having loculicidal capsule one to three locules but differ in having many minute seeds in each locule. In Ericaulaceae and Burmanniaceae the fruits are loculicidal capsules two to three locules rarely one locule with many seeds. The fossil fruit though comparable with the modern monocotylednous families in some ways but differ in many aspects. It shows more resemblances to modern family Liliacceae but could not be comparable to the any of the genus of Liliaceae exactly.

Comparison with reported fossil capsular fruits

The present specimen is compared with reported fossil capsular fruits such as- *Enigmocarpon parijae* (Sahni,1943) is a 6-12 locular fruit with thick spongy wall, with a row of seeds in each locule. *Harrisocarpon sahnii* (Chitaley and Nambudiri, 1973) and *Sahniocarpon harrisii* (Chitaley and Patil, 1972) are similar in having pentalocular fruit. *Daberocarpon gerhardii* (Chitaley& Sheikh, 1973) differ as it is ten locular with single seed in each locule. *Deccanocarpon arnoldii* (Paradkar and Dixit, 1975) vary as it is eight locular with single seed in each locule. *Loculocidocarpon chitaleii* (Kapgate, 1999) having pentalocular fruit but differ in having loculicidal dehiscence. *Chitaleocarpon intertrappea* (Kapgate, 2000) is a seven locular capsule with 2-8 seeds in each locule. *Lythraceocarpon mohgaonese* (Saxena, 2004) is a hexalocular fruit with hexagonal central axis and 2-8 seeds per locule. *Portulacaceocapon jamsavlii* (Bhowal, Narkhede and Meshram,2011) is unilocularmultiseeded capsular fruit. *Wingospermocarpon mohgaoense* (Sheikh and Kapgate 1984) unilocular capsular, winged seed, free central placentation of the seed. *Wingospermocarpon arillies* (Kapgate and Sheikh 2000) is a unilocular, dicot, pedicellate capsular fuit with arillated seeds. The discussion above point out no resembance of the fossil to any of the living families except modern family Liliaceae. The reported fossil fruits also do not compare favourably with the studied fossil. Hence the fossil fruit is named as *Liliaceocarpon paladaunensis* gen.et sp.nov. This has been done on the basis of morphological characters of the fruit. The generic name after resemblance with modern family Liliaceae whereas specific name after the locality from where the specimen was collected.

Diagnosis-

***Liliaceocarpon* gen.nov.**

The fruit measures about 8 mm in length and 3 mm in cross section tapering at both ends. The anatomy of fruit consists of outer pericarp, three locules with two series of 6 seeds in each locule showing axile placentation, well preserved central axis with vasculature, sedds are well preserved with prominent monocot type embryo.

***Liliaceocarpon paladaunensis* gen.et sp.nov.**

The specimen is tricolular, elongated multiseeded, capsular, dehiscent fruit. The fruit measures about 8 mm in length and 3 mm in cross section tapering at both ends. The anatomy of fruit consists of outer pericarp, Pericarp is thick and differ into epicarp, mesocarp and endocarp. Each locule contains 12 seeds in two rows of 6 seeds each. Central axis is thick. Locules are separated by thin septa. Seeds are small and total 36 in all. Embryo is monocot type. Placentation is axile. Vasculature is seen along the central axis. Dehiscence is loculisidal type.

Holotype :SPP / TRF-1. Florida Museum of Natural History, Florida, USA.

Locality : Paladaun, M.P.

Horizon : Deccan Intertrappean Series of India.

Age :?Upper Cretaceous.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:153-156
UGC Approved Journal No 48951

Original Article Open Access

Fungi associated with the flowers of *Spilanthes acmella* (l.) Murr during storage.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gaikwad Deepmala A and Korpenwar Anil N (2018) Fungi associated with the flowers of *Spilanthes acmella* (l.) Murr during storage, *Int. J. of. Life Sciences*, Special Issue, A12: 153-156.

ABSTRACT

Spilanthes acmella (L.) Murr. Flowers are used as a raw material for the preparation of some important drugs for curing various human diseases. During unscientific methods of storage causing fungal contamination. The fungal contamination effect on the chemical composition of raw materials and thereby decreases potency of drugs. Regarding the above fact the present experiment was conducted and observed that the young flowers were showed no incidence of fungi on Blotter Paper Method except *Aspergillus flavus* on PDA. In case of mature flowers the Blotter Paper Method showed incidence of four fungi like *Alternaria alternata*, *A. niger*, *Aspergillus terreus*, *F. oxysporum*, *Fusarium roseum* whereas PDA Method showed nine fungi. The maximum incidences of fungi were reported in stored flowers as compared to young and mature flowers.

Key words : *Spilanthes acmella* , flowers, fungal contamination.

INTRODUCTION

Spilanthes acmella (L.) Murr belongs to the family Asteraceae. Commonly it is known as Toothache plant grown as an ornamental and as a medicinal plant in various parts of the India. The active constituent spilanthol chiefly present in leaves and flower heads, and produce analgesic activity used to numb toothache. The whole plants can be used in the treatment of dysentery and rheumatism. The flower heads of *S. acmella* can be chewed to relieve toothache and also as a haemostatic and analgesic Leng *et al*. (2011). Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, infections of gums Pandey *et al.* (2004). The flower heads and roots have been used for treatment of toothache, scabies, scurvy, infections of throat and gums, paralysis of tongue. The leaves and flower heads contain analgesic, antifungal, anthelminthic, antimalarial, antibacterial, diuretic Ratnasooriya *et al*. (2004), Rani and Murty, (2006), Barman *et al.*(2009), Prachayasittikul *et al.* (2013).

Medicinal plants may be associated with a microbial contaminants, represented by bacteria, fungi and viruses. This microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. The traditional methods of collection, storage and marketing coupled with humid climatic condition make them victim to the fungal contamination. (Masoumeh and Deokule, 2013., Muntanola, 1987) and Durakovic *et al*. (1989) studied that the fungal contaminates has been reported to affect the chemical composition of the raw materials and thereby, decreases the medicinal potency of the plant material whereas mycotoxins produced by these fungal contaminants causes several effects on liver organs, , kidney, genital digestive tract, respiratory organs nervous system, skin etc. The unscientific methods of harvesting, collection, storage of raw materials, post harvest processing, transport and storage of herbal drugs in unhygienic conditions, are the main causes considered to make both, raw materials as well as herbal drugs prone to microbial infections leading to deterioration in safety and quality and can also cause health hazard to consumer in spite to cure the disease Pinkey.(2014), Many researchers have reported that the presence of potential contaminants in herbal preparations viz. Czech *et al*.(2001), Idu *et al*. (2011), Kulshrestha *et al.* (2008), Alwakeel (2008).The manufacturers should ensure the lowest possible level of microorganisms in the raw material, finished dosage forms and the packaging components to maintain appropriate quality, safety and efficacy of the natural products. Okunlola *et al*. (2007).

According to the WHO, about 80% of the population of the world depends on traditional medicine, mostly herbal remedies, for their primary health care needs. Moerman (1996) . Various pathogens adversely affect the medicinal plant parts and decrease the medicinal value of the part. It may be harmful to the human body while using these infected parts as a medicine. Hamayun *et al.(*2004) So present investigation is an attempt to identify the mycoflora associated with the flowers sample of *Spilanthes acmella* (L.) Murr.

METHODOLOGY

Collection of plant material:

Spilanthes acmella (L.) Murr. flowers were collected from different authentic stores of Jalna district in pre-sterilized polythene bags and brought to the laboratory. samples were identified using the Flora of Marathwada Naik, (1998) at Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. Stored flowers were inoculated aseptically on the sterilized petriplates containing Potato Dextrose Agar (PDA) Medium and Blotter Method incubated at25±2°C temperature for 7 days.

Isolation of mycoflora:

Mycoflora was isolated by using Blotter Method and Potato Dextrose Agar (PDA) Medium.

Identification of fungi:

The fungi occurring on plant material in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals Mukadam *et al*, (2006), Similarly confirmation of identification was made at Department of Plant Pathology Laboratory, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

RESULTS AND DISCUSSION

In order to study changes occurring during storage period on flowers, different parameters such as appearance, color, odor and texture was observed after 6,12,18 and 24 months intervals during storage and results are given in the table 1. It is clear from table no.1 that no black spotted cone shape yellow appearance, black pale yellow color, foul odor and britter texture was found in 6 and 12 months storage period. But after the 18 and 24 months of storage periods, severe type of infection of fungi with black spotted cone shape yellow appearance, black pale yellow in color, foul in odor and briter in texture were observed.

In order to study the percent incidence of fungi on flowers (young, mature and stored) were studied with Blotter Paper Method and PDA and the result are given in table 2. It is clear from result that the young flowers were showed no incidence of fungi on Blotter Paper Method and except *Aspergillus flavus* (05) on PDA.

Table 1. Physical changes in flowers of *Spilanthes acmella* under different storage period.

Parameters	Storage period (months)				
	Fresh	6	12	18	24
Appearance	Cone shape Yellow	Cone shape Yellow	Cone shape Yellow	Black spotted Cone shape Yellow	Black spotted Cone shape Yellow
Color	Dark Yellow	Yellow	Pale yellow	Pale yellow	Black Pale yellow
Odor	Odorless	Odorless	Odorless	Foul odor	Foul Odor
Texture	Normal	Normal	Breakable	Briter	Briter

Table 2. Incidence of fungi on flowers of *Spilanthes acmella* from different age.

Fungi	Young		Mature		Stored	
	Blotter	PDA	Blotter	PDA	Blotter	PDA
Alternaria alternata	-	-	05	05	10	10
Aspergillus flavus	-	05	-	15	15	35
Aspergillus niger	-	-	05	10	10	55
Aspergillus fumigatus	-	-	-	-	05	20
Aspergillus nidulance	-	-	-	-	05	20
Aspergillus terreus	-	-	05	10	10	20
Cladosporium species	-	-	-	-	-	30
Fusarium oxysporum	-	-	05	15	-	50
Fusarium roseum	-	-	05	10	10	10
Mucor globsus	-	-	-	-	10	25
Phoma species	-	-	-	-	-	10
Penicillium notatum	-	-	-	15	10	20
Rhizopus stolonifer	-	-	-	-	-	30
Trichoderma viride	-	-	-	15	05	10

In case of mature flowers the Blotter Paper Method showed incidence of four fungi like *Alternaria alternata* (05), *A. niger* (05), *Aspergillus terreus* (05), *F. oxysporum* (05), *Fusarium roseum* (05) whereas PDA method showed nine fungi viz. *Alternaria alternate* (05), *Aspergillus flavus* (15), *Aspergillus niger* (10), *Aspergillus terreus* (10), *F. oxysporum* (15), *Fusarium roseum* (10), *Penicillium notatum* (15) and *Trichoderma viride* (15).

In case of stored flowers, the maximum incidences of fungi were reported as compared to young and mature flowers. In stored flowers, fourteen fungi were reported viz *Alternaria alternata* (10), *Aspergillus flavus* (35), *Aspergillus niger* (55), *Aspergillus fumigatus* (20), *Aspergillus nidulance* (20), *Aspergillus terreus* (20), *Cladosporium sp.* (30), *Fusarium oxysporum* (50), *Fusarium roseum* (10), *Mucor globsus* (25), *Phoma sp.* (10), *Penicillium notatum* (20), *Rhizopus stolonifer* (30) and *Trichoderma viride* (10) on PDA. whereas in case of Blotter Paper Method only ten fungi were observed. Roy, (2003) studied that the frequent occurrence of *Aspergillus, Fusarium* and *Penicillium* species on different crude herbal drugs. Santhosh *et al.* (2011) observed 41 endophytic fungi from 195 samples of healthy leaves and stem of a red listed endangered medicinal plant *Coscinium fenestratum.* The herbal preparations had the presence of fungal contaminants with predominance of *Aspergillus* spp. and *Penicillium* spp. Sumanth *et al*.(2010) who isolated fungal genera from tested spices, found that the most common fungi isolated were *Aspergillus* spp. followed by *Alternaria alternata*, *Cladosporium*, *Curvularia*, *Fusarium* spp., *Helminthosporium* and *Trichoderma* show maximum incidence on Agar plate method.

The fungal deterioration adversely affects the chemical composition of the raw materials and thereby decreases the medicinal potency of herbal drugs,respectively, supporting the findings of present investigations. In general, flowers (young, mature) material showed decrease in the growth and incidence of fungi as compared with stored flowers material of *Spilanthes acmella*. It was found that both the Potato Dextrose Agar (PDA) method and Blotter Paper Method are effective,

routinely and consistently applicable and provide reliable results.

CONCLUSION

The present study suggests that the methods of harvesting, collection, preparing and storage of medicinal plants part must be improved for reducing percentage incidence of mycoflora and mycotoxins contaminations.

Acknowledgement:
The authors express their sincere thanks to principal, Shri Shivaji Science And Arts College, Chikhli for providing the necessary laboratory facilities.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:157-160
UGC Approved Journal No 48951

Original Article Open Access

SEM based pollen morphodiversity studies in some genera of family bignoniaceae

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Rajurkar AV, Tidke JA and Matte RS (2018) SEM based pollen morphodiversity studies in some genera of family bignoniaceae, *Int. J. of. Life Sciences*, Special Issue, A12: 157-160.

ABSTRACT

Pollen morphology of seven genera viz., *Kigellia pinnata, Milligtonia hortensis, Spathodea campanulata, Tabebuia argentea, Tabebuia rosea, Tecoma stans, Tecoma smithii* belonging family Bignoniaceae have been examined by Light and Scanning Electron Microscope (SEM). The pollen grains are medium sized, size ranges from 26.4- 44.95 µm, isopolar, radially symmetrical, tricolporate, oblate-sub-oblate, Sub-oblate to oblate spheroidal, prolate-spheroidal, the sexine ornamentation ranges from reticulate to microreticulate. The present reports give an account of pollen morphological variations in seven genera of Bignoniaceae growing in S. G. B. Amravati University campus which can be used as an identification character for the species.

Key words: Pollen morphology, Bignoniaceae, LM, SEM.

INTRODUCTION

The morphological characters of pollen grains are embodies in the exine and are important criteria in consideration of the taxonomy and inter-relationships of plants at various taxonomic levels. Hyde and Williams (1945), Erdtman (1952) and Nair (1960) studied the pollen morphology in detail in different flowering plant species.

Erdtman (1952) seems to be the first one to study the pollen morphology of the family Bignoniaceae. However, later on the pollen of various genera of the family were examined in relation to taxonomy and phylogeny. Bove (1993) examined the pollen morphology of 33 species of 19 genera of Bignoniaceae native to the south Brazilian Atlantic forest using Light- and Scanning Electron Microscopy. In the present investigation seven genera of family Bignoniaceae has been discriminate based on pollen morphological characters.

METHODOLOGY

The Polliniferous material of eight genera of Bignoniaceae were collected from Amravati University campus and stored in 70% alcohol. The studied taxa were identified from Flora of Marathwada (Naik, 1998). The collected material was crushed with a glass rod in plastic centrifuge tube and crushed material was filtered through fine meshes to isolate pollen grains. The pollen grains were prepared for light and scanning electron microscopy by the standard method described by Erdtman (1960) and Arora and Modi (2008). For light microscopy, the pollen grain were mounted in stained glycerine jelly and observations were made with Trinocular Fluroscence Microscope (Axiostar HBO 50/AC Carl zeiss). For SEM studies, pollen grain were suspended in a drop of ethanol and directly transpired with a fine pipette to a metallic stubs using double sided cello tape and coated with gold palladium in a sputtering chamber (POLARON SPUTTER COATER). The SEM examination was carried out on a LEO electron microscope (LEO 430). The measurements are based on 10 readings from each pollen type by ocular micrometer and the pollen grain size, colpi size, pore size was measured. The terminology used in accordance with Erdtman (1971), Faegri and Iverson (1964), Bhattacharya *et. al.* (2006), Agashe (2006) and Punt *et. al.* (2007).

RESULTS

Description of pollen type :
Family- Bignoniaceae

1. Kigellia pinnata **(Jacq.) DC.Prodr:** Pollen grains, PA 49.2-54 µm, EA 41.4-42 µm, sub-prolate ,radially symmetical, polar outline triangular, equatorial outline elliptic, trizonocolporate, colpi long, narrowly tapering at end,colpi length 37.2 µm, width 18 µm at equator and 6- 7.2 near pole, pori 9.6-10.8 µm in diameter,oval, mesocolpi 21.6 µm, apocolpi 9 -10.2 µm, exine 2.13-2.66 µm thick, sculpturing microreticulate, lumina circular to tetragonal, large at mesocolpi and small at apocolpium ,N3P4C5 [Fig. 1 (LM), Fig. 2 (SEM, Mag. 2.47), Table No. 1].

2. Millingtonia hortensis **L. f. Suppl. :** Pollen grains, PA 43.29 (41-50 µm), EA 44.95 (44-50 µ), oblate spheroidal, polar outline triangular obtuse, eqitorial outline elliptic, colpi faint, tricolporate, colpi length 31 µm, colpi width 4.34- 6.89 µm, pori 3.84 - 4.46 µm wide, mesocolpi 28.14-30.85 µm, apocolpi 16.6-17.23 µm, exine 2.73-3.34 µm thick, sculpturing microreticulate heterobrochate, or reticulate, muri irregularly distributed, lumina oval, elliptic, 0.61-0.91 µm wide, N3P4C5 [Fig. 3 (LM), Fig. 4 (SEM, Mag. 1.63 KX), Table No. 1].

3. Spathodea campanulata **P. Beauv.Fl.Oware Benin :** Pollen grain PA 26.4 - 27.77 µm, EA 36.65-41.65 µm, oblate-sub-oblate ,radially symmetical, polar outline triangular, equatorial outline elliptic, tricolporate, two pore at each colpi, colpi long, narrowly tapering at end, colpi 28.32-29.98 µm long and 2.8-3.2 µm wide, and pori circular 3.32-4.15 µm in diameter, distance between two ori 4.99-7.49 µm, mesocolpi 14.99-16.66 µm, apocolpi 8.33-9.16 µm, exine 2.40-3.39 µm thick sculpturing microreticulate, lumina circular , equally distributed at meso and apocolpium, N3P4C5 [Fig. 5 (LM), Fig. 6 (SEM, Mag. 885 X), Table No. 1].

4. Tabebuia argentea **(Bur.&Schum.)Britt.in Sc. Surv.Porto Rico& Virgin Isl. :** Pollen grains, PA 49.2-54 µm, EA 41.4-42 µm, sub-prolate ,radially symmetical, polar outline triangular, equatorial outline elliptic, trizonocolporate, colpi long, narrowly tapering at end,colpi length 33.43 µm long and 5.2-6 µm wide at equator and 1.5- 3 near pole, pori not distinct, mesocolpi 22.8-24 µm, apocolpi 5.4-5.6 µm, exine 2.08-2.44 µm thick, sculpturing microreticulate, lumina circular to elliptic, equally distributed at meso and apocolpium, N3P4C5 [Fig. 7 (LM), Fig. 8 (SEM, Mag. 5.60 KX) Table No. 1].

5. Tabebuia rosea **(Bertol.) DC.Prodr.** ***:*** Pollen grains, PA 35.7-36.7 µm, EA 32.56-33.73 µm, prolate-spheroidal ,radially symmetical, polar outline triangular, equatorial outline elliptic, trizonocolporate, colpi 22.56 µm long and 5.83-7.54 µm wide, pori circular 3.28-4.97 µm in diameter, mesocolpi 28.25-30.01 µm, apocolpi 6.23-6.72 µm, exine 2.22-3.08 µm thick, sculpturing microreticulate, lumina circular to elliptic, equally distributed at meso and apocolpium, N3P4C5 [Fig. 9-10 (LM), Table No.1].

6. Tecoma stans **(L.) H.B. & K. Nov. Gen. Sp.** ***:*** Pollen grain, PA 33.3, EA 43.29 µm, sub-oblate to oblate spheroidal, medium sized grain, radially symmetical, polar outline triangular, equatorial outline elliptic, obtuse convex, trizonocolporate, colpi long, narrowly

elliptic, colpi length 29.97 µm, width 4.46 µm, pori 3.46-3.54 µm wide, mesocolpi 19.07-20.71 µm, apocolpi 8.97-10.46 µm, exine 2.64-3.20 µm in thickness, sculpturing microreticulate, N3P4C5 [Fig. 11-12 (LM), Fig. 13 (SEM, Mag. 5.10 KX), Table No. 1].

8. Tecoma smithii : Pollen grain, PA 34.4, EA 27.6 µm, sub-prolate, medium sized grain, radially symmetical, polar outline triangular, equatorial outline elliptic, obtuse convex, trizonocolporate, colpi long, narrowly elliptic, colpi length 29.97 µm, width 6-6.4 µm, pori 3.6-4.4 µm wide, mesocolpi 25.6-27.2 µm, apocolpi 4.2-5.4 µm, exine 2.18-2.88 µm in thickness, sculpturing reticulate heterobrochate, microreticulate at colpal area, N3P4C5 [Fig. 14 (LM), Fig. 15 (SEM, Mag.5.28 KX), Table No. 1].

Table 1: Pollen grain characteristics of family Bignoniaceae

Sr. No.	Name of taxa	Pollen grain size (µm)P×E	Pollen shape	Aperture pattern	Colpi/pori size (µm)	Exine ornamentation
1	*Kigellia pinnata*	49.2 × 41.4	Sub-prolate	Trizonocolporate	37.2 × 18	Microreticulate
2	*Milligtonia hortensis*	43.29 × 44.95	Oblate spheroidal	Tricolporate	31× 6.89	Microreticulate heterobrochate
3	*Spathodea campanulata*	26.4 - 27.77	Oblate -sub-oblate	Tricolporate	29.98 × 3.2	Microreticulate,
4	*Tabebuia argentea*	49.2 × 41.4	Sub-prolate	Trizonocolporate	5.2	Microreticulate
5	*Tabebuia rosea*	35.7 × 32.56	Prolate-spheroidal	Trizonocolporate	22.56 ×5.83	Microreticulate
6	*Tecoma stans*	33.3 × 43.29	Sub-oblate to oblate spheroidal	Trizonocolporate	29.97 × 3.81	Microreticulate
7	*Tecoma smithii*	34.4× 27.6	Sub-prolate	Trizonocolporate	29.97 ×6.6	Reticulate heterobrochate

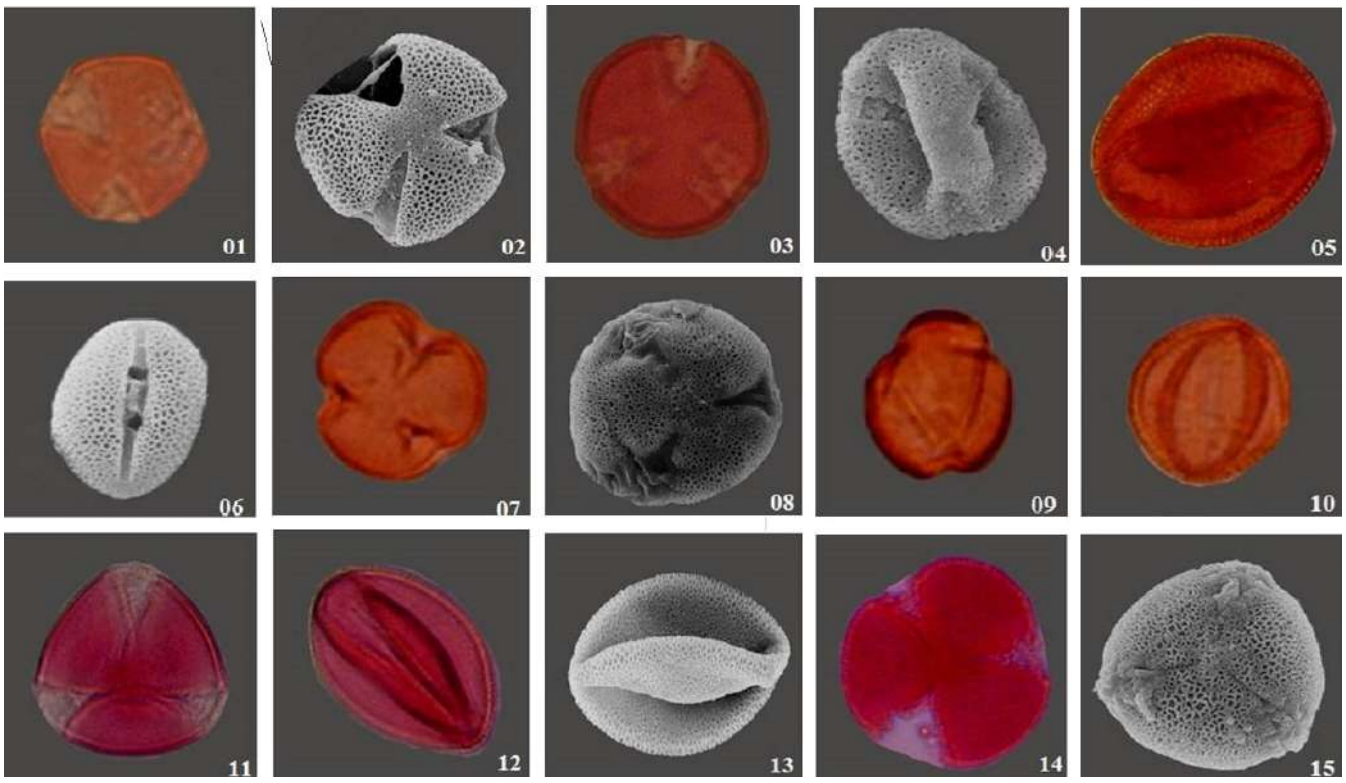

Fig. 1-15: Light and Scanning Electron Micrograph showing structure and exine sculpture of pollen grains: 1-2: *Kigellia pinnata,* **Fig. 3-4:** *Milligtonia hortensis,* **Fig. 5-6:** *Spathodea campanulate,* **Fig. 7-8:** *Tabebuia argentea* **, Fig. 9-10:** *Tabebuia rosea,* **Fig. 11-13:** *Tecoma stans,* **Fig. 14-15:** *Tecoma smithii .*

DISCUSSION

The **Bignoniaceae** family is represented by seven members. The pollen grains are medium sized, size ranges from 26.4- 44.95 µm, isopolar, radially symmetrical, tricolporate, oblate-sub-oblate e.g. *Spathodea campanulata;* sub-oblate to oblate spheroidal e.g. *Tecoma stans, Millingtonia hortensis*; prolate-spheroidal e.g. *Tabebuia rosea*, sub-prolate e.g. *Kigellia pinnata*, *Tabebuia argentea, Tecoma smithii;* the sexine ornamentation ranges from reticulate, microreticulate. No far differences were found among the genera studied only *Spathodea campanulata* having two pori at each colpi while other members shows single pori. Nayar (1990) recorded psilate tectum in *Tecoma stans* by LM studies whereas microreticulate tectum was found in *Tecoma* by SEM observation.

Chelong (2011) reported granular tectum within *Millingtonia* and *Tecoma* by LM studies while the present SEM study reveals microreticulate tectum in *Millingtonia* and *Tecoma.* Bove (1993) reported microreticulate, reticulate sexine ornamentation within the family Bignoniaceae, similar observations were noted regarding sexine ornamentation.

CONCLUSIONS

Pollen morphological characteristics study is an accurate method of relating and differentiating one plant genus to another. The investigation of pollen micro morphological characters suggests intra specific diversity in pollen types of studied genera. From the present findings it is found that qualitative and quantitative micro morphological features of the pollen can be use to discriminate species.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:161-164
UGC Approved Journal No 48951

Original Article Open Access

Effect of gamma radiation on various growth parameters of *Linum usitatissimum* L.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Khirade PD and Dudhe SS (2018) Effect of gamma radiation on various growth parameters of *Linum usitatissimum* L, *Int. J. of. Life Sciences*, Special Issue, A12: 161-164.

ABSTRACT

In present study, dry seeds of Linum *usitatissimum* L. (linseed) were irradiated with gamma rays at different doses levels of 10 kR, 20 kR, 40 kR, 60 kR, 80 kR and 100 kR to examine its effects on growth traits and morphological variation. The experiments was carried out in two sets, one with treated seeds and the other untreated which served as control. Effects of this irradiation were evaluated by studying seed germination, seedling growth, leaf size, plant height and length of internode. Gradual reduction in seed germination was observed from lower doses to higher doses. Shoots were measured longer than roots at every dose and control. Shoot and root immerged from irradiated seeds showed gradual decrease in length except 40 kR where, shoots were measured shorter than roots. Biometric measurement of leaf length showed variation among various doses whereas leaf width is constant at all doses, at 100 kR it was moderately increased. Height of the plant showed variable effect in length. Length of internode was noted almost uniform.

Keywords: gamma radiation, doses, growth parameters, Linum *usitatissimum* L.

INTRODUCTION

Linum usitatissimum L. (linseed) is a cool temperate annual herb with erect stems belonging to Linaceae family. Although there are several utilization purposes, it is cultivated commercially for its seed, which is processed into oil and a high protein stock feed after oil extraction. Gamma rays with different irradiation levels have been found to cause a large range of effects on seeds. Gamma rays have proved to be more economical and effective compared to other ionizing radiations because of their easy availability and power of penetration (Moussa 2006). Marcu (2013) examine the effects of radiation on seeds, it was found that radiation not only impacts the germination potential and actual qualities of the germinated seedlings (such as root and shoot lengths), where germination potential is the percentage of seeds that germinated overall and the time of germination compared to when the seed were planted.

The present study was conducted to examine and investigate effect of gamma radiation (γ rays) on growth and morphological changes such as seed germination, seedling growth, leaf size, plant height and length of internodes of plants.

METHODOLOGY

For present study dried seeds of *Linum usitatissimum* L. (linseed) were used. Mature seeds of linseed were collected and the seeds were kept in zip lock pouches at room temperature for further use. Dry seeds were exposed to radiation at 10 kR, 20 kR, 40 kR, 60 kR, 80 kR and 100kR doses (Source: ^{60}Co, P.G Department of Chemistry, RTM Nagpur university). The control linseed seeds were not irradiated. Effect of gamma radiation was studied on various growth and morphological parameters such as seed germination, seedling growth, leaf size, plant height and length of internodes. The experiments were carried out in two sets, one with treated seeds and the other untreated which served as control.

Irradiated seed were grown to examine percentage of germination. For every dose and control 100 seeds were grown, number of seeds germinated and non-germinated was noted. The percentage of germination was calculated and recorded. One week after shoot and root length in cm were recorded for each dose and control. After one and half month of sowing leaf size, height and internode length were examined for all doses with control. Leaf size was ascertain and recorded by measuring length and width of length in cm. Height and internode length was measured and noted.

RESULTS AND DISCUSSION

The seed germination test after gamma irradiation (10kR-100kR) revealed that the maximum germination percentage was observed in control seedlings. As illustrated in Table-1 and Fig. 1, the final germination percentage decreased with increasing gamma ray doses. At 20kR germination percentage showed inconsiderable change. The maximum decrease of the germination percentage was observed at 100kR. Statistical analysis revealed that exposure at doses higher than 10 kR significantly decrease the seeds germination. The biometric measurements of shoot and roots emerged from irradiated seeds show a significant variation of length (Table 2 and Fig.2). The highest shoot length was observed in the control plants. The gamma rays 40kR imposed a significant impact on the shoot length where more radical length was recorded than shoot. Following exposure to gamma rays, inconsistent shoot length decrease was observed among all irradiations. The maximum decrease of shoot length was observed at 40kR followed by 100kR (Table 2).

The maximum radical length was recorded in the control samples, while the radical length of samples exposed to different doses showed decrease in length. The maximum reduction of radical length was observed at 60kR. Results show that gamma radiation treatment with doses higher than 10kR significantly inhibited the length of the radicular system of plants derived from irradiated seeds. Leaf size biometric analysis revealed highest leaf length at 10kR and maximum width at 100kR than control (Table 3 and Fig.3). While in all radiations decreased leaf length was observed. The leaf length was inconsistent among all treatment.

The maximum reduction in length was recorded at 100kR. Leaf width is almost constant and similar to control except at100kR, where minimal increase was observed. Results revealed gamma radiation treatment with doses higher than 10kR significant to bring increase in length of leaf. Radiations such as 40kR, 60kR, 80 kR and 100 kR are responsible for reduction in leaf length. 100kR increases slight increase in width.

Table 1: Percentage of Seed germination

Treatment	Total no. of. Seed kept for gemrination	Total no. of. Seeds germineted	Percentage of germination
Control	100	89	89 %
10 kR	100	85	85 %
20 kR	100	88	88 %
40 kR	100	79	79 %
60 kR	100	65	65 %
80 kR	100	58	58 %
100 kR	100	56	56 %

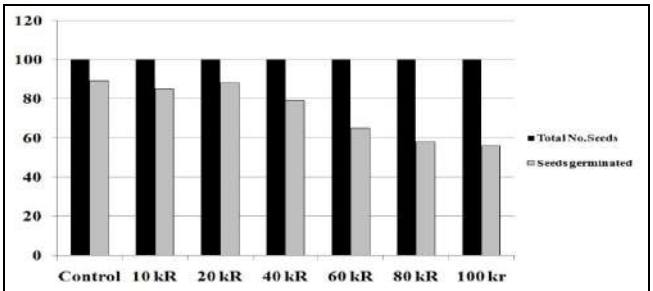

Fig. 1: Percentage of seed germination

Table 2: Biometric measurment of shoot and root emerged from irradiated seeds

Sr. No.	Control		10kR		20kR		40kR		60kR		80kR		100kR	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	14.0	7.9	12.3	8.3	11.5	7.0	6.0	7.0	8.9	4.0	8.0	4.3	7.0	4.8
2	10.7	8.7	12.7	7.6	12.4	6.8	5.0	6.8	8.3	3.6	7.6	4.4	7.2	4.7
3	13.5	8.4	10.7	6.5	10.5	6.5	4.8	6.5	8.5	3.9	7.3	3.9	6.9	4.9
4	12.0	9.4	10.3	7.1	13.2	5.0	5.5	6.3	8.7	3.7	7.4	4.3	7.3	4.4
5	12.3	8.3	11.9	7.2	11.3	6.1	5.9	6.5	8.4	3.8	7.8	4.2	7.1	4.2
Total	62.5	42.7	57.9	36.7	58.9	31.4	27.2	33.1	42.8	19	38.1	21.1	35.5	23
Ave.	12.5	8.54	11.5	7.34	11.7	6.28	5.44	6.62	8.56	3.8	7.62	4.22	7.1	4.6

***S** = Shoot length in cm and **R =** Root length in cm

Table 3: Lenght and width of internode at different kR doses levels of gamma rays

Sr. No.	Control		10kR		20kR		40kR		60kR		80kR		100kR	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	1.3	0.03	1.5	0.03	1.0	0.03	0.9	0.02	1.1	0.04	1.1	0.03	0.8	0.01
2	1.3	0.03	1.4	0.03	1.0	0.02	1.1	0.02	1.0	0.03	1.2	0.03	0.8	0.02
3	1.3	0.03	1.3	0.03	1.1	0.03	1.1	0.02	1.3	0.03	1.0	0.03	1.0	0.03
4	1.3	0.03	1.0	0.03	1.1	0.03	1.2	0.02	1.2	0.04	1.2	0.02	0.7	0.02
5	1.2	0.02	1.2	0.02	1.1	0.02	1.1	0.02	1.1	0.02	1.2	0.03	0.7	0.01
6	1.3	0.02	1.3	0.02	1.0	0.02	1.2	0.02	1.3	0.04	1.1	0.03	0.8	0.01
7	1.2	0.03	1.3	0.02	1.0	0.02	1.2	0.02	1.2	0.04	1.2	0.03	0.7	0.02
8	1.2	0.03	1.2	0.03	1.1	0.03	1.1	0.02	1.3	0.03	1.1	0.02	1.0	0.01
9	1.3	0.02	1.5	0.03	1.1	0.02	1.2	0.02	1.0	0.02	1.0	0.03	0.9	0.01
10	1.3	0.02	1.5	0.03	1.0	0.02	1.2	0.02	1.2	0.03	1.0	0.03	0.9	0.01
Total	11.4	0.26	13.2	0.28	10.5	0.24	11.3	0.20	11.7	0.32	11.1	0.28	8.3	0.15
Ave.	1.27	0.02	1.32	0.02	1.05	0.02	1.13	0.02	1.17	0.03	1.11	0.02	0.83	0.05

***H** = Height in cm and **I =** length of internode in cm

Table 4: Height and length of internode at different kR doses levels of gamma rays

Sr. No.	Control		10kR		20kR		40kR		60kR		80kR		100kR	
	H	I	H	I	H	I	H	I	H	I	H	I	H	I
1	37.5	1.7	28.5	1.3	33.0	1.0	18.0	1.0	31.0	1.1	29.0	1.6	27.0	0.8
2	36.0	1.3	29.0	0.6	31.0	1.0	25.0	1.4	29.0	0.5	28.0	1.6	27.5	1.5
3	36.0	0.9	32.0	1.0	29.5	0.6	27.5	0.6	31.0	0.7	37.0	1.5	29.0	0.9
4	37.0	1.3	31.0	0.7	33.0	1.0	33.0	0.9	33.0	1.0	28.0	1.6	29.0	1.4
5	34.0	0.6	33.0	0.9	26.0	1.4	31.0	0.8	32.0	0.7	31.0	1.4	31.0	1.3
Total	180.5	5.8	153.5	4.5	152.5	5.0	134.5	4.7	156	4.0	153	7.7	143.5	5.9
Ave.	36.1	1.16	30.7	0.9	30.5	1.0	26.9	0.94	31.2	1.3	30.6	1.54	28.7	1.1

***H** = Height in cm and **I =** length of internode in cm

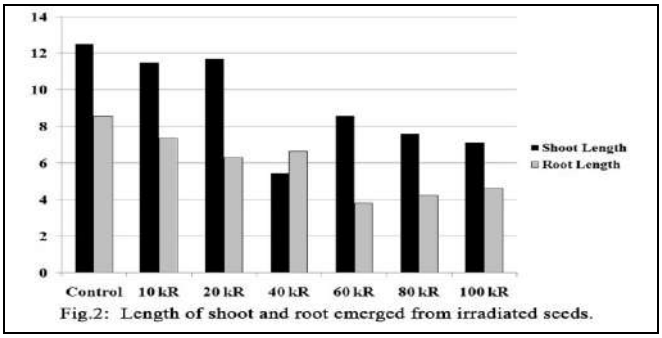

Fig.2: Length of shoot and root emerged from irradiated seeds.

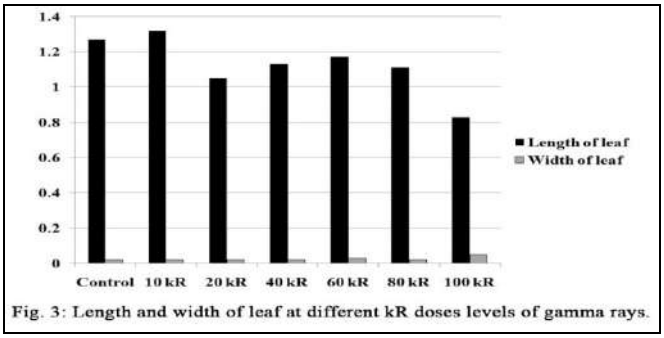

Fig. 3: Length and width of leaf at different kR doses levels of gamma rays.

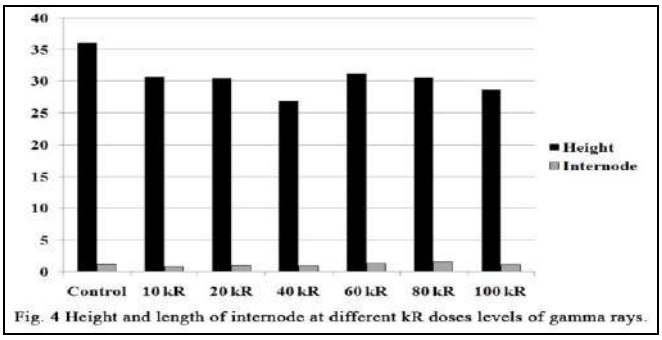

Fig. 4 Height and length of internode at different kR doses levels of gamma rays.

Height of plant and length of internode showed significant variations (Table 4 and Fig.4). Maximum height was observed in control while in irradiated plants the height was variable. Maximum reduction in plant height was observed in plant irradiated at 40kR. Internode length of plant treated at 80 kR was noted maximum. However internode length has minimal variation in length. Biometric analysis revealed that exposure at dose 10kR and higher are significant to bring decrease in plant height. 60kR and 80kR are noticed to increase in internode length.

These results are in accordance with the findings of previous researchers who reported that the seed germination potential of different crops decreased by increasing the irradiation dose. Akgun and Tosum (2004) reported the reduced germination due to inhibitory effect of gamma radiation. Gradual reduction in shoot and root length immerging from irradiated seeds are reported by Delia Marcu et al. (2013) in maize. Yadav (2016) observed different doses of gamma rays produced variable effect in the leaf morphology which did not show dose dependent effect. Irfaq & Nawab (2001) observed gamma treatment (0.1, 0.2, 0.3, 0.4 kGy) of three wheat cultivars caused a delay of the germination process and decrease of the survival percentage and plant height.

CONCLUSION

The present results show that seed treatment with 60Co gamma radiation (10kR-100kR) decreased seed germination, affects the shoot and root growth, moderate change in leaf morphology, decreased plant height and internode. These findings confirm the results obtained by earlier studies that showed the inhibitory effects of plant growth and development exposed to high doses of gamma radiation.

Acknowledgement

The authors wish to express their gratitude to the PGTD Department of Chemistry, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur for providing Gamma radiation facility.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :165-168
UGC Approved Journal No 48951

Original Article Open Access

Phytochemical analysis and antimicrobial activity of *Dendrophthoe falcata* (Linn F.) Etting. leaves.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gond GS, Khirade PD, Dudhe SS and Sharma MA (2018) Phytochemical analysis and antimicrobial activity of *Dendrophthoe falcata* (Linn F.) Etting.leaves., *Int. J. of. Life Sciences*, Special Issue, A12: 165-168.

ABSTRACT

Dendrophthoe falcata (Linn F.) Etting. commonly known as 'Vanda' belongs to family Loranthaceae. This is an evergreen parasitic plant grown on different host plant. This plant species is known to have medicinally important bioactive compounds. Phytochemical analysis is worthy step in detection of phytochemicals. It may add valuable informative data lead to novel drug discovery. Present investigation was carried out to investigate and examine phytochemical constituents and antimicrobial activity of Dendrophthoe falcata (Linn. f.) Etting. In this study extracts of leaves was utilized for phytochemical and antimicrobial screening using standard methods. The screening resulted in the detection of tannins, flavonoids, saponins, terpanoids, reducing sugars, anthraquinone and alkaloids. Leaves methanolic extract have been found to have in vitro antimicrobial properties at different concentrations.

Keywords: Dendrophthoe falcata, phytochemical analysis, antimicrobial screening

INTRODUCTION

Phytochemicals offers medicinal attributes to the plants frequently referred as secondary metabolites because the plants that manufacture them may have little need for them. Solomon, et al. (2013) stated that these phytochemicals are synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds etc. i.e. any part of the plant body may contain bioactive compounds These chemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions (Thilagavathi *et al.*, 2015). Alkaloids, terpenoids, tannins, saponins and phenolic compounds are most important bioactive groups of plants (Edeoga *et al.*, 2005). The species *Dendrophthoe falcata* (Linn F.) Etting. belongs to family Loranthaceae. This genus is rich with different biological active compounds. The present investigation was undertaken to investigate and examine phytochemical

constituents and antimicrobial activity of *Dendrophthoe falcata* (Linn. f.) Etting.

METHODOLOGY

Plant Material: For present phytochemical and antimicrobial activity investigation a plant species *Dendrophthoe falcata* (Linn F.) Etting. having variety of medicinally important phytochemicals is utilized.

Methodology: To carry out phytochemical and antimicrobial activity investigation following methods were adopted.

i) Extensive exploration: Frequent visit to different forest area of Ballarpur, Chandrapur district. Maharshtra, India were made to find out the *Dendrophthoe falcata* (Linn. f.) Etting. during the month of November 2015 to January 2016.

ii) Collection of plant material: Fresh plant of *Dendrophthoe falcata* (Linn. f.) Etting. growing *on Madhuca indica* was collected for further study. Naturally growing plant species under study was photographed along with host species. For the identification purpose plant species under study was photographed along with certain flowering twigs. Certain photographs of the flowers were also taken to make identification easier. During collection leaves along with stems were collected and brought to the laboratory for further study.

iii) Identification of collected plant species: Identification of collected plant species was carried out by referring different floras, books and relevant journal articles. Leaves of freshly collected plant species were selected for further study.

iv) Processing of Plant Material: Leaves were detached from the stems. Detached leaves were washed thoroughly with tap water to remove dust particles followed by distilled water in the laboratory. These leaves were allowed to shed dry for about two weeks. The dried leaves were grinded and sieved. The powder obtained was stored in zip-lock pouches and tested for the presence of various phytochemicals.

v) Extraction: Powdered plant material was subjected to successive solvent extraction. For the extraction Soxhlet extractor (Harborne, 1973) with methanol for 24 hrs method was adopted. The obtained crude mixture was evaporated and stored in closed container in the refrigerator. The condensed extracts were used for preliminary screening of phytochemical constituents.

vi) Antibacterial Activity: For screening of antimicrobial activity two bacterial cultures *Staphylococcus aureus* and *Escherichia coli* were selected for the present investigation.

a) Preparation of microbial inoculums: The fresh microbial cultures were prepared and used during the research period. The Nutrient Broth (NB) was prepared and poured into several tubes. Then pure microbial cultures were collected from the institute and inoculated in the tubes by using inoculation needles or loops. After these tubes were incubated (37^0C for 24-28 hrs for bacteria). After incubation the cultures were used for the experiments.

b) Preparation of nutrient agar medium: 1000ml of Nutrient agar medium is prepared; P^H was adjusted to 6.8. The medium is sterilized by using autoclave at 121 ^{0}C for 15 Ibs pressure for 15 minutes and allowed to cool.

c) Screening for antibacterial activity (agar well diffusion method): The antibacterial activities of the plants were tested against the selected bacterial cultures. The 20 ml of sterilized Nutrient agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 6 mm are made in the medium by using a sterile cork borer, 150 µl of extracts were transferred into separate wells. After these plates were incubated at 37 ^{0}C for 24-28 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around each well.

d) Antibiotic sensitivity test on bacteria (positive control): The antibiotic sensitivity test using standard antibiotics (kanamycin, methicillin and ampicillin) were analyzed by the method of Bauer *et al.,* (1966). The sterilized nutrient agar medium was poured into each sterile petriplates and allowed to solidify.

By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic disc was placed on the

bacterial plates. Then the plates were incubated for 24 hours at 37 ^{0}C. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

RESULTS AND DISCUSSION

The extract subjected to phytochemical screening showed positive tests indicating their presence for Tannins, Saponnins, Reducing sugars, Alkaloids, Terpenoids, Flavonoids and Anthraquinon. Tests for Cardiac glycosides and Phenols of the extract were negative showing their absence in the extract (Fig. 1 and Table I.)

Antimicrobial activity of *Dendrophthoe falcata* (Linn. f.) Etting. leaves methanolic extract have been found to have *in vitro* antimicrobial properties at different concentrations. It was observed that the extract was active against strains of *S. aureus* (Fig. No. 2 and Table II) and was less active against *E.coli* (Fig. No.3 and Table II). The extract exhibited zone of inhibition against *S. aureus.*

The extract was active against *S. aureus* showing zone of inhibition at different concentration as at 100 mg/ ml 15 mm of zone of inhibition was found while at 50 mg/ml observed zone of inhibition was 13 mm (Fig. No. 2 and Table II).

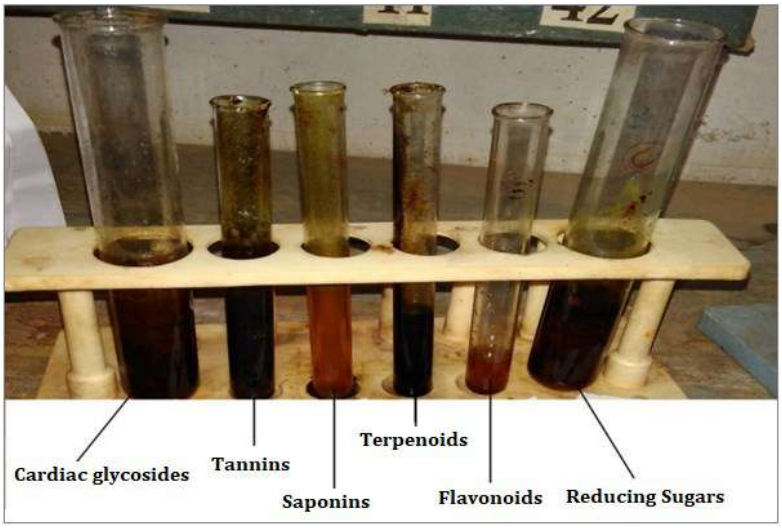

Fig. 1: Test for phytochemical constituents of *Dendrophthoe falcata* (Linn. F) etting. Leaves extracts

Table 1: phytochemical constituents of *Dendrophthoe falcata* (Linn. F) etting. Leaves extracts

Phytochemical	Tests	Inferences*
Tannins	Ferric chloride test	+
Saponnins	Foam test	+
Reducing Sugars	Fehling solution test	+
Alkaloids	Mayers reagent test	+
Terpenoids	Chloroform test	+
Flavonoids	Ammonia test	+
Cardiac glycosides	Glacial Acetic acid test	-
Anthraquinone	Conc. Sulfuric acid test	+
Phenols	Ferric chloride test	-

*** +(Positive) and – (Negative)**

Table 2: Antimicrobial activity at different concentration of leaves extracts

Plant species	Concentraction (Mg/ml)	Zone of inhibitions of bacteria (mm)	
		S. aureus	*E.coli*
Dendrophthoe falcata	100 mg/ml	15 mm	No inhibitions
	50mg/ml	13 mm	No inhibitions

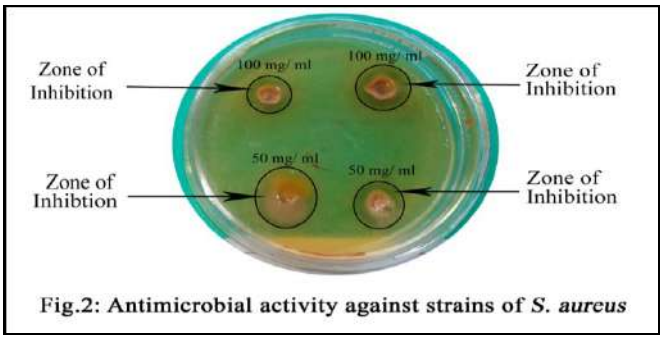

Fig.2: Antimicrobial activity against strains of *S. aureus*

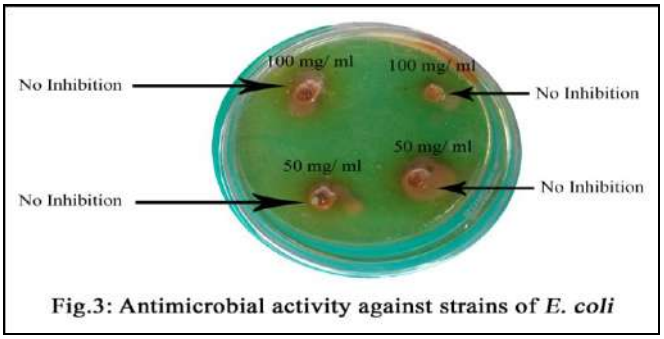

Fig.3: Antimicrobial activity against strains of *E. coli*

The phytochemical analysis and antimicrobial study of *Dendrophthoe falcata* (Linn. f.) Etting. indicates the presence of medicinally important phytochemicals and extract of leaves was active against the microorganisms used. These results are in accordance with the findings of previous researchers.

The phytochemical analysis of *Dendrophthoe falcata* (Linn. f.) Etting. reveals the presence of such important phytochemicals including Tannins, Saponnins, Reducing sugars, Alkaloids, Terpenoids, Flavonoids and Anthraquinon. Sinoria *et al.* (2011) studies *Dendrophthoe falcata* (Linn. f.) Etting. for various aspects and reported the presence of these phytochemicals. Pandey and Dravyaguna (2004) stated medicinal importance of the plant species. They also suggest a drug extracted from this plant species is useful in urinary diseases. Many workers reported the presence of medicinally important phytochemicals in *Dendrophthoe falcata* (Linn. f.) Etting. Some of them mentioned their medicinal properties and uses. The present study reveals the antimicrobial activity against the leaf extract of *Dendrophthoe falcata* (Linn. f.) Etting. The results of this study suggest the extract was active against strains of *S. aureus* while it is less active against *E.coli.* Antimicrobial activity of the plant under study was undertaken by Pattanayak and Sunita (2008). They also found the extract of present plant species is active against microorganisms.

CONCLUSION

The present investigation on phytochemistry and antimicrobial activity of *Dendrophthoe falcata* (Linn. f.) Etting. suggests that the plant has many bioactive compounds. From this investigation and previous literature survey, it may conclude that the plants *Dendrophthoe falcata* (Linn. f.) Etting. is found to be rich in Phenols, Tannins , Saponins, Flavonoids, Alkaloids etc. and shows the antimicrobial properties hence, plants are useful for the medicinal purpose. Therefore, further studies may be carried out to prove the potential of this plant.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:169-172
UGC Approved Journal No 48951

Original Article Open Access

Phytoplankton diversity of Gaurala lake in Bhadrawati, Dist. Chandrapur, Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Nasare PN (2018) Phytoplankton diversity of gaurala lake in Bhadrawati, Dist.-Chandrapur, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A12: 169-172.

ABSTRACT

The present study was carried out on Phytoplankton diversity of Gaurala Lake in Bhadrawati, dist. - Chandrapur, Maharashtra state, India during 2015-2016. The present paper reveals the phytoplankton diversity in Gaurala Lake. During this study, 40 genera of phytoplanktons were recorded, out of 40 genera, 12 genera recorded for cyanophyceae, 23 genera for chlorophyceae and 5 genera for bacillariophyceae amongst three family members, chlorophyceae members found dominant and then cyanophyceae & bacillariophyceae. In case of chlorophyceae members, amongs them *Vaucheria, Cosmarium, Spirogyra, Volvox, Chara and Oedogonium* were found to be dominant. In case of cyanophyceae members, amongst them *Nostoc, Anabaena, Oscillatoria, Anacystist, Microsystis* were found to be dominant. In case of bacillariophyceae, *Diatoms* was found to be dominant. This indicates that the plenty of phytoplanktons are available in the lake and maintaining the ecological balance of the particular lake and will be helpful for the feeding zooplanktons and fishes which will maintain food chain and sustainable ecological balance of the lake.

Keywords: Gaurala Lake, Phytoplankton diversity, Bhadrawati, Chandrapur.

INTRODUCTION

Gaurala Lake is located near Gaurala locality ,near Vinayak Mandir on the way to railway station in Bhadrawati, Dist.- Chandrapur. It is approximately 2 km. from Nilkanthrao Shinde Science & Arts College, Bhadrawati. The present investigation as an attempt to study the phytoplankton diversity of the Gaurala Lake in Bhadrawati, Dist. Chandrapur of Maharashtra state during 2015-2016 . They are of great importance as a source of live food for zooplanktons and fishes. Phytoplankton are the primary producers, which forms the base of an autotrophic food chain.

METHODOLOGY

The samples of phytoplankton from three sampling sites were collected once in a month from the Gaurala Lake in Bhadrawati during 2015-2016. The samples were collected from surface water. The phytoplanktons were counted by drop count method (Lackey, 1957). The phytoplankton species were indentified following Edmondson (1966), Needham and Needham (1978) and APHA (1998). The results were expressed as number of organisms/ml.

RESULTS AND DISCUSSION

During the present investigation, 40 genera of phytoplanktons belonging to cyanophyceae, chlorophyceae and bacillariophyceae were recorded.

Table 1: Phytoplankton diversity of Gaurala Lake in Bhadrawati

Sr. No.	Genera / Species	Months (2015– 2016)				
A	**CYANOPHYCEAE**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
1	*Gloeocapsa sp.*	3	8	14	19	16
2	*Microcystis sp.*	13	18	17	25	14
3	*Nostoc sp.*	15	18	24	40	55
4	*Spirulina sp.*	1	3	10	12	16
5	*Oscillatoria sp.*	19	25	28	34	53
6	*Anacustis sp.*	16	22	29	15	37
7	*Gleotrichia sp.*	6	9	12	4	8
8	*Anabaena sp.*	12	15	19	25	48
9	*Rivularia sp.*	8	9	15	3	15
10	*Scytonema sp.*	3	2	9	4	6
11	*Cylindrospermum sp.*	4	8	12	15	14
12	*Tolypothrix sp.*	4	10	9	7	12
B	**CHLOROPHYCEAE**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
1	*Chlamydomonas sp.*	9	3	12	6	5
2	*Eudorina sp.*	2	6	8	4	10
3	*Scenedesmus sp.*	12	10	11	8	12
4	*Draparnaldia sp.*	8	12	14	18	23
5	*Fritschiella sp.*	9	3	6	8	7
6	*Oedogonium sp.*	14	15	10	23	18
7	*Zygnema sp.*	3	10	8	12	6
8	*Cosmarium sp.*	13	18	23	33	30
9	*Hydrodictyon sp.*	3	14	17	10	12
10	*Spriogyra sp.*	20	18	23	28	14
11	*Vaucheria sp.*	14	20	33	38	35
12	*Chara sp.*	16	14	19	22	24
13	*Nitella sp.*	6	8	4	2	13
14	*Volvox sp.*	12	14	18	30	32
15	*Pediastrum sp.*	8	10	13	18	16
16	*Pithophora sp.*	3	8	1	1	2
17	*Cladophora sp.*	8	10	3	2	8
18	*Protococcus sp (pleurococcus sp)*	4	9	12	16	3
19	Stigeoclonium sp.	5	13	15	19	3
20	Coleochaete sp.	3	8	12	15	18
21	*Chateophora sp.*	4	2	1	8	10
22	*Ulothrix sp.*	3	4	8	20	3
23	*Chlorella sp.*	4	8	3	13	10
C	**BACILLARIOPHYCEAE**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**
1	*Diatom sp.*	18	19	30	35	38
2	*Cyclotella sp.*	3	14	10	6	8
3	*Navicula sp.*	9	2	3	1	1
4	*Nitzschia sp.*	9	3	6	4	1
5	*Rhopalodia sp.*	6	12	3	2	4

*The numbers in table indicates no. of organisms recorded per ml.

Members of cyanophyceae viz *Gloeocapsa, Microcystis, Nostoc, Spirulina Oscillatoria, Anacystis, Gleotrichia, Anabaena Rivularia, Scytonema, Stigonema, Cylindrospermum, Tolypothrix, Oscillatoria* were observed throughout the investigation period, amongst them *Nostoc, Anabaena, Oscillatoria, Anacystis, Microsystis* were found to be dominant. Member of chlorophyceae viz. *Chalmydomonas, Pandorina, Eudorina, Scenedesmus, Draparnaldia, Fritschiella, Oedogonium, Zygnema, Cosmarium, Hydrodicyton, Spirogyra, Vaucheria, Chara, Nitella, Volvox, Pediastrum, Mougeoita, Pithophora, Cladophora, Protococcus, Stigeoclonium, Coleachaete, Chaetophora, Ulothrix, Chlorella,* were observed throughout the study period Amongs them *Vaucheria, Cosmarium, Spirogyra, Volvox Chara and Oedogonium* were found to be dominant (Table 1).

Five members of bacillariophyceae viz Diatom, *Cyclotella, Navicula, Nitzschia, Rhopalodia* have recorded. Amongst Bacillariophyceae *Diatoms* was found to be dominant. Similar type of investigation was done by several workers. Kumawat and Jawale (2003) recorded 59 genera of phytoplankton from a fish ponds at Angale. Out of these 14 genera belonged to chlorphyceae. In the same study, eight genera were observed throughout the year. Somani and Pejaver (2003) also reported 14 genera of Chlorophyceae, in the lake Masunda, Thane, Maharashtra. The species such as *Clostarium, Cosmarium, Oedogonium, Ulothrix, Zygnema, Chara, Nitella* were observed throughout the year. The *Chlamydomonas, Chlorella, Cladophora, Pediastrum, Scenedesmus* were observed only in monsoon months. *Hydrodictyon sp.* was observed only in the month of June.

Tripathi and Pande (1995) observed maximum blue green population during summer months while minimum during winter. Harris and James (1974), Wetzel (1975) observed the occurrence of *Microcystis,* the toxin producing blue green in blooms is a significant feature of tropical waters the species of *Microcystis* such as *M. protocystis, M. incera, M. aeruginosa, M. lotoralits; Oscallatoria, O. princeps, O. limosa, Lyngbya, L. majuscule, Nostoc sp.* and *Anabaena sp.* were found to be toxin producing algal species. Nasare et al (2009) observed six members of Cyanophyceae viz. *Oscillatoria, Micricystis Gleotrichia Anacystis, Spirulina, Agmenelleum* in Khadki lake of Chandrapur District, Maharashtra.

Rao and Raju (2000) observed the Bacillariophyceae species represented by *Melosira, Synedra, Navicula, Nitzschia, Gyrosigma, Cymbella* and Amphora in fish culture pond at Nambur near Guntur, Andhra Pradesh. Pendse *et al.* (2000) observed the Euglenophyceae species *Euglena Phacus* and *Trachelomonas* in percolation tank of Dasane, Maharashtra.

Sirsat *et al.* (2004) recorded 24 genera of Phytoplanktons from fresh water ponds at Dharampuri in Beed District, Maharashtra. Similarly, Pawar *et al* (2006) recorded 61 genera of Phytoplankton from Pethwadaj Dam of Nanded district in Maharashtra. Nafeesa Begum and Narayana (2006) recorded 85 species of phytoplankton from four lentic water bodies in and around Davangarere city, Karnataka.

Nasare et al. (2009 a) observed nine cyanophyean members during winter season. Nasare et al (2009 b) also studied the Phytoplankton biodiversity of Vinjasan Lake in Bhadrawati town of Chandrapur district, Maharashtra state, India. Darshana Bhosale & Nasare (2010) observed Chlorophyceae members as dominant in the reservoir while Englenophyceae members were found scanty. Cyanophyceae and Bacillariophyceae members were also found in adequate numbers. Nasare (2014) observed Chlorophyceae, Englenophyceae, Bacillariophyceae and Cyanophyceae members in Masanghat Lake of Bhadrawati, Dist.- Chandrapur, Maharashtra state, India. during Jan. 2013 to June 2013.

CONCLUSION

It is concluded that the chlorophyceae members were found dominant than the cyanophyceae and bacillariophyceae members. Bacillariophyceae members were less than cyanophyceae and Chlorophyceae members. The cyanophyceae members were in the moderate range. They are of great importance as a source of live food for zooplanktons and fishes. This indicates that the plenty of phytoplanktons were available in the lake and maintaining the ecological balance of the particular lake and will be helpful for the feeding zooplanktons and fishes which will maintain food chain and sustainable ecological balance of the lake.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :173-176
UGC Approved Journal No 48951

Original Article Open Access

Angiospermic dicotyledonous seed from the Deccan intertrappean beds of Singhpur, Madhya Pradesh, India

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Meshram SM and Mohture VM (2018) Angiospermic dicotyledonnous seed from the Deccan intertrappean beds of Singhpur, Madhya Pradesh, India., *Int. J. of. Life Sciences*, Special Issue, A12: 173-176.

ABSTRACT

A well preserved dicotyledonous fossil seed was collected from Singhpur M.P. The seed is polygonal in shape, measuring about 5 mm in length and 2.5 mm in breadth, showing stalk like structure at the top with a slit which might be representing the micropyle. The seed coat is bitegmic having outer integument and inner integument. The embryo is very small and occupies the minimum space of the seed. The seed though shows some resemblances of the present day families like Apocynaceae, Alangiaceae, Bignoniaceae, Boraginaceae, Companulaceae, Compositeae, Loganiaceae, Martyniaceae, Pedaliaceae, Pittosporaceae, Sapotaceae, Solanaceae, Verbenaceae, and Convolvulaceae Polygalaceae, Simaroubaceae, Celastraceae, Rhamnaceae. It has close affinities with the members of the family Polygalaceae. It could not conclusively be traced to any particular genus but it broadly placed under Polygalaceae.

Key words- Dicot seed, Bitegmic, Polygalaceae, Deccan Intertrappean

INTRODUCTION

The present chapter deals with a study of fossil dicotyledonous Seed from the Deccan Intertrappean Beds of Jabalpur, Madhya Pradesh, India. So far few seeds have been reported from the different fossliferous localities of Deccan Intertrappean beds of India. They are *Clusiocarpus arillatus* (Kumar, 1984), *Clausiocarpus indicum* (Kolhe and Wazalwar 1998), from Nawargaon, *Deccanosperma allirata, Ramakonospermus chitaleynsis, Mahabalespermum minutum* (Juneja, 1993) and *Ramakonospermus singhpurii* (Bhowal, 2003). Monocotyledonous phoenicoid seed is reported from Pisdura, Maharashtra by Ambawani and Dutta (2005). *Capparisocarpus nagpurii* (Konde 2012). So the present report of new dicot seed from Singhpur is noteworthy contribution to the knowledge of fossil seeds.

METHODOLOGY

The present work was carried out in Singhpur M.P. The material of seed was exposed on fossil chert in obliquely longitudinal view. It was etched with Hydrofluoric acid and washed under water. Serial peel sections were taken without grinding the material Peels were mounted in Canada balsam and studied. Camera lucida sketches were drawn for its detailed study.

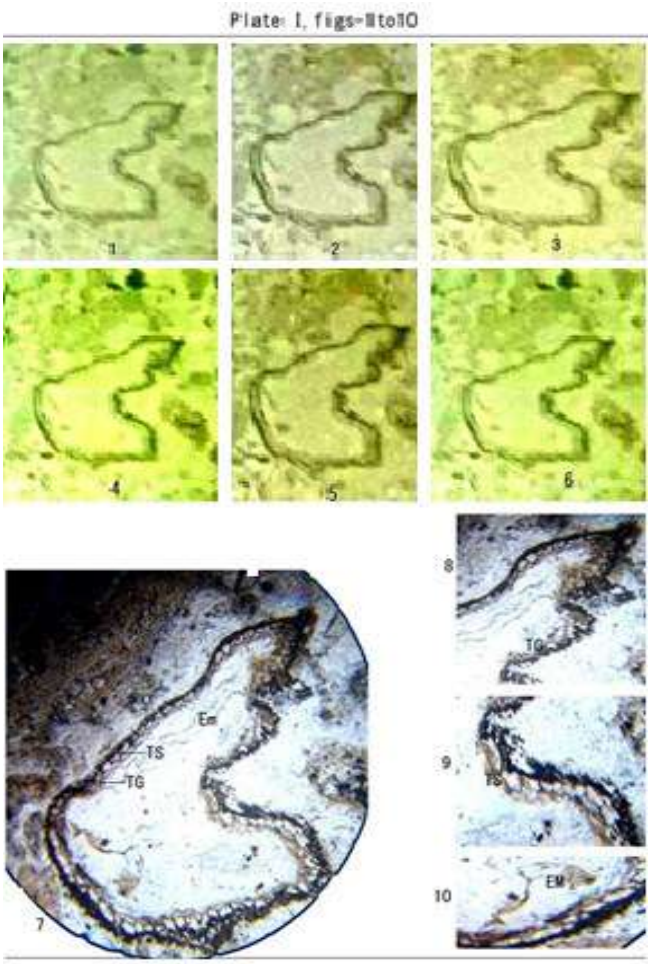
Fig: 1 Satellite image of Singpur Madhya Pradesh India

DESCRIPTION: A seed is petrified, dicotyledonous, bitegmic and endospermic. The seed is differentiated into bitegmic seed coat and large embryo (Plate I. Figs. 1,2,3; Text Figs.1-8) It is polygonal in shape measuring **5 mm** in length and **2.5 mm** in breadth, showing stalk like structure at the top with a slit which might be representing the micropyle. The seed coat is bitegmic having outer integument and inner integument. The embryo is very small and occupies the minimum space of the seed (Plate I. Figs. 1, 2, 3; Text Figs.1-8). The embryo is seen in first few sections and after that it starts disappearing.

SEED COAT: The seed coat is well preserved and differentiated into testa & tegmen. It is bitegmic consisting of testa and tegmen. The seed coat is broad at the upper region (Plate I. Figs. 1, 2, 3; Text Figs.1-8). It measures **640 µm- 340 µm** in width at the broadest and narrowest region respectively.

Seed coat: Seed coat is differentiated into testa and tegmen (Plate I, Fig.9; Text Figs. 2).

1) Testa - It is made up of single layer Consist of a **270 µm** outermost layer thin walled epidermal cells.It is rectangular to polygonal shape and continus. (Plate I, Fig.9; Text Figs. 2-3).



2) Tegmen - It is innermost zone, made up of 3 - 4 celled region. The cells are thick walled, penta & hexagonal in shape. The width varies from **70 µm** to **75 µm** (Plate I, Fig.9; Text Figs. 2-3).

EMBRYO: The embryo is ill preserved. It is not differentiated into parts, but two cotyledons are seen in some section. (Plate I Figs.9-10; Text Fig. 4). Embryo occupies the few space of seed. In between wall of the seed and embryo there is a space all around in this region some thin walled cells are seen.

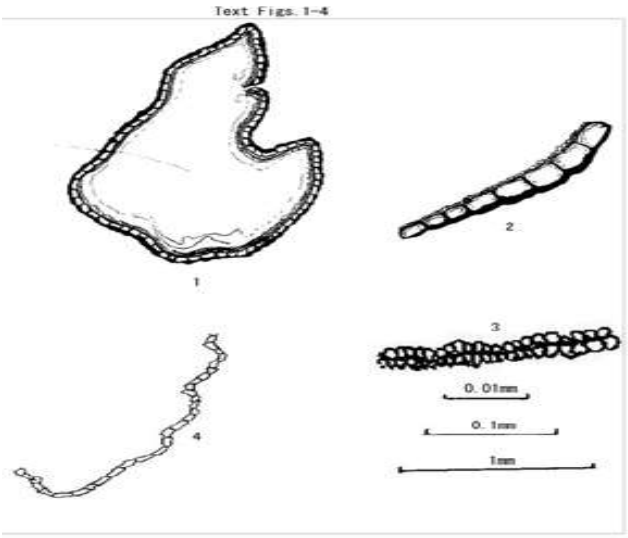
ENDOSPERM: Surrounding the embryo there are thin walled parenchymatous cells which represents the tissue of endosperm. These cells are soft in nature (Plate I, Fig.10).

DISCUSSION & COMPARISON

On the basis of above description seed has certain peculiar characters, which are considered for the identification of seed.

1) Seed is small in size, 5 mm in length.

2) Seed polygonal in shape.
3) Seed coat bitegmic showing presence of testa and tegmen.
4) Testa shows single layer epidermis.
5) Tegmen is made up of 3-4 celled regions.
6) Testa shows well arranged polygonal to elongated cells, some are crushed.
7) Split seen in the seed coat region forming micropyle.
8) Endosperm is not well preserved but some soft cells are seen may be its soft nature.
9) Embryo is ill preserved.
10) Present two cotyledons.
11) Suspensor is not present.



All these characters are of great help in the identification of seeds and find its affinities with seeds of living families. According to Eames (1961) there is a greater reduction of suspensor in angiospermic embryo. In the present fossil seed also, suspensor is completely absent.

The most important characters helpful in the identification of seed is unitegmic seed coat. Testa is differentiated into unspecialized squarish parenchymatous epidermal layer ,middle layer of the testa is also not specialized made up of pent-hexagonal cells.

The studied fossil exhibits certain characters of exotestal seed like the testa have no mechanical tissue as their inner tissues are generally crushe by endosperm or embryo (Corner, 1976). The fossil seed shows embryo with two cotyledons, hypocotyle region, narrow radicle and absence of suspensor. These characters confirm that the seed under investigation is an angiospermic and dicotyledonous in nature which is derived from anatropous ovule. Seed is exarllate. Embryo is large with little endosperm.

After going through the available literature, the standard books of taxonomy and embryology by Rendle (1938); Maheshwari (1950); Hutchinson (1959); Eames (1961); Fahn (1974) were used, and most useful among all by Corner (1976), was of great help in resolving the problem of systematic position of the seed.

Corner (1976) has mentioned about 190 families having unitegmic as well bitegmic seeds, but present paper considered some families showing unitegmic and bitegmic seeds with anatropous ovule like Apocynaceae, Alangiaceae, Bignoniaceae, Boraginaceae, Companulaceae, Compositeae, Loganiaceae, Martyniaceae, Pedaliaceae, Pittosporaceae, Sapotaceae, Solanaceae, Verbenaceae, and Convolvulaceae Polygalaceae, Simaroubaceae, Celastraceae, Rhamnaceae.

Out of these fossil seed shares most of the characters of Pedaliaceae, Martyniaceae, and convolvulaceae. In *Pedaliaceae*, ovules are anatropous, seeds are unitegmic, seed exotestal, exarllate, testa shows presence of palisade, endosperm cellular and embryo is straight. But ,they are quite different like shape and testa single layer therefore it is different from the present seed in minute details like outer integument in seeds of Pedaliaceae is of thick walled lignified cells which are thin walled and parenchymatous in fossil seed.

The seeds of *Martyniaceae*, also have anatropous ovule, unitegmic seed coat, exarllate seed, endosperm cellular and embryo wavy like in fossil seed. But in family Martyniaceae testa is reduced to a sub gelatinous pellicle of large thin walled or sclerotic cells which are not seen (Corner, 1976) in fossil seed. Thus fossil seed differs from the seeds of Martyniaceae. The fossil seed resembles the family *Convolvulaceae* (Corner, 1976) which is widely distributed in tropical and subtropical regions (Hutchinson, 1959, Rendle, 1938) in bearing anatropous ovule with unitegmic seed coat, exarillate seed but the seeds of Convolvulaceae show mesophyll tissue the seed coat and hence it does not correlate with this family.

In Simaroubaceae seed minute to medium size, elongated to elliptical in shape. Seed coat differentiated into Testa and tegmen, present seed also bitegmic but differ in size and shape therefore it is different. In Celastraceae seed obovoid to elongate in shape, seed

medium size. Present fossil seed different to all characters. In Rhamnaceae sp.*Ventilago denticulata*, seed midium to large in size, rounded at the apex, seed coat differentiate testa and tegmen but present fossil seed is small and polygonal in shape therefore it is different. In Polygalaceae seed minute, oval to elliptical in shape with testa and tegmen.Tegmen papery layers and testa thick layers (Corner, 1976) but present seed is polygonal in shape as well as differ in testa and tegmen. In this family shows some resemblances in species *polygala arvensis* like seed size and shape, testa and tegmen thickening. *Polygala arvensis* shows seed having length 3 mm to 7mm. *polygala elongata also shows similar* characters but measurement is different. *Polygala erioptera* shows polygonal shape seed with testa and tegmen. Testa single layer and it is made by rectangular to polygonal parenchymatous cells.

From the above discussion it is clear that fossil seed show close resemblance with the family polygalaceae. The fossil seed under investigation is also compared with earlier reported fossil seeds .The previously reported seed *Clusiocarpus arillatus* (Kumar, 1984) and *Clausicioarpus indicum* differs in seed coat layers. When compared with *Ramkonospemum chitaleyensis (*Juneja, 1993) fossil shows many dissimilarities like embryo is curved and convolute which is not well curved in present specimen as well as seed coat, embryo and endospermic tissues is different therefore it is totally different.

Deccanosperma arillata (Juneja, 1993) differs in having arillate and bitegmic seed. *Mahabalespermum minutum* (Juneja, 1993), *Ramakonospermum singhpurii* (Bhowal, 2003) differ from present seed in well differentiating bitegmic seed coat. Ambwani and Dutta (2006) have reported Phonecoid seed from dinosourian coprolite at Pisdura in Chandrapur district, but present seed dicotyledonous it is not well ellipsoidal it is polygonal in shape. Thus it is the record of bitegmic seed from the Deccan Intertrappean beds of Singhpur. It is different from all other previously reported fossil seeds and show resemblance with the living seeds of living familie, polygalaceae hence named as *Polygalaceaeospermum singhpurii* gen et, sp.nov.

DIAGNOSIS

***Polygalaceaeospermum Singhpurii* gen. nov.:** Seed small polygonal, bitegmic, dicotyledonous. Seed coat with testa and tegmen only. Embryo small well preserved with cotyledons, micropyle seen. Seed coat with testa and tegmen differentiated into outer single layered epidermis made by parenchymatous cells. Tegmen made by in soft parenchymatous cells. Endosperm tissue seen.

***Polygalaceaeospermum singhpurii* gen. et sp.nov.:** Small ovoid unitegmic seed measuring **5 mm** and **2.5 mm** diameter **in** size. Seed cavity oval in shape is differentiated into outer broad testa and tegmen not differentiatd tegman. Testa measures **90 µm** in thickness differentiated into outer single layered epidermis, middle layer, embryo well preserved with two cotyledons measuring **3060 µm** in length and **1200 µm** in breadth in size. Endosperm tissue seen.

Holotype : SMM / Dicot Seed 2/ Deposition at Botany Department, Institute Science, Nagpur.
Locality: Singhpur, M.P. India.
Horizon: Deccan Intertrappean series of Central India.
Age: Upper Cretaceous?

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:177-180
UGC Approved Journal No 48951

Original Article Open Access

Physico-chemical status of farmland soil in Warora, dist: Chandrapur (Maharashtra), India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Tiple Neha Ramesh, Dakhane Vimal P (2018) Physico-chemical status of farmland soil in Warora, dist: Chandrapur (Maharashtra), India, *Int. J. of. Life Sciences*, Special Issue, A12: 177-180.

ABSTRACT

Soil is a natural body of mineral & organic material differentiated into horizons, which differ among themselves in their morphology, Physical, Chemical & biological Characteristics. Plants depend on the soils for their nutrients, water & mineral supply. The soil type is a major factor for determining what types of plants will grow in any area. The physico-Chemical study of soil is based on various parameters like pH, Electrical Conductivity (EC), total organic Carbon (OC), Available Nitrogen (N), Available Phosphorous (P_2O_5), Available Potassium (K_2O), Exchangable Calcium & Magnesium. Three soil samples were collected from three sites of farmland for soil analysis i.e. Sembal Village, Marda Village, and Ekarjuna village which are located near warora. Physical and chemical analysis of soil have done by different methods. Estimation of total Nitrogen (Kjeldahl method), Available Phosphorous (Bray's & Olsen's method), Available potassium (Flame photometric method) & exchangable Calcium & Magnesium (EDTA titration method). Fe, Mn, Zn, Cu determined by DTPA method. The results Shows that, samples taken have various parameters like EC, pH, OC, N, P, K, Ca, Mg, and Fe, Mn, Zn, Cu. The results depend on quality of soil samples.

Keywords- soil analysis and its methods, Kjeldahl, Flame photometric, Bray's, EDTA and DTPA Method.

INTRODUCTION

Soil is the surface on the earth crust where Geology & Biology meet & the land surface that provides a home to plant, animal & microbial life. (Pelczar *et al.*,1993). Joffee (1949) reviewed the soil is a natural body of mineral & organic material differentiated into horizons, which differs among themselves as well as from underlying materials in their morphology, physical make-up, Chemical composition & biological Characteristics. Solanki and Chavda (2012). Soil types are a major factor

for determining what types of plants will grow in a certain area as plants use inorganic elements from the soil such as nitrogen, potassium and phosphorous. Nitrogen and Phosphorous are not available to the plant directly. They are incorporated in the organic material. Potassium is present in elemental or exchangeable form. Calcium & magnesium interfere in the soil activity as well as activate several plant enzyme systems. The deficiency of any of these elements has retarding effect on the growth of plants. The most significant discovery was that of the german chemist Justus von leibig (1840) showed that the growing plant obtained element calcium, potassium, sulphur and phosphorous from soil. For the first time he showed that plants obtained their carbon supply from carbon dioxide in air & not from soil. The elements of micronutrients are used in field a very small quantity or in a trace amounts. They are iron, zinc, manganese and copper but they are important as major elements in plants nutrients. For maximizing quality and productivity of crops, all these essential nutrients must present or supplied in balanced form.

The availability of mineral nutrients is controlled by the chemical and physical properties of the soil. The cation exchange properties of the soil clay and organic matter regulate the availability of the cation nutrients. Availability of organic sources of N is dependent upon mineralization of the N to the inorganic forms, ammonium and nitrate. The solubility of most micronutrients is affected by soil, pH and organic matter content to assess the sufficiency of the mineral nutrients for optimum plant growth. For sustained high crop yield, the application of nutrients is required. Efficient use of applied nutrients depends upon the timing and methods of nutrient application. The chemical and physical properties of the soil which determine methods of application and soil management practices are best suited for a given soil (Kamprath and Watson 1980).

Mitscherlich *et al*. (1925) conducted extensive studies on the effect of quantities of nutrients in soils on dry matter yields and found that a simple exponential function could relate one to the other. The function can be fitted to observe yield resulting from incremental applications of fertilizer and provides yield response curve that describes expected yield as a continuous and smooth function of nutrient availability. Nitrogen occurs in several forms as a Nitrate (NO_3^-) and Nitrite (NO_2^-) anions, ammonium (NH_4^+) and organic compounds. Adequate supply of These elements are associated with the plant growth & the deep green plant color. The nitrogen deficient soil has stunted plant growth and shows signs of chlorosis. Phosphorous is occurs in soil in both organic and inorganic form. The organic form is more important for the crop nutrition. The supply of P at the early vegetative growth phase strengthens its reproductive parts and formation of seeds. Deficiency will lead to discoloration of older leaves and leaf edges. Potassium is present in the soil in different form. The requirement of plant for K is high because plants absorb it in higher amount than other nutrients. The deficiency of K leads to chlorosis or necrosis. Calcium is present in the soil either as soluble Ca_2^+in complex form or a free calcium carbonate ($CaCO_3$) and act as plant nutrient at the same level as N, P and Mg and pH regulator. Magnesium is a main constituent of chlorophyll molecule, related to metabolism of phosphorous, activates number of plant enzymes and absorbed by plant roots as Mg^{++} ion. If the soil is Mg deficient, the plant grown in such soil will become pale yellow and then turns brown and necrotic.

MATERIAL AND METHOD

Three sites were selected for soil analysis ie. Farmland of Sembal Village, Marda Village, and Ekarjuna village which are located near Warora. Soil samples were collected from three sites of Warora taluka. Each soil samples were taken from a depth of 15 – 20 cm in a quadric manner. After collection, the soil samples were spread for air – drying. After proper drying large stones and other similar objects were removed. Then the soil was ground with the help of mortar and pestle to break up aggregates and the crumbs. After that soil pass through 2mm sieve and stored in a clean polythene bags and labelled properly with necessary information of field. Physico-chemical parameter of soil samples were analysed by using different methods. pH was measured by using pH meter, EC determined (conductivity meters), OC (Colourimeter), Available Nitrogen (Kjeldahl method), Available phosphorous (Bray`s method for acidic soil, olsen`s method for neutral and alkaline soil), Available potassium (flame photometric method), Exchangeable Ca and Mg (EDTA titration method) and Fe, Mn, Zn, Cu determined by DTPA method.

Sample no.	Name of different places
Site - 1	Farmland of Sembal village
Site - 2	Farmland of Marda village
Site - 3	Farmland of Ekarjuna village

RESULT AND DISCUSSION

Analysis of soil samples showed that the value of pH ranges from 6.9 - 7.4 indicating that the soil are neutral to slightly alkaline. The highest pH was recorded in site 1 (7.4) and lowest site 2. (6.9) pH greatly affects solubility of minerals and another parameter. The measurement of EC gives the concentration of soluble salt in the soil at any temperature. The variation of EC is due to the higher concentration ions in solution and directly related to soluble salt concentration. The value of EC ranges from 0.19 – 0.49 dSm^{-1}. The highest value recorded in site 2 (0.49 dSm^{-1}) and lowest in the site 1 (0.19 dSm^{-1}). Wagh, et. al, (2013) state that soil with EC below 0.4 dSm^{-1} are considered saline while soil above 0.8 $dSm^{-1}$are severely saline. Organic carbon ranges from 0.22 – 0.85%. The value of OC was recorded highest in the site 3 (0.85%) and lowest in the site 2 (0.22%). In the colorimeter method, organic matter is oxidized with chromic acid, standard value of OC < 0.50, medium 0.50 – 0.75 and high > 0.75 (Datta *et al.,* 1962).

Available nitrogen helps the plants for rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops. In the present investigation nitrogen value ranges from 69.8 - 153 kg/ha. It was maximum in the site 3 (153 kg/ha) and lowest in the site 2 (69.8 kg/ha). It can be applied to the soil in the form of urea. Farmers are advised to use biofertilizers like Rhizobium, Azotobactor and Nitrogen solubilizing bacteria. Ranges of Phosphorous was 14.1 – 50.1 kg/ha and potassium 553 – 276 Kg/ha. Maximum P was found in the site 1 (50.1 Kg/ha) and lowest in site 2 (14.1Kg/ha). Maximum K was reported in the site 3 (553 kg/ha) and lowest in the site 2 (276 Kg/ha). Availability of phosphorous is medium while availability of potassium is very high due to excessive potassium fertilizer. Calcium ranges from 31.5 – 32.8 Kg/ha and Magnesium ranges (6.66 – 23.6 Kg/ha). Maximum value of Ca was reported in the site 3 (32.8 kg/ha) and lowest in the site 2 (31.5 Kg/ha). Present investigation of Mg value was highest in the site 1 (23.6 kg/ha) and lowest in the site 2 (6.66 kg/ha).

Micronutrients like Fe and Zn are less 25 – 30 kg/ha Ferrous sulphate and Zinc sulphate can be apply to the soil. The Fe ranges from (0.35 – 0.73 Kg/ha), Mn (3.02 – 4.33 Kg/ha), Zn (0.22 -0.24 Kg/ha) and Cu (0.35-0.53) Kg/ha. The soil at Munipallein Gunter district of A. P. was clay loam with pH 7.9, EC 0.7 dSm^{-1}, Available N, P and K, 155 - 66 - 195 kg/ha respectively (Ramani and Pillai, 1992). Jadhav et al (1990) reported at Pune was loam texture, slightly alkaline in reaction, pH 8.2, low available nitrogen (65.65 kg/ha), medium in available P (26.74 kg/ha) and rich in available K (462.36 kg/ha) for pearl millet.

Indian soil range of an available micronutrients status of Fe 0.8 -196 mg/kg, Mn 0.2 -118 mg/kg, Zn 0.2 – 6.9 mg/kg and Cu 0.1 - 8.2 mg/kg (Singh, 1999). In Vidarbha, 5014 soil samples analyzed, available Fe, Mn, Zn, and Cu status in soils. Out of these, Fe is low 12% samples, medium in 48% samples and high in 40% samples. Fe deficiency was observed in Maharashtra, particularly Nagpur district, out of 524 soil samples analyzed for Fe and observed that Fe was found to be 8% low, 49% medium and 43% high. The available Fe varied from 3.10 to 9.34 ppm with mean value of 3.58 ppm. (Patil *et al*., 2004). Giri (2007) reported soil from Thane district pH 6.6% , moisture content 8.3% different mineral present in the soil 10 kg Cu/ha, 25 kg Fe/ha, 15 kg Mn/ha, 5 kg Zn/ha, 30 kg Ca/ha and 35 kg Mg/ha for growth of Fenugreek and Mustard.

Table-1 Physico- chemical analysis of soil

Soil Sample	PH	EC dSm^{-1}	OC %	Macronutrients (Kg/ha)					Micronutrients (ppm)			
				N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Site-1	7.4	0.19	0.48	73.6	50.1	431.96	32.8	23.6	0.49	4.33	0.22	0.47
Site-2	6.9	0.49	0.22	69.8	14.1	276.0	31.5	6.66	0.73	3.92	0.24	0.53
Site-3	7.3	0.24	0.85	153	36.9	553.0	32.5	16.2	0.35	3.02	0.22	0.35

EC (dSm^{-1}), OC(%), N, P, K, Ca, Mg (kg/ha), Fe, Mn, Zn, Cu (ppm).

CONCLUSION

From the above results, concluded that, the essential information's of the soil status were known, that helps in maintaining the physical condition of soil and help in providing proper mineral nutrients. Nutrient analysis is the measurement of nutrients present in the soil which is removed from the soil using an extracting solution. Most of the farmers are using excessive chemical fertilizers and the too much dose of such fertilizers in soil. So therefore, Soil testing needs in determination of such requirements, which helps in balanced fertilization for future to avoid deficiency/ toxicity of different plant nutrients and helpful to microbial population in soil. Thus, the recommendation for application of plant nutrients and their doses depends on soil fertility status. This information will helpful to farmers to decide the problems related to soil nutrients, amount of fertilizers to be added to soil to make production economic.

Acknowledgement:
Thanks to Principal and teaching staff of Janata Mahavidyalaya, Chandrapur for given their inspiration and co-operation. Again, Thanks to Principal, Head, Department of Botany, Dr. Ambedkar college of Arts, Commerce and Science, Chandrapur, Gondwana University, Gadchiroli for their valuable suggestions.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:181-185
UGC Approved Journal No 48951

Original Article Open Access

Seed Mycoflora of *Brassica carinata* and Biochemical Changes in Protein, Oil, Starch and Sugars under the Seed Storage

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Ghugal SI (2018) *Seed Mycoflora of Brassica carinata and Biochemical Changes in Protein, Oil, Starch and Sugars under the Seed Storage*, *Int. J. of. Life Sciences*, Special Issue, A12: 181-185.

ABSTRACT

The seeds of *Brassica carinata* were stored and isolated twenty two seed storage fungi in seed samples by agar plate method and blotter method throughout the year on a monthly basis. Major predominant fungi were *Alternaria alternata, Aspergillus flavus, A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum* and *P. Oxalicum.* Seed sample evaluated for the crude protein, oil, starch and sugars on monthly basis of one year storage and shows significant decreased subsequently with an increase in storage period. It was observed that the percentage change over control in protein, oil and starch was -31.79 %, -24.09 % and -52.74 % respectively at the end of year. Total sugar, reducing and non-reducing sugars were found to be decreased till 8th months of a year of the storage period but subsequently increased at the end of storage period.

Key words: Storage fungi, Protein, Oil, Starch.

INTRODUCTION

Brassica carinata (or Abyssinian mustard) almost certainly originated in North East Africa. Due to its limited distribution to Ethiopia and neighbouring countries, this *Brassica* oilseed has little exposure to modern plant improvement. It is high oil yielding with light brown seed coat colour. This oilseed crop grows in rabi season and the flowering and maturity period is similar to other oilseed Brassicas. Large number of fungi is known to bring about several biochemical changes in oilseeds and degrade seed constituents (Rai and Saxena, 1980). The seed mycoflora associated with the seeds may be pathogenic, weak parasites or saprophytes and may be external or internal. The present study deals with storage fungi associated with the seeds of *Brassica carinata* and also changes in protein, oil, starch and sugars due to the association of seed fungi.

METHODOLOGY

Brassica carinata seed samples were selected for experimental study and collected from different oilseed *Brassica* growers of North India and also from Department of Botany, RTM Nagpur University. Seed samples were bulked together and selected randomly for further investigations.

Isolation of fungi was done by both blotter as well as agar plate method as recommended by ISTA (1966). The seed sample was stored in small cotton bags under normal room temperature condition for one year. After every month 400 seeds were taken out randomly and isolated the storage seed mycoflora.

To study the effect of fungi on the seed constituents, the seeds were stored in separate cotton bags in a glass desiccator maintained at 96 percent relative humidity, prepared by saturated salt solution of sulphuric acid (Johnston and Booth, 1983). The control seeds (surface sterilized and dried) were kept in separate desiccators at a relative humidity of 96 percent. At an interval of every 30 days, the seed samples were taken out randomly. The fungal mycelium was removed by washing the seeds gently in running tap water and then dried at 60 ^{0}C for 48 hours. Both the infected as well as the control seeds were dried and powdered separately in a grinder which was followed by a quantitative estimation of nitrogen, protein, oil, starch and sugars (reducing, non-reducing and total sugar). Nitrogen percentage and crude protein estimation was calculated by micro-kjeldahl method suggested by Davys and Pirie (1969). To calculate the crude protein percentage, Nitrogen percent per gram was multiplied by the factor 6.25 (Sadasivam and Manikam, 1992). Oil percentage was determined with the help of Oxford 4000 NMR (Nuclear Magnetic Resonance Spectroscopy Analyzer) in a 2 ml assembly at Department of Botany, Nagpur. The mode was introduced, tuned and calibrated with pure oil.

For quantitative determination of sugars and starch, 0.5 – 1.0 g of dried, powdered seed sample was taken in round bottom flask. 125 ml of 50 % ethyl alcohol was added to it and the mixture was refluxed for 4 hours with an air condenser. The contents were kept overnight and centrifuged at 3000 rpm for 10 minutes. The supernatant was evaporated to few ml by heating gently. This extract was diluted to 100 ml with distilled water. From this aqueous extract, 50 ml was taken for reducing sugar and 50 ml was kept for non- reducing sugar estimation. The residue after centrifugation was preserved in deep freezer for starch analysis (Agrawal *et al*. 1992). The amount of reducing sugar was determined by Phenol-Sulfuric Acid suggested by Dubois *et al*, (1956).

RESULTS AND DISCUSSION:

Table 1 represents the data on *Brassica carinata* seeds exhibited the association of 22 fungal organisms of which *Alternaria alternata, Aspergillus flavus, A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum* and *P. oxalicum* were recorded throughout the year. *Fusarium chlamydosporum* and *Penicillium multicolor*, which were confined to winter season only. The fungal organisms chiefly confined to summer season were *Aspergillus nidulans, Aspergillus glaucus*, and *Rhizopus nigricans*. *Aspergillus versicolor, Chaetomium bostrychodes, Phytophthora undulata, Penicillium notatum, Penicillium purpurogenum, Pythium sp.* and *Syncephalastrum sp.* were associated sporadically.

Table 1: Incidence of Seed Mycoflora

Occurrence of storage fungi			
Throught out the year	**Summer months**	**Winter months**	**Sporadically**
Alternaria alternate, Aspergillus flavus A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum,P. oxalicum	*Aspergillus nidulans Aspergillus glaucus Rhizopus nigricans*	*Fusarium chlamydosporum Penicillium multicolor*	*Aspergillus versicolor, Chaetomium bostrychodes, Phytophthora undulata Penicillium notatum, Penicillium purpurogenum, Pythium sp, Syncephalastrum sp*

Table 2: Changes in Protein and oil contents in *B. carinata* seeds due to fungal association during storage.

Incubation Period	Protein Percentage		% Change in Protein over control	
(Months)	a*	b*		
0	30.54	30.54	0.00	0.00
1	30.02	30.18	-1.70	-1.18
2	30.02	30.18	-1.70	-1.18
3	29.76	29.81	-2.55	-2.39
4	29.50	28.99	-3.41	-5.08
5	28.98	28.65	-5.11	-6.19
6	28.98	25.26	-5.11	-17.29
7	28.72	23.96	-5.96	-21.55
8	28.46	22.40	-6.81	-26.65
9	28.20	21.88	-7.66	-28.36
10	27.94	21.09	-8.51	-30.94
11	27.42	20.83	-10.22	-31.79

a* - control seeds. b* - infested seeds.

Table 3: Changes in starch, total, reducing and non-reducing sugars in *B. carinata* seeds due to fungal association during storage

Period of incubation (Month)	Percent Starch	% Change in starch over control	Total Sugars (TS) (mg/g)	% Change in TS over control	Reducing Sugars (RS) (mg/g)	% Change in RS over control	Non-reducing Sugars(NRS) (mg/g)	% Change in NRS over control
0 a*	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
b*	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
1	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
	57.08	-3.03	3.76	-8.01	2.37	-1.25	1.39	-17.61
2	58.87	0.00	3.80	-6.93	2.39	-0.42	1.41	-16.18
	55.81	-5.19	3.46	-15.26	2.36	-1.50	1.10	-34.80
3	58.81	-0.10	3.73	-8.74	2.38	-0.67	1.35	-20.21
	55.35	-5.97	2.87	-29.64	2.35	-1.92	0.52	-69.00
4	58.79	-0.14	3.70	-9.38	2.33	-2.63	1.37	-18.97
	55.30	-6.07	2.83	-30.13	2.28	-4.84	0.57	-66.03
5	58.71	-0.27	3.81	-6.78	2.33	-2.92	1.48	-12.27
	39.40	-33.07	2.91	-28.66	2.04	-14.86	0.87	-48.25
6	58.69	-0.30	3.83	-6.29	2.27	-5.13	1.55	-7.94
	34.50	-41.39	2.99	-26.87	1.66	-30.59	1.32	-21.58
7	58.67	-0.34	4.16	-1.89	2.25	-6.26	1.91	13.46
	33.64	-42.86	3.05	-25.23	1.53	-36.02	1.52	-9.90
8	58.62	-0.42	4.17	2.03	2.33	-2.63	1.83	8.65
	33.35	-43.35	3.34	-18.20	1.80	-24.88	1.54	-8.71
9	58.59	-0.47	4.20	2.87	2.35	-1.80	1.85	9.48
	33.41	-43.25	3.83	-6.29	1.97	-17.65	1.85	9.84
10	58.55	-0.54	4.56	11.68	2.41	0.42	2.15	27.68
	33.47	-43.15	4.14	1.40	2.21	-7.93	1.93	14.64
11	58.48	-0.67	4.63	13.30	2.45	2.38	2.17	28.81
	27.82	-52.74	4.76	16.65	2.52	5.30	2.24	32.78

a* - control seeds. b* - infested seeds.

Table 2 shows the protein content in control and infested seeds. In the first month of storage period, it was recorded as 30.54%. Then, it gradually declined till the end of storage period. In the storage period of 1 to 11 month the protein contents were 30.18, 30.18, 29.81, 28.99, 28.65, 25.26, 23.96, 22.40, 21.88, 21.09 and 20.83% respectively as compared to 30.54% in control. At the end of storage period, in surface sterilized seeds, percent change in protein over control was -10.22% and in case of infested seeds it was -31.79%.

The oil content was 33.04% oil at the beginning of storage and declined its oil content regularly for one-year storage. At the end it showed 25.08% oil content with -24.09% change over control and that was -2.12% in surface sterilized seeds.

Table 3 indicates the quantitative changes in starch, total sugars, reducing and non-reducing sugars occurring during one year of storage period. Seeds (control and infested) were assayed on 0 day showed 58.87% starch found at start of storage. After interval of every month there was depletion in starch rapidly in infested seeds and reduced to 27.82% with 52.74% decrease over control. The same case happened with surface sterilized seed but the depletion was very small found to be 0.67% decreases over control at the end of storage.

Total sugar observed at 0 month of storage was 4.08 mg/g reduced rapidly up to 4 month of storage i.e. 2.83 mg/g with 30.13% decrease over control in infested seed. However, the reduction in total sugar observed till 9th month with 6.29% decrease over control and then it increased during 10th and 11th month. At the end of storage period, it was 4.76 mg/g with 16.65 % increase over control in infested seed. In surface sterilized seed there was increase in total sugar at the end of storage, found to be 4.63 mg/g.

In the beginning of the storage, the reducing sugar was observed 2.40 mg/g. Rapid depletion was observed till 7th month, however, the reduction in reducing sugar in infested seed was observed up to 11th month of storage i.e. 2.21 mg/g with 7.93% decrease over control. At the end of storage there was increase in reducing sugar in infested seed was found to be 2.52 mg/g with 5.30% increase over control. In surface sterilized seed the increase was observed at the end of storage i.e. 2.45 mg/g (+2.38% change over control).

1.69 mg/g non-reducing sugar was found at the start of storage, it shows depletion up to 8th month of storage in infested seed, after it shows rapid increase till the end of one year storage, found to be 2.24 mg/g with 32.78% increase over control in infested seed. In surface sterilized seeds the increase was +28.81% over control.

The nitrogen requirement for the growth of fungi comes from nitrates, ammonium and organic sources especially the amino acids. Proteins are the combination products of enzymes and amino acids. The nitrogen sources are good for growth and reproduction also. Nitrate nitrogen that is generally good for sporulation in several fungi (Bilgrami and Verma, 1978). Lalithakumari *et al* (1971) and Yadav et al (2014) reported that *Aspergillus flavus* reduced oil content of the groundnut remarkably. Similar were the findings of Rai and Saxena (1980) who reported that *Aspergillus flavus* was more effective in reducing oil content of Indian mustard. Agarwal (1965) and Prasad and Singh (1983) observed decrease in oil content due to fungi at higher relative humidity. Utilization and reduction of starch content by *Aspergillus parasiticus* shown by Singh and Sinha (1985) in arhar seeds while Prasad et al (1987) studied the quantitative biochemical change (protein, starch and carbohydrates) in cereal seeds under different storage condition. Singh *et al* (1973), showed reduction in starch content in sunflower seeds and infected groundnut seeds. In the present study, reduction in the total sugar content was observed in initial phase of storage and increase at end the of storage period. This may be due to its preferential utilization by some of the fungal species infesting the seeds. Preferential utilization of sugars by fungi also been reported by Cochrane (1958), Gupta and Gupta (1984), Bilgrami *et al* (1979), Bilgrami and Verma (1978). Similar results were obtained in case of reducing and non-reducing sugar.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:186-188
UGC Approved Journal No 48951

Original Article Open Access

Tissue culture studies in *Celosia argenteal*. var. Argentea

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Nirpane Sachin and Pandhure Narayan B (2018) Tissue culture studies in Celosia argenteal. var. Argentea, *Int. J. of. Life Sciences*, Special Issue, A12: 186-188.

ABSTRACT

Kurdu *i.e.Celosia argentea* L. is a herbaceous plant belongs to family Amaranthaceous. Traditionally the plant material is used for the treatment of jaundice, gonorrhea, wounds and fever. The leaves are also used for the treatment of inflammations, fever and itching. The seeds are bitter, useful in blood diseases, mouth sores. They are efficacious remedy in diarrhea. Hence,*in vitro* techniques for multiplication of *Celosia argentea* was developed using apical buds and shoot tip as an explant.

Key words: *Celosia argentia,* Multiplication. MS media,

INTRODUCTION

Kurdu, i.e. *Celosia argentea* L. is a herbaceous plant belongs to family Amaranthaceae. It is Annual erect herbs, simple or with many ascending branches. Leaves 2-15 x 0.1-3.2 cm, lanceolate-oblong to narrowly linear, acute to obtuse, shortly mucronate with the excurrent midrib, glabrous; lamina of the leaves from the center of the main stem tapering below into an indistinctly demarcated, slender petiole, to 2 cm long; upper and branch leaves smaller, markedly reducing. Inflorescence a dense many-flowered spike, 2.5-20 x 1.5-2.2 cm, white to pink, terminal on the stem and branches, peduncle up to c. 20 cm long; bracts and bracteoles lanceolate or the lower deltoid, 3-5 mm, hyaline, more or less aristate with the excurrent midrib, persistent. Perianth segments 6-10 mm, narrowly elliptic-oblong, acute to rather blunt, shortly mucronate, margins hyaline. Filaments very delicate, free part subequalling or exceeding the staminal sheath, sinuses rounded with very minute intermediate teeth; anthers and filaments creamy to magenta. Ovary 4-8-ovulate, style filiform, 5-7 mm long; stigmas 2-3, very short.Capsule 3-4 mm, ovoid to globose; seeds c. 1.25-1.5 mm, lenticular, black, shining, very finely reticulate.

Medicinal and phytochemical properties: *Celosia argentea* is used traditionally for the treatmentof jaundice, gonorrhea, wounds and fever. The leaves are used for the treatment of inflammations, fever anditching. The seeds are bitter, useful in blood diseases, mouth sores. They are

efficacious remedy in diarrhea (Kirtikar, 1935). Based on ethno botanical practice the plant was investigated for anti-inflammatory (Patil *et al*., 2003), anti–pyretic (Bhujbal *et al*., 2006) anti diabetic, (Thangarasu *et al*., 2002), anti-bacterial and diuretic properties (Patel *et al*., 1993). Plant is also found to be useful in cancer. Even though plant is considered as weed but it is seasonal. Looking towards its medicinal properties it was decided to undertake in vitro studies in *Celosia argentia*.

METHODOLOGY

Preparation of Explants:

Celosia argentea was collected from campus of Dr. Babasaheb Ambedkar Marathwada University campus and Over-Jatwada area of Aurangabad, Maharashtra. The explants were washed carefully in running tap water for 5 minutes and followed by distilled water for 5 minutes. Explants were surface sterilized for 5 minutes with 0.3% mercuric chloride, followed by three subsequent rinses with sterilized double distilled water, in a laminar air flow. Explants were dissected into small pieces and inoculated aseptically in culture vessel and test tube on sterilized MS medium.

Culture media:

Murashige and Skoog (1962) media was supplemented with various concentrations of auxins and Cytokinins. MS medium was fortified with 3% sucrose and gelled with 2.5% Clerigel. pH of the medium was adjusted up to pH 5.8 after addition of growth regulators. The media were autoclaved under 15 psi and 121° C for 20 minutes. After autoclaving, the vessels containing the media were transferred to laminar air flow for inoculation.

Culture conditions:

After inoculation, culture tubes and vessels were transferred to culture room under a 10 h photoperiod supplied by cool white fluorescent tube lights and 25 ± 2^0C temperatures. At least five replicates were raised for each treatment.

Data record:

Data was recorded after 30days. Mean (μ) values with the standard error (S.E.) were calculated from five replicates each for callus induction, shoot multiplication and shoot length.

RESULT AND DISCUSSIONS

Surface sterilization of explants is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue (Conger 1987). Therefore, the explants were excised in proper size and shape, surface sterilized and aseptically inoculated on MS medium. MS medium was fortified with different concentrations with BAP 1.0, 1.5, 2.0, 2.5, 3.0, mg/l and IAA, (0.2mg/L) produced maximum average percentage of shoot multiplications. Combinations of BAP and IAA were better for induction of shoots *in vitro* as compared to any other combination of growth regulators. It is noticed that *Celosia argentea* has produced maximum *in vitro* shoots with apical shoots as an explant. Explants viz. apical shoots and axillary buds were tried in MS medium supplement with 3% sucrose 2.5% clerigel in combination with growth regulators viz. BAP and IAA as shown in table 1. Maximum induction of shoots was recorded in MS medium fortified with 0.5to 2 mg/l BAP and keeping 1 mg/L, IAA constant using shoot tip and axillary bud as an explants. With increase in concentration of growth regulators i.e. BAP there was subsequent increase in induction of shoot (Table 1) and subsequently decline in the induction of callus (Plate 1.). Shoots recorded were healthy. Taking apical shoots as n explant and with 0.5 mg/lit of BAP and 1.0 mg/lit IAA more callus was recorded along with low frequency of shoot induction. With increase in concentration of BAP there was subsequent increase in frequency for shoot induction.

Table.1 Effect of BAP and IAA on Multiplication in *Celosia argentea*

Sources of Explant	Growth regulators Mg/l		Frequency of callus	No of multiple shoots induced
	BAP	IAA		
Apical Shoots	0.5	1.0	+++	1.05±0.21
	1.0	1.0	++	1.5 ±0.12
	1.5	1.0	+	7.05±0.10
	2.0	1.0	++	2.09 ± 0.12
Axillary Bud	0.5	1.0	+++	0.9 ±0.11
	1.0	1.0	+	4.5 ±0.19
	1.5	1.0	++	2.05±0.12
	2.0	1.0	++	2.04± 0.12

**After 35 days, mean ± SE of 5 replicates.*

Plate.1 Callus with shoot Initiation

Plate.2 Multiple shoots with roots

Highest rate of shoot induction was recorded in medium fortified with 1.5 mg/lit BAP and 1.0 mg/lit. IAA. In case of axillary bud as an explant similar pattern was recorded but rate of shoot formation was less. Highest number of shoots was recorded on MS fortified with 1 mg/lit BAP and 1 mg/lit IAA. There was similar pattern for callus induction with axillary shoot as an explant (Plate.2).

Similar results were reported for callus initiation and shoot multiplication *in vitro*, using various explants, in different plants (Pandhure *et al*. 2010) in *Solanum nigrum* using shoot tip and axillary buds. Proliferating shoot cultures were established by repeatedly sub culturing the mother explants on the hormone free medium. Repeated sub-culturing was said to be one of the methods of maintaining juvenility (Franclet *et al*., 1987) which was proved through results recorded during these studies. During the present piece of work highest numbers of shoots were recorded on MS with 2 mg/it BAP and 1 mg/lit IAA. Experimental results of Devendra and Sandeep Reddy, (2011) indicated that nodal explants have high competence for shoot induction in *Eclipta alba.* This is further confirmed from our studies, from the fact that Shoot tip explants have more recalcitrant capacity than other explants.

CONCLUSION

Even though *Celosia argentea* is considered as weed but looking towards the utility of plant material in various disorders, only *in vitro* methods are capable to make provision of fresh plant material. Hence multiplication of this plant through *in vitro* method makes this technique viable as well as valuable.

Acknowledgements:
Authors are grateful to Professor and Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.), India for providing all necessary facilities and encouragement for the present research work.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:189-191
UGC Approved Journal No 48951

Original Article Open Access

Anatomical changes induced by glyphosate herbicide in *Hyptis suaveolens* L.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Dudhe SS and Khirade PD (2018) Anatomical changes induced by glyphosate herbicide in *Hyptis suaveolens* L, *Int. J. of. Life Sciences*, Special Issue, A12: 189-191.

ABSTRACT

The weed *Hyptis suaveolens* L. belonging to family Lamiaceae. It is growing in Maharashtra especially in vidarbha region and found growing luxuriantly on boundary of crop fields, on sides of railway tracks and road sides. Plants already grown in fields were sprayed with aqueous concentration of glyphosate herbicide at various concentration like 100-3000 ppm. 3000 ppm considered to be lethal dose for plant. Anatomical changes like desiccation of epidermal cells, lacunae were found in cortical cells of stem, leaf showed mesophyll cells of leaf lamina was desiccated and destruction of palisade and spongy cells, and petiole showed remarkable changes at lower to higher concentration like lacuna formed at phloem region, distortion and disorganization of cortical cells while root showed epidermis and cortical cells distorted due to cambial activity, as compare the control.

Key words: Herbicide, Glyphosate, Anatomical, *Hyptis suaveolens* L.

INTRODUCTION

Plants on the earth is a great asset to mankind, out of 2,50,000 plant species present in the world, nearly 200 species are found to be prominent weed causing severe losses in agricultural systems. Weeds are unwanted and undesirable plant, it interferes with the utilization of land and water resources and this affects human and animal welfare. Unwanted vegetation flourishes in field crops, forestry, industrial sites, railway lines, air field, water ways and non-cropped lands create several problems. Great crop losses also occur due to weed about 20 to 100 percent. The natural growth aggressiveness and high adaptability of weed always makes them winners in the competition race. Soil, water and mineral nutrients are basic need of crop and weed. Weeds take more water due to large extension of root system and absorption of more nutrients from soil. Weeds harbor insects and pests during off season and then later attack to the crop field after sowing and damage them. Several weeds grow in grassland and are hazardous to animals, as it contains more amounts of glucosides, lactones, alkaloids, oxalates, coumarins and tannins which are lethal to animals.

Thousands of sheep and beef cattle killed in U.S.A. every year due to presence of *Hologeton glomeratus*, a poisonous weed in crop field. Traditional approaches for weed control are manual method and mechanical method. Manual method by using handweeding, digging, cheeling, sickling and mowing and mechanically by using tillage, hoeing, inter row cultivation, ecofallow system, burning, flooding, mulching like this way weed control were taking place, but it is not so effective because it is expensive and time consuming practices. The success of biological weed control is so directly or indirectly action on crop plants. But mechanically and biologically urgently weed management does not take place so there is the last, quick and economically beneficial method is to control weed by chemically.

Employing chemicals for weed control referred as chemically weed control method, it is commonly referred as herbicides, weedicides or agrochemicals, it constitute the principal component of weed management. Glyphosate is a non-selective, systemic herbicide that can control most annual and perennial plants. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants. Glyphosate is strongly adsorbed to soil particles, which prevents it from excessive leaching or from being taken-up from the soil by non-target plants. It is degraded primarily by microbial metabolism, but strong adsorption to soil can inhibit microbial metabolism and slow degradation.

Glyphosate is profitable not only in situations, where labor scarce and expensive but also where labor is plentiful and cheap because glyphosate can be applied for weed control in crop rows were cultivation is possible, pre-emergence weed control, avoid root damage during manual weeding, control many perennial weeds and weed control by ecofriendly and ecoenvermentally. Glyphosate is extensively tested for health and safety, low-cost, effective weed control, glyphosate herbicide economically and effectively controls broadleaf weeds growing in between rows of fruit and vegetable fields and on orchards floors. The weedicide is primarily used for weed control in many crops.

Monsanto discovered and held the patent for glyphosate, and was for many years, the only company that manufactured and sold this herbicide. The patent expired in 2000, however, and already several other companies are making and selling glyphosate formulations. Some of the current trade names include: Roundup Ultra®, Roundup Pro®, Accord, Honcho, Pondmaster, Protocol etc. Over 400 herbicides have been developed and registered in the world for weed control in agricultural and nonagricultural systems. By keeping these properties of glyphosate in mind this work has been undertaken.

METHODOLOGY

Hyptis suaveolens L. belonging to family Lamiaceae. It is weed growing in Maharashtra especially in vidarbha region; it is found growing luxuriantly on boundary of crop fields, on sides of railway tracks and road sides. Plants already grown in field were sprayed with aqueous concentration of herbicide glyphosate at various concentrations like 100-3000 ppm by aspee-poly sprayer of 1 liter capacity by making randomly designed plots of size approximately 2/2 square feet at the evening at low temperature. For anatomical study the plant parts like stem, leaf, petiole and root were collected and fixed in F. A. A. (Formaline: Acetic Acid: Absolute Alcohol) solution for 24 hours stored in 70% alcohol. Plant material embedded in paraffin wax and sections were cut at 8-10 microns and stained with safranine- light green and mount in DPX. Microphotographs of treated and control plants were taken.

RESULTS AND DISCUSSION

Plants sprayed with glyphosate showed anatomical changes from 400 ppm concentration, desiccation of epidermal cells at nodal region, pith parenchyma becomes meristematic and cortical cells distorted. At 1000 ppm showed phloem distortion. Swelling of leaf lamina, mesophyll cells and spongy parenchyma distorted at 400ppm, illdeloped vascular and transfusion tissue were recorded at higher concentration. Petiole showed remarkable response at lower concentration to higher concentration, at 100 lower concentration cambium strip divide and lacunae formed at phloem region while at higher concentration 1500 ppm distortion and disorganization of epidermis and cortical cells. Root did not showed prominent remarkable changes at 100ppp except 2500ppm where cortical cells crowed due to cambial dividing activity, epidermal cells desiccated and distorted.

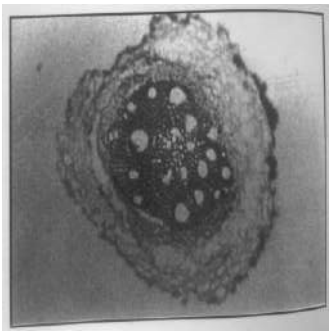

Fig 1. T.S. root - control

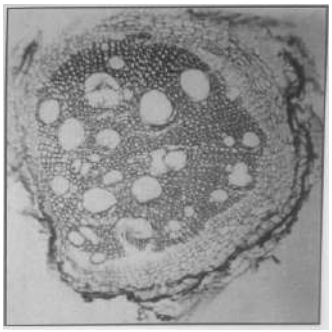

Fig 2. T.S. of root at 2500 ppm

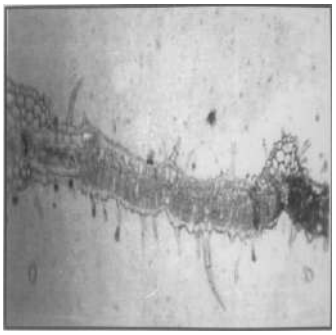

Fig 3. T.S. of root at 2500 ppm

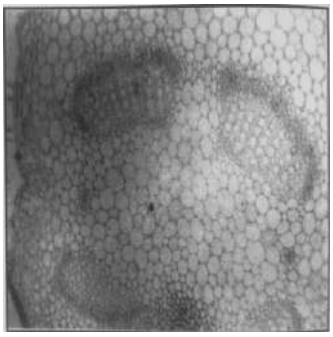

Fig 4. T.S. petiole - control

Herbicide glyphosate induces anatomical changes after spray on *Hyptis suaveolens* L. plant of stem, leaf, petiole and root, Schrubbers et al. (2014) in *Coffea Arabica.* In stem proliferation of cambium strip and phloem, epidermal breakage, lacuanae noted in phloem region, similar results were stated earlier by Tulankar (1998) in *Amaranthus lividus* this result might be due to meristematic activity of pericycle and lacunae due to toxicity of herbicide.

Leaf showed reduction in size of vascular bundle, proliferation of parenchyma, reduced mesophyll cells irregular in shape found. Earlier worker Canal et al. (1990) reported disorganization of vascular bundle, similar result were reported by Tulankar (1998) in *Amaranthus lividus.* Leaf anatomical changes showed plasmolized cells, epidermal disruption, distorted cells, hyperplesia, cell collapsing and necrotic tissue reported by Lailla et al. (2016). Petiole of weed showed notable abnormalities desiccation of cortical cells, destruction of phloem region, epidermal breakage, earlier Tulankar (1998) in *Amaranthus lividus.* In root, proliferation of cambium and phloem resulted destruction of cortex, similar results reported by Vaughn and Dukes (1986) in *Glycine max.*

CONCLUSION

Herbicide glyphosate induce proliferation of phloem in cortex ultimately ruptures the epidermis. Proliferation of secondary phloem in cortical region leads desiccation of root. In leaves depletion of chloroplast results in desiccation. These changes might be due to the physiological and biochemical toxicity of glyphosate.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:191-194
UGC Approved Journal No 48951

Original Article Open Access

Evaluation of phytoconstituents of *Geodorum densiflorum* (Lam.) Schltr by using UV-VIS and FTIR Techniques.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Theng PA, Korpenwar AN and Borkar SU (2018) Evaluation of phytoconstituents of *Geodorum densiflorum* (Lam.) Schltr by using UV-VIS and FTIR Techniques, *Int. J. of. Life Sciences*, Special Issue, A12: 192-194.

ABSTRACT

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug. The present investigation conducted to produce Fourier transform-infrared (FT-IR) and ultraviolet-visible (UV-VIS) spectrum profile of *Geodorum densiflorum*. Fourier transform-infrared (FT-IR) and ultraviolet-visible (UV-VIS) spectrum profile of leaves extract in chloroform was used for evaluation. FTIR evaluation was used to detect the characteristic peak values and their functional groups. FTIR evaluation of leaves powder extract in chloroform revealed the presence of Hydrogen bonded alcohols, phenols, Alkanes, Aliphatic esters, Alkenes, Nitro compounds, Bromides and Aromatics groups. UV-VIS profile of leaves powder extract in chloroform showed the peaks at wavelength 426, 491, 536, 611 and 671 with the absorption 2.04, 2.078, 1.67, 1.37 and 2.18 respectively.

Key words: *Geodorum densiflorum,* UV-Vis, FTIR spectrum, leaves, chloroform.

INTRODUCTION

Medicinal plants are important source of inexpensive and practical drugs for people throughout the world. Medicinal plants could be used for therapeutic purpose or which are precursors for synthesis of useful drugs (Nathan *et al*., 2012). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). Many data on the phyto-pharmacology have showed medicinal plants capacities in certain area of pharmacology (Osadolor *et al*., 2011) and researcher are also beginning to realize the role of medicinal plants in health care delivery (Kolawole *et al*., 2011).

Orchids comprise five subfamilies and approximately 870 genera occurring on all vegetated continents and even some Antarctic Islands (Dressler, 1981, Chase *et al*., 2003). *Geodorum densiflorum* was belongs

into family Orchidaceae. Fourier Transform Infrared spectroscopy (FTIR) gives a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an advance method to characterize and identified functional groups (Gunasekaran, 2003).

METHODOLOGY

Collection and extraction of plant material: The fresh leaves material of *Geodorum densiflorum* was collected from Amba Barwa forest, Jalgaon Jamod tehsil, district Buldhana (M.S.). Leaves material was washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in airtight bottles. About 25 gm powdered plant material weighed accurately and extracted in Soxhlet apparatus by using chloroform as solvent.

Spectroscopic analysis:
About 10 mg pure solute obtained after evaporation of solvent was used for in fourier transform infrared spectroscopic evaluation. The dried 10 mg powdered extract was mixed with KBr salt and encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powder sample of each plant specimen was loaded by using a Perkin Elmer Spectrum RX1, FT/IR spectrometer, with wave number from 4400 to 450 cm^{-1} having a nominal resolution of 1 cm^{-1}. For each spectrum 64 runs were collected and averaged. Sample was placed in sample chamber and spectra were taken ATR mode. Results were plotted against wave number verses percent transmittance. *Geodorum densiflorum* leaves powder extract in chloroform was examined under UV and visible light for immediate evaluation. Plant sample extract was centrifuged at 3000 rpm for 10 minutes and filtered through filter paper (Whatman No.1) under high pressure of vacuum pump. The sample was diluted to 1:10 by using same solvents. The extract was scanned in the wavelength range from 190- 1100 nm using EQUIP- TRONICS (EQ-826) and the peaks were detected.

RESULT AND DISCUSSION

The FTIR spectrum was used to identify the functional group of different phytoconstituents based on the peak values in region of infrared radiation (4400- 450 cm^{-1}), present in chloroform extracts of leaves powder. FTIR analysis was used to detect the characteristic peak values and their functional groups. FTIR spectrum of leaves represented in fig. [1] And peak value and functional group in table [1]. FTIR analysis of leaves powder extract in chloroform revealed the presence of Hydrogen bonded alcohols, phenols, Alkanes, Aliphatic esters, Alkenes, Nitro compounds, Bromides and Aromatics groups. Phenols are of immense significance as they protect the human body from the oxidative stress, which cause many diseases including cancer, cardiovascular problems and ageing (Robards *et al*., 1999). The carboxylic acid is a functional group plays a cardinal role in living system as well as in drug design (Ballatore *et al*., 2013).

They protect the plant against water loss, avoid the leaching of important mineral by rain and protect against microorganism and harmful insects (Riederer and Markstadter, 1996). Alkenes (Ethylene) were used for artificial ripening of fruits (Bleecker and Kende, 2000).

Table 1: FTIR spectral peak value and functional groups obtained for chloroform extract of *Geodorum densiflorum* leaves.

Peak value (in cm^{-1})	Functional group	Bond	Group frequency (in cm^{-1})
3424,52	Hydrogen bonded alcohols, phenols	O-H Stretching	3600- 3200
2918,21	Alkanes	C-H Stretch	2970- 2850
1737,43	Aliphatic esters	C=O Stretch	1750- 1730
1611,51	Alkenes	C=C stretch	1680- 1600
1514,55	Nitro compounds	N=O	1550- 1490
1462,39	Alkanes	C-H	1470- 1340
1165,44	Alcohols, carboxylic acid, esters, ethers	C-O stretch	1300- 1000
888,56	Alkenes	C-H bend	1000- 650
729,59	Aromatics	C-H 'oop'	900- 690 (s)
522,62	Bromides	C-Br	650- 510

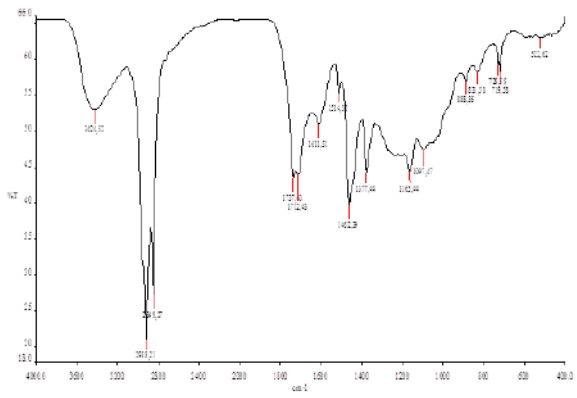

Fig.1: FTIR spectrum of chloroform extract of *Geodorum densiflorum* leaves

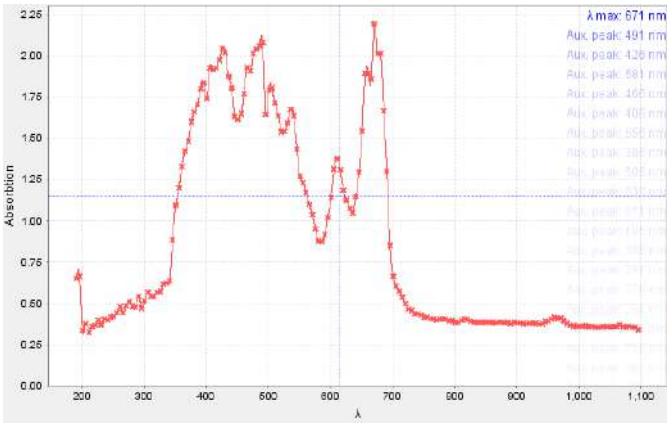

Fig. 2: UV-VIS spectrum of chloroform extract of *Geodorum densiflorum* leaves

Table 2: UV-VIS spectrum profile of *Geodorum densiflorum* leaves powder extract in chloroform.

Wavelength (λ) in nm	426	491	536	611	671
Absorbtion	2.04	2.07	1.67	1.37	2.18

The UV-VIS profile of *Geodorum densiflorum* leaves powder in chloroform was selected at a wavelength of 190 to 1100 nm. The peaks were obtained in the range of 400- 700 nm wavelength. The extract showed peaks at the wavelength of 426 nm, 491 nm, 536 nm and 611 nm and 671nm with absorption at 2.04, 2.07 and 1.67, 1.37 and 2.18 nm respectively. The result of UV-VIS analysis of leaves powder extract in chloroform was mentioned in table- [2] and figure- [2].

CONCLUSION

The results of the present study showed that *Geodorum densiflorum* leaves displayed novel phytochemical markers responsible for many biological activities. FTIR spectrum was helpful to judge medicinal materials from the adulterate and even evaluates the quality of the medicinal plant materials.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:195-197
UGC Approved Journal No 48951

Original Article | Open Access

New Plant Records for the Chandrapur District of Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Dudhe NS, Srinivasu T and Dudhe SS (2018) New Plant Records for the Chandrapur District of Maharashtra, India, *Int. J. of Life Sciences*, Special Issue, A12: 195-197.

ABSTRACT

The following plant records from the Chandrapur District of Maharashtra State during the field survey. The author collected some uncommon taxa from the different localities during the preparation of digital database of dicot plants of Chandrapur District, which were not recorded so far in the early floristic documentation. Four species belongs to four different families, were collected, identified and recorded as new additions to the existing floristic record of Chandrapur District. The species are Nopalea cochenillifera Salm. Dyck. (Cactaceae), Glochidion ellipticum Wight. (Euphorbiaceae), Ficus palmata Forsk. (Moraceae) and Hygrophila erecta Hochr. (Acanthaceae). A taxonomic description along with Electronic Herbarium were prepared for each taxon.

Keywords: New plant records, Electronic Herbarium, Chandrapur District.

INTRODUCTION

Chandrapur district comprising 15 talukas namely Chandrapur, Ballarpur, Bhadravati, Warora, Bramhapuri, Chimur, Nagbhid, Pombhurna, Sindewahi, Mul, Saoli, Gondpipari, Rajura, Korpana and Jivati having very rich in biodiversity and known as 'district of forest'. It lies between 18° 41' and 20° 50' north latitudes and 78° 48' and 80° 55' east longitudes and has an area of 11417 sq km. The climate follows a typical seasonal weather pattern. The peak temperature are usually reached in May-June and can be high as 50^0c.The onset of Monsoon is usually from July and extends up to September Month, with monsoon peak during July to August. After monsoon the average temperature varies between 27^0C and aprox 6-7^0 through December and January. The plant wealth of the Chandrapur district is known through publications of several researchers (Tiwari1990; Patil 1991; Moghe 1992; Malhotra and Moorthy 1992; Chavan *et al*., 2011;. Deshmukh *et al.,* 2012; Shende *et al.*, 2012; Rathor *et al.*, 2013; Dudhe and Srinivasu 2013; Wadekar *et al.*, 2013 and Dudhe *et al.*, 2016) However recent urbanization and industrialization has affected

the flora and fauna of Chandrapur and its surroundings a lot. From biodiversity and conservation point of view it is very necessary to explore existing floristic structure of Chandrapur district to update and revise the earlier data.

Herbarium is the collection or depository of dried plant specimens. Herbarium serves as vital link for various disciplines of biology not only to provide information about plants from the preserved specimens but also to give insight, the changes occurred in the existing plant biodiversity with past once from time to time. However, there are several disadvantages like insects attack, biodegradation of specimens, high maintenance cost, and availability of plant information. With the advent of computers, digital cameras this problem can be overcome easily can make herbarium i.e. electronic herbarium.

Electronic herbarium defined as high resolution virtual images of plant specimen in digital format (Srinivasu, 2005) is prepared by selecting various morphological characters (> 200) with a number of possible variable states as a model. This work is done using software, DELTA (Descriptive Language for Taxonomy) (Dallwitz *et al*.2000) is a flexible and powerful method of recording taxonomic descriptions for computer processing is used for organizing a database on dicot plants. During the preparation of digital database of Dicot plants of Chandrapur district, these four plant species found to be new to this region.

METHODOLOGY

Exploration for collection of dicot plants were made during research work in different places of Chandrapur district, 4 species of 4 different families were reported new for this area, collected from their natural habitat and details of taxonomical description entered into the computer after identification and authentification of specimen with the help of floras [Flora of Maharashtra State: Dicotyledons Vol I and II (Singh et. al., 2000, 2001), Flora of Maharashtra (Almeida,1998,2001and 2003), Flora of British India (Hooker, 1885), Flora of Chandrapur and Gadchiroli district Ph. D. thesis, Nagpur University Nagpur (Moghe, 1992) and Ethnobotanical studies of Chandrapur and Gadchiroli district Ph. D. thesis, Nagpur University Nagpur (Tiwari,1990)] the digital images are attached after processing to the respective plant description in the database.

RESULT AND DISSCUSION

Author collected four specimens belonging to families Cactaceae, Euphorbiaceae, Moraceae and Acanthaceae from research area were reported new addition to the Chandrapur district. The specimens are enumerated below. The flowering and fruiting seasons, ecology, localities in the district of the plants also cited in the text.

Nopalea cochenillifera* (Linn.) *Salm.Dyck.

Citation: Cact. Hort. Dyck. ed. 2, 64, 1850; *Cactus Cochenillifea* Linn., *Sp. Pl*. 468, 1753; *Opuntia cochenilliefera* Mill, Gard. Dict. ed. 8, 6, 1768; Almeida, *Fl. Maharashtra* 2: 336, 1998; Singh *et al.,Fl. Maharashtra State* (Dicot) 2: 83, 2001.

Succulent shrub. Stem erect, flattened, jointed, modified into phylloclade, areoles appears in leaf axil consist of long spines covered with tiny bristles having hooked strikers. Flowers solitary, axillary 7-7.5 cm long, rotated, perianth 4-5 whorl outer sepaloid, thick inner thin, pink, ovate, mucronate, petaloid, spiral; stamens many, epipetalous on equal filaments 2-3 cm long , lobes oblong, pink; carpel thick, ovary syncarpous, inferior, unilocular, with parietal placentation, style long, stigma 6 lobed. Fruit fleshy 1 celled berry with numerous seeds, scarlet red.
Place of collection: Aksapur.
Status of plant in Nature: Wild.
Flowering & Fruiting period: October-December.
Uses: Fruits are edible.

***Glochidion ellipticum* Wight.**

Citation: *Sp. Pl*. 453, 1753; Hook. f., Fl. Brit. Ind. 5:239, 1887; Cooke*, Fl. Pres. Bombay* 2:576, 1907; Ugemuge, *Fl. Nagpur Dist.* 328, 1986; Almeida, *Fl. Maharashtra* 4: 323, 2003; Singh *et al*. *Fl. Maharashtra State* (Dicot) 2; 887, 2001.

Tree, branched; stem cylindrical, reddish brown, alternate branch. Leaves alternate, oblong, obtuse base, midrib prominent, green above, pale beneath, glabrous, 8-12cm long, 2.5-5 cm broad; petiole short; stipule triangular. Flower axillary cluster; male: 0.8 cm long-0.5 cm broad, pedicel 0.5 cm long; tepals 3+3, outer large, ovate, acute, yellowish green, inner 3, oblong, acute; anthers 3, free. Female flower 0.8 cm long, 0.4 cm

breath, style conical, 6 toothed at the apex, sepals small. Capsules 0.8 cm long, 4-lobed. Seeds orange- shiny.
Place of Collection: Somnath**.**
Status of Plant in Nature: Wild**.**
Flowering and Fruiting period: February- October.

***Ficus palmata* Forsk.**

Citation*: Kngb.,* ex Hook. f.*, Fl. Brit. Ind*. 5:530, 1888 Almeida, *Fl. Maharashtra* 4: 371, 2003; Singh *et al*. *Fl. Maharashtra State* (Dicot) 2; 939, 2001.

Spreading deciduous, tree, without aerial roots. Leaves entire, undulate oblong-ovate, shortly acuminate, petiole long, channeled; stipules ovate/lanceolate, pubescent. Receptacle in axillary pairs, globose, whitish; bract 3, rounded, very small. Male flowers few; sepals 4; stamen; 1 Gall & female flowers, sessile; sepal 3-4; style in the female longer than the Gall flower. Achenes smooth.
Common name: "Pakari, pipri, pakar".
Place of collection: Ballarpur.
Status of plant in Nature: Wild.
Flowering & Fruiting: January-April.

***Hygrophila erecta* (Burm.f.)Hochr.**

Citation: in Candollea 5:230, 1934 Burm. f. *Fl. Ind.*135, 1771.Hook. f., *Fl. Brit. Ind.* 4:4, 1884; Clarke in Hook.f., *Fl. Brit. Ind.*4:408, 1885; Almeida*, Fl. Maharashtra* 4: 49, 2003; Singh *et al. Fl. Maharashtra State* (Dicot) 2: 590, 2001.

An erect herb. Stem obtusely quadrangular, nodes swollen. Leaves lanceolate or elliptic, narrowed at both ends, margin undulate. Flowers in axillary whorls, sessile, bi lipped, pale purple; stamens 4, didynamous; gynoecium bicarpellary, style long; capsules much exceeding the sepal,linear-oblong; Seeds numerous.
Place of collection: Rajura and Dopdala colony.
Status of plant in Nature: Weed
Flowering & Fruiting period: September-November.

CONCLUSION

Beside tremendous development towards floristic study of Maharashtra and Chandrapur district but still some alien species regularly introduced due to modernization and urbanization this study will help to identify and mention the new records and conserved.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :198-200
UGC Approved Journal No 48951

Original Article Open Access

Preliminary phytochemical analysis of *Antidesma ghaesembilla L.*

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Matte RS and Kale MC (2018) Preliminary phytochemical analysis of *Antidesma ghaesembilla L.*, *Int. J. of. Life Sciences*, Special Issue, A12: 198-200.

ABSTRACT

Antidesma ghaesembilla commonly known as Jondhurli it grows gregariously on open grasslands and scattered in mixed forest. Plantation can be raised both on irrigated and dry lands. Root suckers are freely produced and help in vegetative propagation. The plant is traditionally reported to possess astringent, bitter, alterative, aphrodisiac, anthelmintic, antibacterial and anti-asthmatic properties. As per phytochemical investigation, the ether, methanol and aqueous extract used for testing various chemical compound.

Keyword: *Antidesma ghaesembilla, Phytochemical*, Traditional aphrodisiac, anthelmintic.

INTRODUCTION

India is sitting on a goldmine of well-recorded and traditionally well-practiced knowledge of herbal medicines, therefore, any scientific data on such plant derivatives could be of clinical importance. *Antidesma ghaesembilla* widely distributed throughout Inda. It holds an important place because of its medicinal and other miscellaneous uses. Antidesma ghaesembilla of economic value. It is one of the most beautiful tree has been put off some useful purpose. Is extensible used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. Commonly it is used as tonic, astringent, aphrodisiac and diuretics. A large shrub or small tree. Leaf – broadly elliptical or obovate, rounded, petiole long, stipules long, pubescent, acute. Flower – greenish – yellow, sessile, in paniculata spikes, across, hairy, pubescent. Fruit- drupe, dark purple when ripe.

METHODOLOGY-

The plants collected during the tours. The entire plant or its parts i.e. stem, root, leaves, bark, fruits were used for the phytochemical studies.

The plants were washed properly with distilled water, chopped in small pieces and dried in shead. After drying they are granded in powder which was later kept in polythene bags. This was later used for the phytochemical analysis.

Procedure

The procedure of Chhabra et.al., (1984) was adopted here. Qualitative detection of the compounds was done by soaking 10g powder of plant material in 100ml of petroleum ether. After 24 hours, petroleum ether was distilled off and the residue was dissolved in 25ml ethanol and divided in to two portions (A) and (B). Portion A divided in two parts (A.1&A.2). Portion (A.1) of the extract was tested was tested for alkaloidal bases and volatile oils. The other portion (A.2) was saponified with 5ml of alcoholic potassium hydroxide(0.5N) by refluxing on water bath for 90 minutes. The alcohol was distilled off and residue was redissolved in hot distelled water (10ml). The non-saponifiable (A.2.1) was extracted in ether (3x5ml) and tested for presence of carotenoids, steroids/triterpenoids. The alkaline aqueous solution was acidified (pH 3-4) with concentrated hydrochloric acid and extracted in ether (3x10ml). This ethereal solution (A, 2.2) was tested for coumarins, emodins, fatty acids and flavonoids.

The plant residue marked (B) which was exhausted with ether, was extracted with hot methanol(100ml) and kept overnight for extraction by facilitated diffusion technique (Keen, 1978) on a orbital shaker at 150 rpm. The methanol extract was decated off in another flask and it was reduced to 1/3 of its volume under vacuum at 40^0 C. It was divided in two portions (B.1&B.2). Portin (B.1) was tested for alkaloida salts, reducing compounds and tannins. The other remaining portion (B.2) was hydrolysed with hydrochloric acid (5ml 10%) by refluxing on water bath for 30 minutes. Contents were cooled and after adding water (10ml), extracted with ether (3x10ml). The ethereal solution(B.3)was tested for anthracene glycosides, coumarins, flavonoids, steroids and triterpenoids. Acidic solution (B.4) was tested for anthocaynin and anthocyanidin.

The plant residue marked (C), exhausted with ether and methanol, was extracted with hot distilled water(100ml) and kept overnight to ensure complete extraction. The water extract was reduced to 1/3 ot its volume under vacuum and divided into two portions. the portion (C.1) was tested for alkaloidas salts, ployosed, polyuronoids, reducing compounds, saponin, starch and tannin. The portion (C.2) was hydrolysed with hydrochloric acid (5ml 10%) by refluxing on water bath for 30 minutes. Contents were cooled and after adding water (10ml) extracted with ether (3x10ml). The ethereal solution (C.3) was tested for anthracene glycosides, coumarins, flavonoids, steroids and triterpenoids. Acidic soulution(C.4) was tested for anthocaynin and anthocyanidin.

RESULT AND DISCUSSION

Preliminary phytochemical screening of the presence of various phytocompounds is tabulated in the table 1, and 2. The maximum number of phytocompounds were seen in the ether extract which showed the presence of Alkaloids, Coumarins, Emodins, Fatty acids and Flavonoids, whereas the presence of Alkaloids, Anthocyanin and Coumarins presence in the methanol extract, on the other hand Anthocyanin, Flavonoids and Polyuronoids are presence in the aqueous extract. After surveying all the available paper, journals and books about plant *Maytenus senegalensis.*

Table 1: Preliminary Phytochemical Screening of : *Antidesma ghaesembilla*

Parts used	Alkaloids			Anthocyanin/ Anthocyanidin		Anthracene Glycoside		Anthroquinone
	Ether	Methenol	Water	Methanol	Water	Methanol	Water	
Leaf	++	-	-	+	+	-	-	-
Stem	+	-	-	+	+	-	-	-
Flower	+	-	-	-	+	-	-	-

Table 2: Preliminary Phytochemical Screening of: *Antidesma ghaesembilla*

Parts used	Carotenoids	Coumarins			Emodins	Fatty Acids	Volatile oils
	Ether	Ether	Methenol	Water	Ether	Ether	Ether
Leaf	-	+	-	-	-	-	-
Stem	-	-	+	+	-	-	-
Flower	-	-	-	-	-	+	-

We can certainly conclude that, a number of compounds can be isolated by means of different extraction procedure following their through characterization and optimization. Study of pharmacological activities with different extract, which show that the compounds have beneficial effects against a number of diseases.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:201-203
UGC Approved Journal No 48951

Original Article Open Access

Intramural airborne mites from poultry farm in Nagbhid, MS, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Shende AN and Korpenwar AN (2018) Intramural airborne mites from poultry farm in Nagbhid, MS, India, *Int. J. of. Life Sciences*, Special Issue, A12: 201-203.

ABSTRACT

Airborne mites are the main material found in intramural dust. Dust is fine dry powder and it consists of various particles. Dust mites found in poultry dust are allergens causing allergy in sensitive individuals. Some of them have also been found to cause diseases in poultry birds. The mites were picked up from intramural dust of poultry farm. Air sampling was done by using Tilak Air Sampler. The dust from poultry form was collected and scanned under binocular microscope during study period June 2016 to May 2017.The mite Dermatophagoides pteronyssinus were in more number followed by *Dermanyssus farinae* which is actually associate with poultry birds i.e. chicken mites. The observation revealed mites found exhibiting seasonal fluctuations. Further investigation would include exploring more biodiversity of mites in intramural environment. Allergen load of mites in dust samples and clinical investigation.

Keywords: - Airborne mites, Allergen, Intramural, Poultry, Nagbhid.

INTRODUCTION

Mites are established in environment as cosmopolitan in occurrence immanent distributed all over the world. Mites are four-legged belonging to phylum Arthropoda and class Arachnida. They prefer humid condition as suitable environment rich in organic matter. Many species of mites are known to be present in stored food products such as cereal grains. Some of them are minute enough that they are suspended in air; therefore, investigation of mites also forms an important part in the field of Aerobiology (Shende and Korpenwar, 2018).

The intramural dust mite has maximum nutritional and environmental adaptability. Some are found in birds like chick, fowl, duck, pigeon etc., causing various infections in birds- externally and internally. The mites found in poultry dust are allergens causing allergy in sensitive individuals. It also results into aerobiopollutants (Jogdand *et al*., 2007). Some of them are very tiny and light weights therefore are suspended in breeze, and forms exclusive part of Aerobiology. It takes 20 minutes to 2 hours for them to settle back down out of the air. The activities that create airborne

mites are spreading of straw, wood shaving by hand, placing out trays of chicks, transferring of hen, ruffling of feathers. Some are predatory. Most of the mites are ectoparasite. They feed upon blood, shed skin and dandruff etc. Mites are contaminants in fungal and other culture media (Bansod *et al*., 2013; Damle, 2013).

The airborne mites were also trapped by usingTilak Air Sampler. It was first introduced, used and published by Tilak et.al. (1969). Kern in 1921 was first to discover house dust mite as an allergen. Domestic mites feed on variety of material and they prefer protein rich substance. Some like moldy substrates. The poultry workers expose to airborne dust particles is substantial and produce occupational respiratory diseases may develop permanent breathing problems, and they are unable to work.

The main morphologic characters are the possession of four pairs of legs. (Spieksma, 1997). They are found intramural and in house, sheds of cattle's, poultry farm, stores house. Significant role of house dust mites responsible for health hazards such as respiratory allergy in sensitive individuals (Talib and Hare, 1985). It not only affects the individual working in the poultry but also the poultry birds and has affected the growth of birds and lying of the eggs. People working in the poultry farm breathe in many different airborne particles which together are called poultry dust. The activities that create airborne mites are spreading of straw/ wood shaving by hand, placing out trays of chicks, transferring of hens into cages and also ruffling of feathers and other activities of the poultry birds.

Spieksma 1991) in his research found that HDM are very often the cause of allergic rhinitis and asthma in sensitive people. Some mites have been found to cause allergy in sensitive victims and is potential allergens (Jogdand, 2007).

METHODOLOGY

The 'Volumetric Tilak air sampler' (Tilak and Kulkarni, 1970) is an electrically operated device was fixed in middle of the poultry farm It is located in Nagbhid tehsil (between 19.30'N & 20.45'N latitude and 78.46'E longitude) of Chandrapur district of Maharashtra at the height of 1.5 meter from ground level and runs continuously from June 2016 to May 2017. Fourteen slides were prepared from Vaseline coated cello tape on drum by impingement process, cello tape removed from rotating drum of the sampler at the end of 7th day respectively.

Airborne fungal spores and mites were observed qualitatively and quantitatively recorded and identified by using the standard literature and reference materials. The mites per cubic meter were calculated by the following formula: spores/Mites/m3 = No. of same type of spore/mite X 14(Where 14 is the conversion factor for Tilak Air Sampler). Permanent slides are prepared from cellotape mounts in melted glycerine jelly. Add a drop of melted glycerine jelly over the tape by a dropper. Put a rectangular cover slip and press it to remove the air bubbles. The mounted slides were scanned by Binocular research microscope and microphotographs were captured by using microcamera which directly attached to the microscope.

Identification:
These mites had been identified according to the key given by Fain (1957) & the criteria of Hughes (1961) and other available literatures.

RESULT

The occurrences of airborne mites in the slide of Tilak air sampler were observed and identified.

Table 1: Percentage contribution of airborne mites from friend's poultry farm during study period June 2016 to May 2017**.**

Sr. No.	Types of Mites	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	Total	%
1.	*Dermatophagoides pteronyssinus*	56	80	56	42	14	28	42	14	-	-	14	-	346	29.67
2.	*Dermatophagoides farinae*	28	56	42	80	28	14	28	14	-	-	-	28	318	27.27
3.	*Cheyletus eruditus*	56	42	70	42	14	14	0	28	-	-	00	14	280	24.1
4.	*Fuscuropoda agitans*	28	42	56	42	28	-	14	12	-	-	-	-	222	19.03

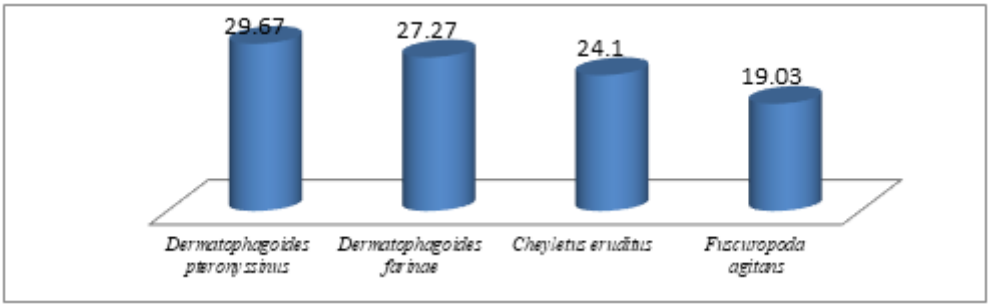

Fig. 1: percentage peculiarity of airborne mites of poultry farm from study period

The body of the mite *Dermatophagoides pteronyssinus* is small and oval; it is broader in middle and narrow at both ends. The general body structure has two parts i.e. Gnathosoma and Idiosoma. Eyes are absent, the gnathosoma has pedipalp. The first pair of leg is directed forward.

Dermatophagoides farinae was first found by Hughes in 1968. The First pair of leg is directed forward and is curved. First leg is expanded laterally. Anterior dorsal shield only about 1.4 times longer than width.

Cheyletus eruditus is a common predatory mite. The mites were numerous in the present investigations. It has modified mouth parts.

Dermanyssusgallinae is an important pest of domestic birds, especially chickens in all parts of the world.

CONCLUSIONS

The present investigation have been revealed four intramural airborne mites *Dermatophagoides pteronyssinus* (29.67%), *Dermatophagoides farina* (27.27%), *Cheyletus eruditus* (24.1%) and *Fuscuropoda agitans* (19.03%) were observed in slides which is made Tilak Air Sampler in friends poultry farm.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:204-206
UGC Approved Journal No 48951

Original Article Open Access

Habitat destruction of local hedge plants of agricultural field by the modernization of agriculture in saoli region.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Watakhere Devilal N and Kale MC (2018) Habitat destruction of local hedge plants of agricultural field by the modernization of agriculture in saoli region, *Int. J. of. Life Sciences*, Special Issue, A12: 204-206.

ABSTRACT

Saoli region is basically an agriculture zone where majority of people survive on rice farming. In this area agricultural field is surrounded by the barbed wire fencing instead of natural fencing by hedge plants. There are several hedge plants which includes medicinal, ecological and wild fruit and vegetable plants. The present investigation focuses on the current status of hedge plants due to habitat destruction by modern agricultural techniques. The outcome of this research will open the debate on classical farming versus modern farming and also make awareness among the people about importance of hedge plants in nature.

Keywords: Saoli, Habitat, Hedge plants, Modernization, Barbed Wire Fencing.

INTRODUCTION

Habitat destruction occurs when a natural habitat, such as a forest or wetland, is altered so dramati- cally that it no longer supports the species it originally sustained. Plant and animal populations are destroyed or displaced, leading to a loss of biodiversity. Habitat destruction is considered the most important driver of species extinction worldwide (Pimm and Raven 2000). Humankind has dramatically transformed much of the Earth's surface and its natural ecosystems. This process is not new it has been ongoing for millennia but it has accelerated sharply over the last two centuries. Today, the loss and degradation of natural habitats can be likened to a war of erosion. Few habitats are destroyed entirely. Very often, habitats are reduced in extent and simultaneously fragmented, leaving small pieces of original habitat. In concert with habitat loss, habitat fragmentation is a grave threat to species survival (Laurance et al. 2002; Sekercioglu et al. 2002). Globally, agriculture is the biggest cause of habitat destruction. Other human activities, such as mining, clear cut logging, trawling, and urbanization also destroy or severely degrade habitats.

In developing nations, where most habitat loss is now occurring, the drivers of environmental change have shifted fundamentally in recent decades. Instead of being caused mostly by small-scale farmers and rural residents, habitat loss, especially in the tropics, is now substantially driven by globalization promoting intensive agriculture and other industrial activities. Destruction and fragmentation of natural habitats are the 2 most important factors in the current species extinction event (Groombridge 1992). Loss and fragmentation of habitat result in reduced population sizes, which increases the probability of extinction by demographic and/or environment (Burkey 1995).

A "hedge" is a living wall composed of plants around farm, garden and home lawns. A hedge is a line of closely spaced shrubs, climbers and sometimes trees, planted and trained to form a barrier or to mark the boundary of an area. The development of hedges over the centuries is preserved in their structure. The first hedges enclosed land for cereal crops during the Neolithic Age (4000–6000 years ago).Hedge is work as a decorative as well as security purpose. Hedge plants can give a higher level of security if farmers select shrubs or small trees that have thorns such as *Acacia sp*., *Caesalpinia sp*., *Bombax ceiba* etc. Hedges used to separate a road from adjoining fields or one field from another. Hedges also serve as wind breaks to improve conditions for the adjacent crops.

In India farmers were primarily planted natural hedge plants around the agriculture farm for security reason.as time goes on hedge plants uses increased from security to different purposes. Hedge plants are used by farmers as vegetables for instance several species of Fabaceae and Cucurbitaceae (***Lablab sp***., ***Memordica sp***.), for medicinal purpose - e.g. Abrus, Acacia, Caesalpinea etc., hedge plants is use as a fruit in villages for example ***Zizyphus sp***.,as a timber plant e.g. ***Acacia nilotica,,*** as fuel wood and uses goes on. Hedges acts as a wind breaks. Possibly the most salient point for many people, they work as an effective wind break but, unlike a fence, they allow some wind to pass through their foliage. When an 80mph wind blows. It bangs into a solid fence panel the pressure on the wood is immense. A hedge will allow that wind to pass through its foliage and slow it down which in turn protect the crops. Hedging is very vital for our wildlife, especially insects who need to travel from garden to garden. Fences stop these lovely creatures from getting to food, shelter and breeding sites but hedges allows it. Birds will take shelter in hedges and bees will collect pollen and nectar from flowering varieties. Indirectly it increases the biodiversity of nature. Now a days due to modernization of agriculture, hedge plants around the farm is replaced by barbed wire fencing for higher security reason.it directly destruct the habitat of hedge plants. The goal of this study was to assess the habitat loss on population of hedge plant and extinction from Saoli town and nearby villages.

METHODOLOGY

Study Area:

Saoli is a tahsil in chandrapur district of Maharashtra state. Location of Sawali is $20^{0}06'41"$N, $79^{0}47'21"$E. Sawali is come under the rice cultivation belt of Maharashtra. People of this village basically depend upon the rice farming. The main occupation is rice farming. Saoli is surrounded by large agricultural fields. The farming in this area is still not that much developed though the fencing of agriculture fields are barbed wire fence.It indirectly impact the habitat of hedge plants. The selected agricultural fields for study are from Saoli town, Malpiranji Village, Khedi Village, Sindola village, and Chakpiranji village. Figure 1 shows the location of the study area.

Fig 1: Location of study area

There are different methodologies proposed by ecologists for sampling of angiosperms. The most important and widely used method for a general assessment is belt transect method. The random sampling method in the selected area. The transect method was followed, and accordingly, transects or straight lines were marked starting from the base of the study area to the end of the Agricultural fields in each selected site. The length of a transect was 500 m to 1 km in each of the selected habitat. This is the standard scientific method followed by various workers in

Table

SN	Agriculture Site	GPS Location	Number of agriculture field surveyed in each site (500mt each)	Number of Hedge plant fencing present
01	Saoli	20⁰06'41"N 79⁰47'21"E	10	-
02	Chakpiranji	20⁰04'46"N 79⁰47'49"E	08	++
03	Khedi	20⁰04'31"N 79⁰46'40"E	07	++
04	Malpiranji	20⁰05'16"N 79⁰47'33"E	07	++
05	Shindola	20⁰04'19"N 79⁰48'57"E	08	-
Total	05		40	06

Note: +, - sign indicates the presence and absence of hedge plant fencing, number of + indicate The number of site where hedges present

respect of phytosociological studies (Cottam and Curtis 1956; Ralhan et al. 1982; Saxena and Singh 1982; Lu et al. 2004; Nautiyal 2008)

Observation: The main focus of this study is to assess the habitat of hedge plant is destroyed or not after doing transect method following observation has done:

Data Collection: The experimental site are selected randomly from each direction keeping in Saoli as center. Malpiranji is in north , Sandola is in south, Khedi is in west and Chakpiranji is in east with respect to Saoli as a center spot. Within each site agricultural field is surveyed. Number of field is depend upon the area of village. Total forty fields has been surveyed. Out of forty only six field has hedge plant fencing. The economical background of farmers also collected. Out of total maximum farmers has more than 5 acre of lands and those have a hedge plant fencing have less than 2 acre land.

RESULT AND DISCUSSION

The data indicates that loss of habitat of hedge plants is huge. Only six field out of forty has a natural hedge fencing it shows that marginalized farmers are still have natural hedge plant fence as they cannot afford barbed wire fencing. As the income of farmers raised this trend of natural hedge will decrease. The trend of use of modern equipment for agriculture is found in those villages which is nearer to city. The modernization of agriculture is causing more threat to habitat of hedge plants. This study shows the status of hedge plants near Saoli region.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:207-209
UGC Approved Journal No 48951

Original Article Open Access

Pollution controlling aquatic plant species from salim Ali lake, Aurangabad

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Ahuja Sangeeta and Pandhure Narayan (2018) Pollution controlling aquatic plant species from salim Ali lake, Aurangabad, *Int. J. of. Life Sciences*, Special Issue, A12: 207-209.

ABSTRACT

Water pollution is serious problem now days. Among various remedies to control water pollution naturally, pollution tolerant plant species plays their vital role in controlling water pollution. The present paper deals with the identification of pollution conrolling aquatic plants growing in the water environment of Salim Ali Lake, Aurangabad. Knowledge of the qualitative and quantitative composition of water is the first step to reveal the nature of the particular environmental problem. One of the most important environmental areas is the quality of life giving water. During the investigations it was observed that 42 aquatic plants species, out of which 22 species belonged to Dicotyledons and 12 plant species of monocotyledons representing 24 families. Dicot dominates over monocot in the ration of 5:3. These plants can be utilized for removal of the heavy metal pollutants from the polluted water bodies without disturbing the lives of other flora and fauna. It may be concluded that these aquatic plants, which employ solar energy, can be utilized heavy metals from waste water for the scavenging for water purification.

Key words: Pollution controlling plant species, Salim Ali Lake, Aquatic Plants.

INTRODUCTION

Water is one of our basic natural resources. It is essential for life in both the biochemical and biophysical senses and its influences are both internal and environmental. It is not only the most abundant single substance in the biosphere but probably is the most remarkable as well. The water environment can generally be characterized as a dilute, aqueous solution, containing a large variety of organic and inorganic chemical species, dissolved and in suspension, and including a variety of plant and animal life. Knowledge of the qualitative and quantitative composition of water is the first step to reveal the nature of the particular environmental problem. One of the most important environmental areas is the quality of life-giving water.

Now a day lakes are degraded by both natural and anthropogenic activities, which deteriorate their quality, and push them to the bank of extinction. In this process of unplanned human developmental activities initiated the need of suitable conservation strategies. Normally, lakes perform the functions directly related to their physical, chemical and biological integrity to decide quality status of water. The present piece of research work is initiated on pollution status at Salim Ali Lake by interference and increase in the population of phytoplankton and microbe. Salim Ali Lake is popularly known as Salim Ali Talab or Abari Houd and located near Delhi Gate Aurangabad. It is situated in the northern part of the city. During the Mughal period it was known as Khiziri Talab. Later on it has been renamed after the great ornithologist, naturalist Salim Ali and also known as birdman of India. Salim Ali Lake comprised a rare and rich biodiversity spot within the city. Salim Ali lake is very much interesting with regards to vegetation because of the fact that the floristic compositions of this locality are mixed type having both terrestrial and aquatic which are yet to be explored. No systematic and extensive floristic works on this lake have been done except for a few scattered reports. The present paper deals with the selection of pollution tolerant aquatic plants growing in the water environment of Salim Ali Lake, situated in Aurangabad District of Maharashtra.

METHODOLOGY

The present work is based on the results of extensive systematic field studies of the plants of this area for a period of three years (May 2014April 2017) under minor project funded by Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Field trips were made once in a week converting the entire area with a view to find out the aquatic plant species and their ecological features. Field observations were recorded like habit, habitat, association and frequency in the field, available local names, as well as flowering and fruiting periods of the investigated taxa. The plants have been identified from fresh materials with the help of different Floras (Naik; Cook). The collected specimens were then poisoned, pressed and dried. After drying, the plants were mounted on the herbarium sheets and labeled properly for future use.

RESULTS AND DISCUSSION

The biota in the surface water is governed entirely by various environmental conditions that determine the selection of species as the physiological performance of the individual organisms. The primary production of organic matter, in the form of phytoplankton and macrophytes is more intense in lakes and reservoirs than in rivers. The physico-chemical properties of freshwater bodies are characteristic of the geochemical, climatic, geomorphological and pollution conditions (largely) prevailing in the drainage basin and the underlying aquifer. In contrast to the chemical quality of water bodies, which can be measured by suitable analytical methods, biological quality is a combination of both qualitative and quantitative characterization. The sample collected should be small in volume, enough to accurately represent the whole water body.

Plate1. Aeschynomene American

Plate.2. Eichhornia crassipes

During the present investigation minimum and maximum along with average values of physico-chemical parameters of the water temperature was recorded. This plays important role in controlling the occurrence and abundance of blue-green algae, planktons and Macrophytes. The dissolved oxygen content was the highest during water at all 03 stations of lake as agreed with earlier workers.

In present study, biological oxidation demand fluctuated directly with water temperature and pH of all 03 stations lake. During present investigation all three samples of water of these collecting sites was alkaline in nature. The concentrations of nitrate and phosphate were greater at all 03 stations of lake. The concentration of nitrate, phosphate and sulphate indicated the higher concern of pollution at all 03 stations of lake. The abundance of blue-green algae during winter and summer confirmed the earlier observations Moore, et, al 1980.

Salim Ali Lake has been situated in the north of Aurangabad city. Sewage and effluent from Cidco, Hudco and other areas have been added in this water body which makes it polluted. Municipal Corporation has taken efforts to make it pollution free but pollution has not been controlled. Ecosystem has got its mechanism to control the pollution. During preliminary investigations it was observed that some plant species are growing luxuriantly in Salim Ali Lake. Hence it was decided to work on plant which tolerates the pollution. During the investigations 44 aquatic plants were recorded out of which 18 species belongs to Dicotyledons and 16 species of monocotyledons representing 27 families. The species documented were *Aeschynomene American, Alternanthera sessilis Ceratophyllum demersum, Bacopa monnieri, Ceratophyllum demersum, Eichhoruia crassipes, Hydrilla verticillata, Leersia hexandra, Pistia stratiotes, Vallisneria spiralis* and different species of *Cyperus*. Plants like *Pistia stratiotes, Eichhornia crassipes* and *Hydrilla verticillata etc.* These plants remove pollutants from water body and grow well. These plants can be utilized for removal of pollutants and heavy metals from the polluted water bodies without affecting its flora and fauna. These species are also found effective for accumulation of heavy metals and to control water pollution.

DISCUSSION

Plants like *Wolffia, Lemna* and *Spirodela* of the family Lemnaceae have also been utilized as fresh fish feed and they resulted in good fish production. These plants can grow fast in the sewage effluents or in rich organic pollutant water-bodies, which can act as biological filter in sewage effluent. Some of the aquatic plants like *Pistia stratiotes, Eichhornia crassipes*, and *Hydrilla verticillata,* have already been proved to be as Hg (II) and Cr (VI) accumulators. These plants can be utilized for removal of the heavy metal pollutants from the polluted water bodies without endangering the lives of other flora and fauna. By considering the data it was concluded that, physico-chemical parameters and pollutions tolerant genera and some species of blue green algae confirmed in Salim Ali Lake. Their presence indicates that the water on the verge of pollution. Nature takes its care as its own which could be indicated through presence of aquatic plant species like *Aeschynomene American, Alternanthera sessilis Ceratophyllum demersum, Bacopa monnieri, Ceratophyllum demersum, Eichhoruia crassipes, Hydrilla verticillata etc.* Further studies in this matter advocated

Acknowledgement:
The Authors are grateful to Dr. Babasaheb Ambedkar Marathwada University Aurangabad for providing funds through minor project to carry out present research works.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:210-211
UGC Approved Journal No 48951

Original Article Open Access

Abutilon theophrasti Medik. (Malvaceae): New distributional plant records to Chandrapur District, MS, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Shende Mukund B and Deshmukh Umakant B (2018) *Abutilon theophrasti* Medik. (Malvaceae): New distributional plant records to Chandrapur District, MS, India, *Int. J. of. Life Sciences*, Special Issue, A12: 210-211.

ABSTRACT

During floristic survey an interesting plant of family Malavaceae was collected from Sonegaon village of Chimur Tahsil , Chandrapur District of Maharshtra state. After critical observation it was identified as *Abutilon theophrasti* Medik. It is reported as a new distributional plant record for Chandrapur district. A brief description with photograph, notes on occurrence and distribution of this taxon are provided for easy identification.

Keywords: Malavaceae, *Abutilon theophrasti* Medik. new, record, Chandrapur District

INTRODUCTION

The genus *Abutilon* Mill. is represented by an approximately 200 recognized species distributed in tropical and subtropical countries (Sivarajan and Pradeep,1996). This genus having characters like tri to multi seeded mericarp, lack of an epicalyx and dorsal wings in mericarps and presence of an endoglossum differs it from other closely related generas of Malvaceae (Esteves and Krapovickas,2002). *Abutilon theophrasti* Medik. (Malvaceae) originally described from India in 1787.It is commonly called as Velvet Leaf, Button Weed, Butter Print,Indian Mallow ,Abutilon Hemp and Chinese Jute (II' in 1949 and Riedl,1976). According to Vavilov (1951) and Li (1970) China is stated to be the origin of *A. theophrasti*.

Roxburgh (1832) described *A. theophrasti* as *Sida abutilon,* as 'a native of various parts of India , though not common'. He reported velvet seed was received from Peking (*sic*) and cultivated in the (then) Bengal province as a substitute for hemp and flax. According to Voigt (1944) *A. theophrasti* was grown in former East India Company's Botanical Garden, Calcutta, and in the Serampore Botanical Garden in the late 18th and early 19th centuries. Hooker (1875) reported 12 species of *Abutilon* from India and *A. theophrasti named* as *A. avicennae, A. theophrasti* as one of the seven

Indian *Abutilon* species of economic importance and it is believed it to be native to northwest India, Sind (now in Pakistan) and Kashmir, with its distribution extending to North Asia, South Europe, and North America (Watt,1889).

Total 18 species of *Abutilon* has been reported from India (Kumar, 2001 and Singh *et al*. 2002). Total 11 species of it has been reported from Maharashtra (Almeida, 1996) and only 2 species reported from Chandrapur District. (Malhotra and Moorthy, 1992). An occurrence of *Abutilon theophrasti* Medik. (Malvaceae) from Sonegaon village of Chimur Tahsil, Chandrpur district shows new distributional records to flora of Chandrapur district of Vidharbha Region. (Maharshtra State)

Taxonomic Treatment:

Abutilon theophrasti Medik. Malv. 28. 1787; Borss. in Blumea, 14: 166. 1966; Paul in Sharma et al. Fl. India 3: 274. 1993; Almeida, Fl. Mah. 1: 103. 1996.

Fig. 1: Habit of *Abutilon theophrasti* Medik in Agriculture field.

Description: A herbaceous annual, covered with fine tomentum intermingled with a few villi. Leaves 7-10 cm long, orbicular-chordate, acuminate, dendate, villose on both surfaces, hispid along the nerves. Petiole 7-8cm, hispid. Stipule large, oblique, broadly ovate-lanceolate. Inflorescence a terminal leafless panicle. Pedicel short, solitary, axillary, jointed below the middle. Calyx hispid, deeply 5-parted nearly to the base; segment ovate-lanceolate. Petals 5, yellow, hardly exceeding the sepals, staminal tube very short. Ripe fruits cylindrical, truncate, umbilicate, longer than the persistent calyx. Carpel's 15-20, oblong, truncate, hispidulous or pubescent, dehiscing along the dorsal suture, each 3-seeded, with 2- long horizontally spreading ciliolate awns. Seeds covered with tufts of stellate hairs. (Fig.1)

Habitat: Weed in Agriculture Field.

Flowers and Fruiting- February – April.

Distribution In Maharashtra- Nasik, Nandur-Madhmeshwar, Sonegaon Village of Chimur Tahsil, Chandrapur District (Now it is collected by Umakant Deshmukh on dated 27 Feb. 2018, from Sonegaon village, GPS location N20°47.5' E79°41.1'-of Chimur Tahsil (Chandrapur Distrct). Herbarium specimen deposited at P.G.Department of of Botany Janata Mahavidyalay , Chandrapur (Voucher No.215) .

Acknowledgement:
The authors are thankful to Dr. Ashok S. Jiwatode, Secretary, Chanda Shikshan Prasarak Mandal, Chandrapur. Dr. M. Subhash, Principal and Dr. Y. B Gedam Head, Department of Botany, Janata Mahavidyalay, Chandrapur for providing laboratory facilities.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018L 212-214
UGC Approved Journal No 48951

Original Article Open Access

Science behind the traditional food, *UGADHI PACHADI*

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Rikkula Roja Prakash (2018) Science behind the traditional food, *UGADHI PACHADI*, *Int. J. of. Life Sciences*, Special Issue, A12: 212-214.

ABSTRACT

People lives in Telangana, Andrapradesh, and some areas of Maharashtra and Karnataka celebrate their new year on Ugadhi. Ugadhi Celebrate on chaitrashudhaekadashi, in this special day they make ugadhipachidi as a prashadam for their deity, ugadhipachadi has to be prepared, almanac will kept before they deity in the prayer room. After general puja people have to take ugadhipachadi as prasadam with empty stomach and listen panchangasravanam. The science behand this traditional food is to improve immunity and to prepare their psychology for future problems. This food is made from six different items that is mango, neem flower, tamarind, jaggery, salt, red chili powder which represent our life with many experience like surprises, sadness, disgust, happiness, fear and anger that has to be accepted through that year. All items in this food have medicinal value which helps sustain during seasonal changes.

Keywords: UgadhiPachadi, Psychology, Medicinal Use.

INTRODUCTION

Science is a Latin word which means to know details about the things. In this paper as studied about traditional *food UgadhiPachadi or Ugadhicharu or Ugadhi chutney*. Which is prepared on the *Ugadhi* festival as a Prasad for their deity. Ugadhi festival celebrated as a new year in Telangana, Andra Pradesh, Karnataka and some areas of Maharashtra. Ugadhi is the beginning of New Year for this area it is the first festival of next year. *Ugadhi i*s celebrated on *Shukla Paksh, Padyami, Chaitra masam* which is the first season (*vasantruthu*) according to the lunar calendar that signifies to new life. In this special day people wake up make a early morning, take bath, wear new cloths, decorate their houses with mango leaves, neem flowers and rangoli. After the decoration elders of the house prepared ugadhipachadi for prasadam. After general Pooja *ugadhipachadi* and *almana*c kept before theire deity in the prayer room. After puja people have to take *ugadhipachadi* as a Prasad with empty stomach and listen amanac*panchang*sravanam. sravanam which for caste good and bad for all the zodiac signs throughout that year, among the months. *Chaitra masam* is the first month, in a stars Ashwini is a first nakshetra and

thitipadyami comes first. People belives that after lord krishnas death, the lord brahma created this kaliyug.1. The first day of kaliyug is celebrated as ugadi. From that day ugadhi is celebrated as a new year and gives ugadhipachadi as a *prasadam.*

There is a certain significance to ugadhi being the new year and not like the first of January, in terms of what is occurring in the planet and the human physiology and mind on this day. Ugadhi follows the lunisolar calendar which has direct connection with the way a human body is made. The Indian calendar is very significance not just culturally but scientifically because it connects people with moments of the planet. Lunisolar calendar include two things that is *sourmana* (themovment of sun with other stars and *chandramana* (the movment of moon according to the earth). *Chandramana*ugadhi is the beginning of new year as a per the lunisolar calendar, largely followed by the Indian people for millennia. From this day the tilt of the globe renders the north hemisphere to receive the highest amount of the suns energy after the 21st days form the ugadhi. Though it may be uncomfortable for humans in terms of the temperature and soaring. In the preparation for this hottest period of the year in tropical latitude they take ugadhipachadi as a remedy. It helps to sustain in this hottest period.

Ugadipachadi made with six different ingredients like mango, tamarind, jaggary, salt, chili and neem flowers. Which obtain naturally during that season, which represent six different shades of life and have medicinal properties. Which include improve immunity during seasonal change, for high energy and antioxidants. Psychologically this six different taste represent the six different feelings like happiness, sadness, angry, disgust, surprises, and teste of life.

METHODOLOGY

It is a general *prasadam* with many uses. The preparation is very simple like a salad. All requirements of this recipe is seasonal, easily available.

Requirements:

- ✓ One cup of finely chopped unripe mango pieces
- ✓ One cup of tamarind fruit pulp
- ✓ One cup of neem flowers \
- ✓ Half tea spoon chili powder
- ✓ Half tea spoon salt
- ✓ Half cup of jaggery
- ✓ One cup of water

PROCEDURE

- Take a big bowl to mix all the ingredients
- Add a one cup of water in a bowl and add one cup of tamarind pulp and mix it well.
- Add half cup of jaggery and mix it until jaggery is melted.
- Now add one cup of finely chopped unripe mango pieces, neem flowers and add half tea spoon of chili powder and salt.
- Now use your hand to blend everything just crush them with your hand to bring out the taste out.
- After preparation first kept before deity and give all the family member and surrounding people to eat as a prasadam.

DISCUSSION

Ethnobotany is the scientific study of the traditional knowledge and customs of people conceiving plants and their medicinal usethe present ethnobotanical paper deals with traditional food and their medicinal use. The *ugadhipachadi* made with jaggery, tamarind, mango, chili, salt and neem flowers and gives six different taste that is sweetness, sourness, tanginess, spicy, and saltiness and bitterness, which represent six different feelings of life. The festival begins with new joy, happiness marks the beginning of new life. Consuming this chutney represent our life with many experiences like happiness, surprises, anger, fear, disgusting that has to accepted through this year. Apart from being tasty dish. The *ugadhipachadi* is significant in other ways.

Bitterness: The bitterness taste of the pachadi comes from the neem flower. The bitter taste are the unhappy moments of life. Bitter moments are also a part of life and so it should not be forget on.

Sweetness: The sweet taste of the *pachad*i comes from the jaggery which represent happiness of life .

Tangy: The tangy taste of pachadi come from unripe mango pieces. Which represent surprises of life, the person should always prepared for it.

Spicy: The spicy taste comes from the red chili powder. It represent angry moments of life.

Salty: The salty taste come from the salt which represent the taste of life

Sour: Tamarind adds the sour taste to the pachadi. It represent the disgust moments of our life. Which along with other flavors lives worth living.

Scientific reasons behind the *ugadhipachadi* is; eating this pachadi with neem, raw mango, tamarind it helps body prepare us to fight with infections and help to improve immunity and sustain during seasonal changes.
Neem flower contain many chemical compounds that help in fight against skin allergies, kill bacteria and intestinal worms. The compound present in a neem flowers have been divided into to major classes. Isoprenoids containing protomeliancins, Limonoids, Azardirone, Limbin, Salanin and non-isoprenoids like proteins flavonoids, coumarins and tanins. (Prabhakaranrao and satyanarayan 2014*)*

Tamarind has long been considered a natural laxative and its dietary fiber eating tamarind us fruit or as a spicy can increase the efficiency of your digestive system. Tamarinds is also bilious substance meaning that stimulates the activity of bile which can help dissolved food faster and juices to speed up the digestion. It help in chronic constipation. Tamarind fruit contain mainly tartaric acid, sugar and vitamins which help in digestion (Devid AND Samson 2014*)*

Mango is an antioxidants, anti-allergic, rich health booster. Mango is rich source of vitamin C. especially when it unripe. The amount of vitamin c in mango also contributes to supporting the body to absorb calcium making the bones strong. Vitamin c rich fruit can help the body fight against infection and reduce the risk of blood disease because this nutrients help in increase the elasticity of blood vessels promoting the formation of new blood vessels. (Shabana, & Rajlaxmi 2010*)*
Jaggery effectively cleans different body parts and prevent anemia

Chili powder contains vitamin A and C, can help individual fight infections. chili powder is an excellent source of vitamin E, is a good for skin and hair. Salt balance the sugar level in the blood and prevent dehydration.

CONCLUSION

Ugadhipachadi is simplest home remedy which in a home and gives many benefits. psychologically it makes positive environment for the people at home and help to sustain in different flavors of life like a consular. Medicinally it is the best remedy during seasonal change. It is better to eat ugadhipachadi up to Shri Rama Navami to get better heath from climatic changes.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:215-225
UGC Approved Journal No 48951

Original Article Open Access

Morpho-histological studies on the adrenal gland in male and female bat, *Taphozous Kachhensis* (Dobson)

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Bansod Deepak S and Chavhan Pankaj R (2018) Morpho-histological studies on the adrenal gland in male and female bat, *Taphozous Kachhensis* (Dobson), *Int. J. of. Life Sciences*, Special Issue, A12: 215-225.

ABSTRACT

It was aimed to study the size, weight and shape differences between right and left adrenal glands, the cortico-medullary ratio of the adrenal glands in bat *Taphozous kachhensis*. The specimen of *Taphozous kachhensis* were collected from Ambai Nimbai,45 kilometers from Bramhapuri (M.S.). Many collections were made during the breeding season so as to coincide with the time of reproductive cycle. These bats are very sluggish in nature after collection they were sexed and were brought to the laboratory. Weight recorded with digital balance before they were sacrificed. The Adrenal were dissect out and fixed in alcoholic Bouin's fluid. After fixation for 24 hr tissue were washed with 70% ethanol. For the histological examinations, after fixation, tissue samples were dehydrated, cleared, and embedded in paraffin. Haematoxylin and eosin staining method was used to examine tissue sections. The size and weight of the gland were different from male to female bats. The adrenal gland is composed of two distinct cell layers, the cortex and the medulla. The medulla is composed of chromaffin cells that produce the hormones epinephrine and nor-epinephrine. The mean diameter and the weight of the Right and left adrenal gland is varies during different phases of the reproductive cycle. The cortico-medullary ratio in male was 65.57% cortex and 34.43% medulla (1.9:1) and in female 58.84% cortex and 41.16% medulla (1.43:1). Medullary tissue of adrenal glands was more in female whereas the cortical tissue was more in male and this suggests that the adrenal medulla hormones production was more in female bat and similarly, cortical hormone secretion was more in male bat.

Key words: Adrenal gland, Cortex, Medulla, Cortico-medullary ratio, bat.

INTRODUCTION

Adrenal gland is one of the most important glands of the endocrine system. These paired glands maintain the homeostasis and play the key roles in response the stress (Humayun *et al*., 2012, Randall *et al*., 2002, Freeman, 1985). The function of the adrenal gland is different according to their cortical and medullary cells. A variety of hormones are produced

from this gland which are very important to maintain the everyday life. Most of the mineralocorticoids and glucocorticoids are produced from adrenal cortex and medulla secretes norepinephrine and epinephrine (Humayun *et al*., 2012). Stress as well as hyper and hypofunction of the adrenal gland is known to suppress reproduction in mammals. Different factors are responsible for the weight, length, width and thickness of the adrenal glands. Unlike to the mammals, in chicken the cortico-medullary tissues are intermingled to each other (Ghosh *et al*., 2001). The light microscopic observations of adrenal gland are known in few species of bat, *Eptrsicus fuscus* and *Anatrozous pallidus; Megaderma lyra lyra* (Bhima Rao Shankar 1975); *Miniopterus schreibersii* (Planel and Guliham, 1961);*Rousettus leschenauti* and *Pteropus giganticus* (Sapkal,1977); *Cynopterus sphinx* and *Taphozous longimanus*; *Taphozous melanopogon* (Lawory and Lall, 1986); *Hipposideros lankadiva* (Seraphim, 2004), *Hipposideros lankadiva* (Dhamani, 2004)and *Taphozous longimanus* (Nerkar, 2007). The study of weight, size and shape of right and left adrenal gland in male and female bat show the significant difference in *Taphozous melanopogon* (Lawory and Lall, 1987). Therefore, this experiment was done to study the size and shape differences between right and left glands, the cortico-medullary ratio.

METHODOLOGY

In the Bat, histomorphometric analysis was carried out at the light microscopic level on the right and left adrenal of male and female bat during various phases of reproductive cycle. The glands were fixed in Bouin's solution and embedded in paraffin wax. The entire gland was cut in serial section at 6µm. Three of these were selected, one from the middle of the gland and two from the poles. They were stained by Haematoxylin and eosin (H and E) to identify the main cell types. The histological sections were analyzed to determine the volume densities of the various components of the gland. The mean volume densities (Vv) were then calculated for the components of the gland. The analysis of the glandular components included the connective tissue, cortex, medulla, blood vessels and nerves. Since the counting was carried out under X10 objective lens, it was decided not to be considered chromaffin tissue and ganglion as two separate entities, but to consider both of them as adrenal medulla. Each complete section was analyzed field by field using an objective X10. Depending on the area of the section, the number of fields ranged from 5 to 15 field per sections.

RESULTS

Gross anatomy of the adrenal glands of male and female bat: The adrenal glands of the bat *Taphozous kachhensis* can be distinguished into two zones an outer cortex and inner medulla. The cortex is completely encircles the medulla. The medulla in this species consists of epitheloid cells arranged in smaller groups surrounded by blood capillaries. Adrenal glands of female bat, *Taphozous kachhensis* during pregnancy are larger than those of other phases. While in male bat, *Taphozous kachhensis* the weight is more during sexually active period. The weight of adrenal gland (mean ± SEM) during different phases of reproductive cycle is represented in Table.1

A. Histology of male adrenal gland

Histology of adrenal gland during sexually quiescence period: Histomorphological findings show that left adrenal gland of male *Taphozous kachhensis* is always larger vascular than right. Light Microscopic studies of adrenal gland of sexually quiescence bat *T. kachhensis* shows that it is round to oval in shape. It is enclosed in collagenous capsule which sends variable deep trabeculae into cortex; the capsule shows enrich adrenal plexus supplying branches to the gland. Just below the capsule the gland has two distinct noticeable zonations, the cortex and medulla (Fig-1). The medulla is present centrally and completely surrounded by cortex. On the basis of histological observations of steroidogenic cells, the cortex is further divided into three major zones; zona glomerulosa, zona fasciculata and zona reticularis (Fig- 2).

Zona Glomerulosa: Zona glomerulosa is the smallest zone of the adrenal cortex present beneath the capsule measuring (73µ) in thickness during the sexually quiescent period of reproductive cycle. This zone consists of polyhedral glomerular cells arranged in rounded cells and generally found in group of 3 - 7 cells or acini. These cells have darkly stained nucleus, either centrally or eccentrically placed. Chromatin clumps are seen in the nucleus scattered towards central or peripheral region. The cytoplasm takes acidic stain and eosinophilic in nature. A few lipid vacuoles are observed in the cytoplasmic matrix and blood capillaries are also observed between the clusters of acini. Zona glomerulosa is compactly associated with zona fasciculata and hence no identifying separation is observed in between these two zones (Fig- 3).

Table. 1: Mean adrenal gland weight with SEM, diameter of various zones of adrenal gland during the different phases of reproductive cycle of male and female bat, *Taphozous kachhensis.*

Reproductive Phase	Weight of the right adrenal gland of female bat Mean weight ± SEM (n = 5)	Mean weight of right adrenal gland of male bat (mgs) ±SEM	Weight of the left adrenal gland of female bat Mean weight ± SEM (n = 5)	Mean weight of left adrenal gland of male bat (mgs) ±SEM
Sexually inactive	1.22±0.058	0.87±0.012	1.43±0.045	0.92± 0.009
Preparatory	1.50±0.071	1.04±0.009	1.87±0 .051	1.12± 0.005
Sexually active	2.02±0.086	1.09±0.006	2.36±0.076	1.16± 0.034

Table 2: Mean adrenal gland diameter of various zones of adrenal gland during the different phases of reproductive cycle of Male and Female bat, *Taphozous kachhensis*.

Period	Diameter in (µ)				
	Whole Gland	Zona glomerulosa	Zona fasciculata	Zona reticularis	Medulla
	Mean diameter	Mean diameter	Mean diameter	Mean diameter	Mean diameter
Female					
Sexually inactive	750 ± 9.82	90 ±1.96	300 ± 0.96	90 ± 2.10	270 ± 3.53
Sexually active	930 ± 2.11	105 ± 2.54	130 ±2.10	95 ± 1.10	600 ± 2.10
Male					
Sexually quiescence	891 µ	73 µ	206 µ	69 µ	543 µ
Sexually active	1072 µ	81 µ	221 µ	75 µ	695 µ

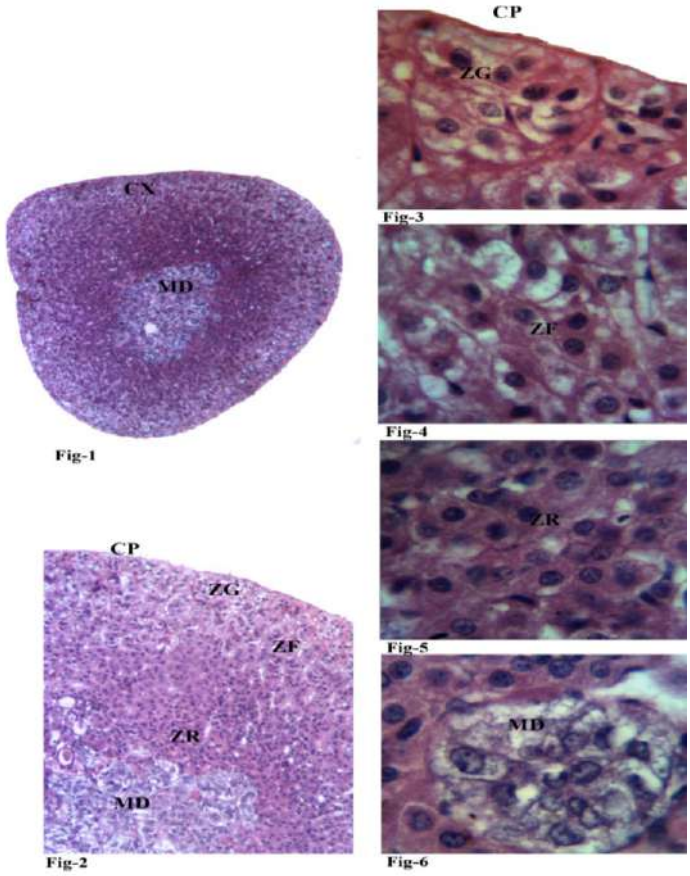


Fig- 1 : Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the presence of capsule (CP) covering the gland, outer cortex (CX) and inner medulla (MD). X 100

Fig- 2 : Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the outermost capsule (CP). Note the cortex differentiated in to outer small zone, zona glomerulosa (ZG), middle long cell cords of zona fasciculata (ZF) and inner zona reticularis (ZR) and innermost centrally placed medulla (MD). X 400

Fig- 3 : Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle shows the presence of outermost capsule (CP) followed by small acini like group of cells of zona glomerulosa (ZG). X 1000

Fig- 4: Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing cords of zona fasciculata (ZF). X 1000

Fig- 5 : Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing cords of zona reticularis (ZR). X 1000

Fig- 6 : Light micrograph of transverse section of adrenal gland during sexually quiescence period of reproductive cycle showing inner medullary zone (MD) surrounded by many small blood vessels (BV) and capillaries. X 1000

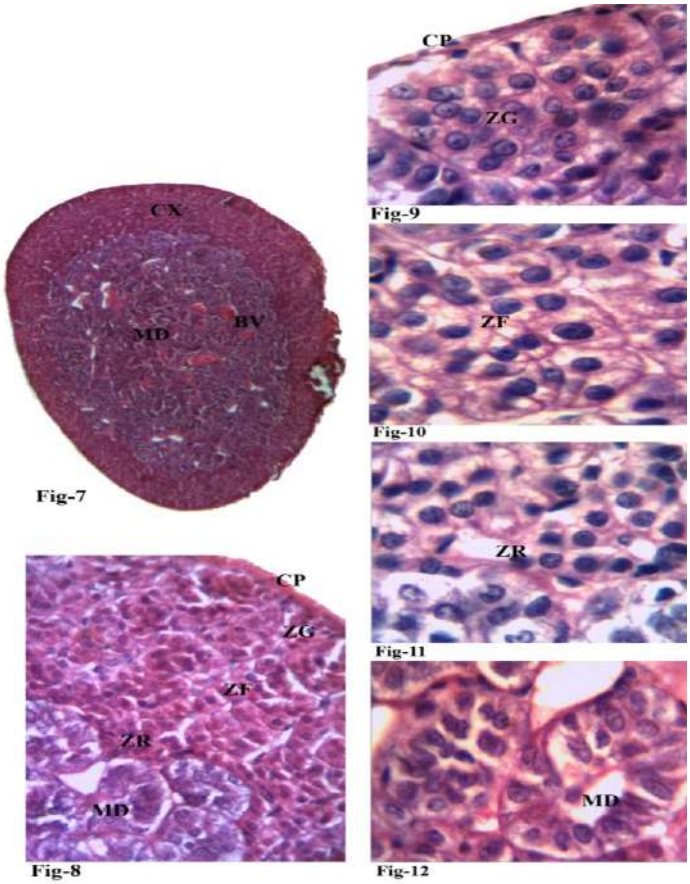


Fig- 7: Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the presence of capsule (CP) covering the gland followed by hypertrophoid cortex (CX) and inner medulla (MD). X 100

Fig- 8 : Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the cortex differentiated in to outer small zona glomerulosa (ZG), middle hypertrophoid zona fasciculata (ZF) and inner zona reticularis (ZR) and innermost centrally placed medulla (MD) with blood vessel. X 400

Fig- 9 : Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle shows the presence of outermost capsule (CP) followed by small acini like group of cells of zona glomerulosa (ZG). X 1000

Fig- 10 : Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing hypertrophoid cell cords of zona fasciculata (ZF) with vacuolations. X 1000

Fig- 11 : Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing cells of zona reticularis (ZR). X 1000

Fig- 12 : Light micrograph of transverse section of adrenal gland during sexually active period of reproductive cycle showing enlarged medullary zone (MD) with blood vessels (BV). X 1000

Zona Fasciculata: Zona fasciculata is largest cortical zone present in between zona glomerulosa and zona reticularis measuring about (206µ) in width during the sexually inactive period of reproductive cycle. This zone shows presence of polyhedral cells arranged in straight column and forms a group of 2 - 5 cells. The cords present in this zone are arranged in a redial manner that runs towards the medulla in the center of the gland. The cords of the zona fasciculata are separated from each other by means of straight venous sinusoids. Comparatively the cells of this zone are larger and vacuolated as compare to the cells of zona glomerulosa and zona reticularis.

Each cell contain large nucleus with scattered chromatin clumps mostly near the peripheral region. Eccentrically, single nucleolus is prominently visible in nucleoplasm. Large numbers of lipid vacuoles are seen in this zone which gives spongy appearance to the cytoplasm, which is basophilic in nature. Formation of cords and spongy cytoplasm are thus characteristics feature of this zone (fig- 4).

Zona Reticularis: Zona reticularis is the innermost zone of the cortex. It is present just below the zona fasciculata on one side and is adjacent to the medullary zone, on another side measures in (69µ) in thickness.

In this zone, each cell disposed in anastomosing cords of varying shape and size. Each cell has basophilic cytoplasm with large vesicular nucleus either centrally or eccentrically placed. Dense chromatin material is seen near the peripheral region of nuclei. Cytoplasm shows few lipid vacuoles. Lipid droplets are very less comparative with zona fasciculata. Sinusoidal capillaries occupy the interstices of the cords of the zone (Fig- 5).

Medulla: Medulla is the central zone of adrenal gland, covered from all the sides by outer cortical zones having diameter (543µ). It shows distinct demarcation from the cortical zone. This zone consists of numerous short cords of 2-3 cells and few groups of 6-14 cells which are separated by sinusoids and capillaries. Each cells of the medulla are darkly stained, having large spherical to irregular shape nuclei. Single prominently visible, darkly stained nucleolus is also observed eccentrically in the

nucleoplasm. Chromaffin clumps are also seen towards the periphery of nucleus. Cytoplasm is basophilic and granular (Fig- 6).

ii) Histology of adrenal gland during sexually active period: The bat *T. kachhensis*, during sexually active period shows very typical and remarkable changes. Morphological studies reveal that, the size of adrenal gland during this period increases as compared to sexually quiescent period. The shape also changes from oval or circular during inactive period to oblong elliptical during active period (Fig- 7). The mean diameter of adrenal gland (1072µ) during sexually active period is more than that of the sexually quiescence period (891µ) (Table- 6 and Histogram- 10).

During active phase, male bat shows vigorous spermatogenesis in their testis and seminiferous tubules are seen packed with spermatozoa. The histological study of adrenal gland during sexually active period shows that the gland is enclosed in a collagenous capsule which sends variable deep trabeculae into the cortex. Below the capsule gland shows two distinct zones, cortex and medulla. During this phase medulla occupies larger area as compare to the medulla of sexually inactive bat (Fig- 7). The cortex shows three distinct zones as seen in the sexually inactive bat; viz. zona glomerulosa, zona fasciculata and zona reticularis (Fig- 8).

Zona Glomerulosa: Zona glomerulosa is the outermost small zone. The average diameter of zona glomerulosa of adrenal gland (81µ) during sexually active period is larger than the average diameter of zona glomerulosa of sexually quiescence period (73µ) (Table- 6 and Histogram- 10). Structurally similar cellular composition is observed in this zone as observed during sexually quiescence period of reproductive cycle. This zone is made up of thick radially arranged long cords or acini. The cell cords or acini are round to elongate in shape. Most of the acini are separated from each other by sinusoids. The acini consist of group of 7-10 large polyhedral cells. Plasma membrane is not clearly observed. The cells have large, eccentrically placed, darkly stained and rounded to oval shaped nucleus. Nucleolus is centric or eccentric in position. Cytoplasm is lightly stained and eosinophilic. The cytoplasm is vacuolated and lipid vacuoles are more than that observed in the cells of glomerulosa during the quiescence period (Fig- 9).

Zona Fasciculata: It is an intermediate zone present beneath the zona glomerulosa and is the largest zone of the cortex present between the zona glomerulosa and zona reticularis. The average diameter of zona fasciculata of adrenal gland (221µ) during sexually active period is larger than the average diameter of zona fasciculata of sexually quiescence period (206µ) (Table- 6 and Histogram- 10). This zone consists of small columns of single or double row of alternately arranged and radially oriented cells. These cells are cuboidal and low columnar types. During this phase the hypertrophied cells of zona fasciculata show spongy cytoplasm with large number of lipid vacuoles. Nucleus is centrally situated, spherical in shape, darkly stained with well developed nuclear membrane having dense chromatin clumps at the periphery. Each cell shows spongy cytoplasm due to the presence of large number of lipid vacuoles. Darkly stained nucleolus is visible eccentrically in the nucleoplasm. Cytoplasm is eosinophilic. Lipid vacuoles in the cells of zona fasciculata are increased during sexually active phase than those found in the cells of sexually inactive phase. Many sinusoids and blood capillaries are seen in the cell cords of this zone (Fig- 10).

Zona Reticularis: It is the innermost zone of the cortex without any clear demarcation. The zona reticularis is present below the zona fasciculata and above the medulla. The average diameter of zona fasciculata of adrenal gland (75µ) during sexually active period is larger than the average diameter of zona reticularis of sexually quiescence period (69µ) (Table- 6 and Histogram- 10). This zone possesses polymorphic cells with lightly stained cytoplasm and vesicular nuclei. Chromatin clumps are seen at the periphery of the nuclei and a single nucleolus is also observed. The cytoplasm shows presence of few lipid vacuoles. The lipid vacuoles are also seen as vacuolations but these are less than those found in the cells of zona fasciculata of the active phase but more than the cells of zona reticularis of sexually quiescence phase of reproductive cycle. Few blood capillaries are also observed in this zone (Fig- 11).

Medulla: Medulla is the innermost region of the adrenal gland. Medullary zone of sexually active bat occupies largest area as compared to the medulla of sexually quiescent bat. During the active breeding period the diameter of medulla (695µ) is larger than the diameter of medulla during sexually quiescence period (543µ) (Table- 6 and Histogram- 10). Entire medulla is surrounded by cortex. It consists of many groups of 4-8

large cells which are separated from various sinusoids. The polymorphic cells of medulla contains spherical or irregular shaped, darkly stained, eccentric placed nucleus with one or two nucleoli in nucleoplasm. Nuclear membrane is well developed with chromatin clumps near periphery. Cytoplasm is granular and basophilic in nature (Fig-12).

B. Histology of female adrenal gland

The histological changes in the adrenal gland of the *Taphozous kachhensis* during different phases of reproductive cycle are presented in figure

i) Adrenal gland during sexually active period

Adrenal gland of *Taphozous kachhensis* is round to oval in shape and encloses in a connective capsule made up of spindle fibrous cells. (Fig.13). Thin strand of connective tissue or trabeculae extend from capsule between the columns of cell in the cortex. Beneath the capsule gland have two distinct zonations the cortex and medulla. On the basis of the observation on histology of the steroidogenic cells cortex is further divided in to three zones namely zona glomerulosa, zona fasciculata and zona reticularies. (fig.14)

Zona glomerulosa: This is the smallest zone of adrenal cortex measuring 90µ during the estrous period. This zone consists of small spherical cells that are compactly arranged and appear acinus like group of cells. These cells have lightly stained cytoplasm with small nuclei with clear nucleolus. Chromatin clumps are observed. Cytoplasm is eosinophilic and granular. Vaculation is observed in the cytoplasm of some cells. Acinar membrane is well defined. (Fig 15).

Zona fasciculate: This is the widest cortical zone observed in the adrenal cortex measuring 300µ during estrous period. Zona fasciculata consist of large polyhedral or cuboidal cells arrange in cords. Cytoplasm is eosinophilic and vacuolated due to the presence of lipid droplets. Nucleus is darkly stained. Zona fasciculata merges with zona glomerulosa above and zona reticularies below. The cords are separated from each other by connective tissue and blood capillaries. (Fig 16).

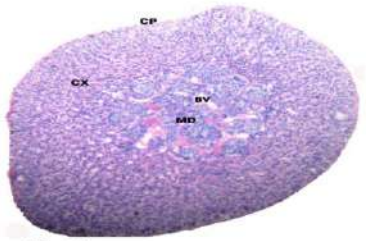
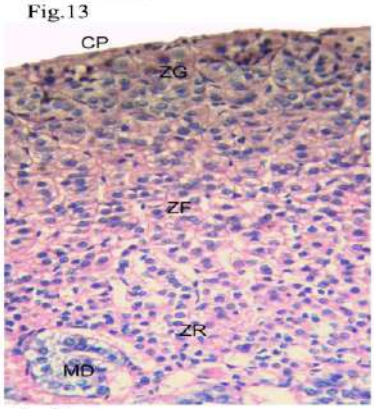


Fig.13



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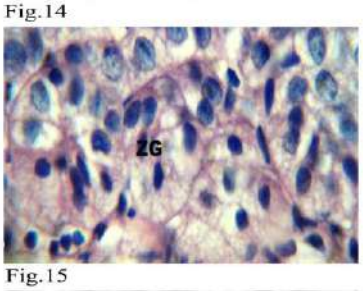

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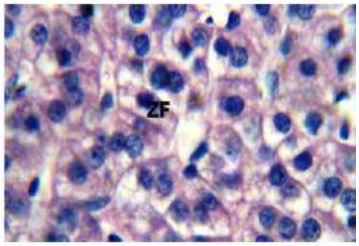

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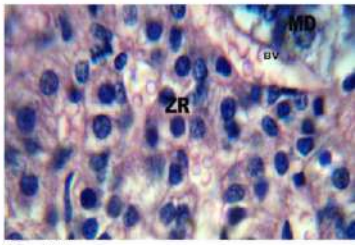

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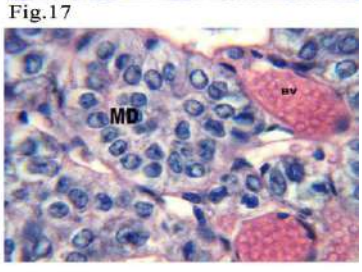


Fig.18

Fig. 13 Transverse section of adrenal gland during sexually active period showing cortex (CX) and medulla (MD). Note the oblong shape of adrenal gland. X 40

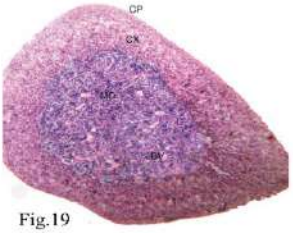
Fig. 14 Transverse section of adrenal gland during sexually active period showing zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularies (ZR) and medulla (MD). X 1000

Fig. 15 Transverse section of adrenal gland during sexually active period showing vacuolated cells of zona glomerulosa (ZG) with darkly stained spherical nuclei. X 1000

Fig.16 Transverse section of adrenal gland during sexually active period showing elongated cell cords of zona fasciculata (ZF) with vacuolated cell cytoplasm. X 1000

Fig. 7 Transverse section of adrenal gland during sexually active period showing the cell of zona reticularis (ZR) are in the form anastomosing cords and having varying degree of shape and size. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in the network of cell cords of zona reticularies. X 1000

Fig. 18 Transverse section of adrenal gland during sexually active period showing medulla(MD) consist of cells arranged in irregular strands or short cords surrounded by blood capillaries. Cells contain darkly stained vesicular nucleus. Cytoplasm of cell is basophilic and granular and in some cells Vaculation is observed. X 1000



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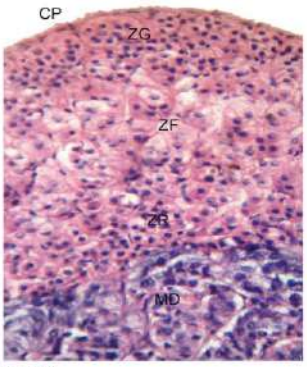

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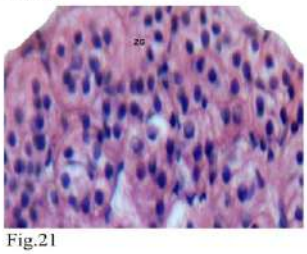
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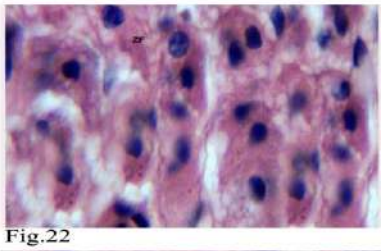
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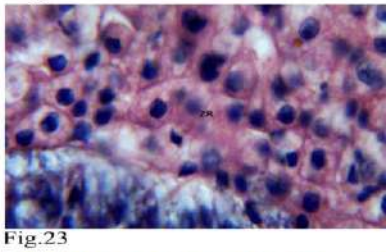
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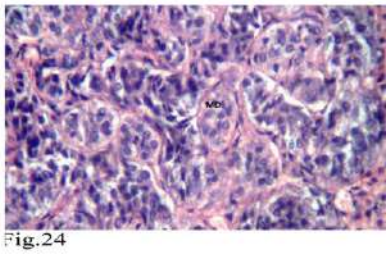
Fig.24

Fig. 19: Transverse section of adrenal gland during sexually inactive showing cortex (CX) and medulla (MD). Note the triangular shape of adrenal gland. X 40

Fig. 20: Transverse section of adrenal gland during sexually inactive showing zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularies (ZR) and medulla (MD). X 1000

Fig. 21: Transverse section of adrenal gland during sexually inactive showing elongated cells of zona glomerulosa (ZG) with darkly stained nuclei and vacuolation in cytoplasm. X 1000

Fig.22: Transverse section of adrenal gland during sexually inactive showing cells of zona fasciculata (ZF). cells are hypertrophied and nucleus is vesicular, round and darkly stained. The cytoplasm is eosinophilic and more vacuolated. X 1000

Fig. 23: Transverse section of adrenal gland during sexually inactive showing the cell of zona reticularis (ZR). Cell cytoplasm is eosinophilic with darkly stained nucleus and more vacuolated as compare to estrus. X 1000

Fig. 24: Transverse section of adrenal gland sexually inactive showing well developed medulla (MD) consists of cells arranged in acini surrounded by blood capillaries. X 1000

Zona reticularis: This zone is present just below the Zona fasciculata and measure about 90 µ during estrous period. The cell of Zona reticularis are in the form anastomosing cords and having varying degree of shape and size. Each cell has eosinophilic cytoplasm with vesicular nucleus. Blood spaces are observed in the network of cell cords of zona reticularies. (Fig 17).

Medulla: Medulla is clearly demarcated from the cortex in *Taphozous kachhensis.* It measure about 270µ during the estrous period of the reproductive cycle. Medulla consist of cells arranged in irregular strands or short cords surrounded by blood capillaries. Cells contain darkly stained vesicular nucleus. Cytoplasm of cell is basophilic and granular. In some cells Vaculation is observed (Fig 18).

ii) Adrenal gland during sexually inactive period

Adrenal gland of *Taphozous kachhensis* is oval to elongated in shape. Cortex is reduced in size and medulla extensively developed.(Fig 19&20).

Zona glomerulosa: Zona glomerulosa is made up of acinar structure. Vaculation are more pronounced in the cytoplasm. Zona glomerulosa measure about 105µ. Cell cytoplasm is lightly stained with round nucleus.(Fig 21).

Zona fasciculate: Zona fasciculata increases in diameter and measure about 130 µ. Zona fasciculata merge with zona glomerulosa above. It is in the form of cords which are not radially arranged and irregular in distribution. Cell cytoplasm is lightly stained. Cytoplasmic vacuolation is more pronounced.(Fig 22).

Zona reticularis : Zona reticularis is measure about 95µ in diameter. Cells are large with faintly stained nucleus observed in the center of the cell and shows prominent nucleolus. Lipid vacuoles are seen in the cells. (Fig 23).

Medulla: Medulla is very large and measure about 600µ in diameter. It is made up of group of cells and surrounds by blood capillaries. The compactly arranged group of cells during early pregnancy is seen scattered during late pregnancy. Cell cytoplasm appears granular and vacuolated. (Fig 24).

DISCUSSION

Morphometric data on the adrenal gland of different species are scarce. In the camel the cortex occupied about 74% of the gland (Ali, 1987). According to Cronshow *et al*. (1974), the duck cortex constitutes 68.2%, the medulla 28.6% and vascular space 3.2% of the total area. This confirmed the present study in which it was observed that bat cortex occupies 64% of the gland during sexually active. In *Taphozous kachhensis*, the size and weight of left adrenal gland is always higher than the right adrenal gland during different phases of the reproductive cycle. Similar observations are reported in *T. melanopogon* (Lawory an Lall, 1987), *H. lankadiva* (Dhamani, 2004), *Taphozous longimanus* (Shende, 2009), *T. kachhensis* (Chavhan et al., 2011, Bansod and Dhamani, 2013) and *P. giganteus giganteus* (Papadkar and Dhamani, 2012). The weight of the adrenal gland increases during estrus and is maximum during early pregnancy in female, it decreases at mid-pregnancy. However, it again increases at late pregnancy. In male the weight of the adrenal gland is lowest during sexually quiescence period, increases from preparatory (recrudescence) period and the highest during sexually active period. Similar observations are reported in bat *Herpestes auropunctatus* (Tomich, 1965), *T. melanopogon* (Lowry and Lall, 1986) and *P. giganteus giganteus* (Papadkar and Dhamani, 2012), *T. kachhensis* (Chavhan et al., 2011, Bansod and Dhamani, 2013). The relative weight of adrenal gland remains more or less constant in adults in both the sexes of Indian mongoose *Herpestes edwardsii*, however the right adrenal gland is observed to be smaller than the left adrenal gland. This is also related with the present findings in *T. kachhensis.* The cortex and medulla of adrenal gland of bat, *Taphozous kachhensis* are clearly marked. Similar observations were made in the bat, *M. schreibersii*, *V. pipistrellus* (Saidapur and Nadkarni, 1976), *R. leschenaulti* (Sapkal, 1978), *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *Cynopterous sphinx* and *Taphozous longimanus* , *R. leschenaulti*, *T. melanopogon* (Lowry and Lall, 1986), *Taphozous longimanus* (Nerkar, 2009), *M. lyra lyra* (Sonwane, 2010), *P. giganteus giganteus* (Sapkal, 1978; Papadkar and Dhamani, 2012), *T. kachhensis* (Chavhan *et al*., 2011, Bansod and Dhamani, 2013).

The adrenal cortex is divided into three distinct zones viz. zona glomerulosa, zona fasciculata and zona reticularis. A distinct zones of the cortex is observed in *M. schereibersii* (Planel et al., 1961), *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *V. pipistrellus* (Saidapur and Nadkarni, 1976), *P. giganteus giganteus* and *R. leschenaulti* (Sapkal, 1978), *Cynopterus sphinx*, *H. lankadiva* (Dhamani, 2004) and *P. giganteus giganteus* (Papadkar and Dhamani, 2012). The zona reticularis is absent in the adrenal gland but it is present in the form of islets of cortical cells in the medullary region in *T. melanopogon* (Lowry and Lall, 1987) and *T. longimanus* (Shende, 2009; Nerkar, 2009). The adrenal gland of *T. kachhensis* during sexually quiescence period is oval to elliptical in shape and is enclosed by thick capsule. Just below the capsule lies a small cortical zone, the zona glomerulosa. It consists of polyhedral glomerular cells which appear group of 3-7 acini or cells. Similar structure is also observed in the zona glomerulosa of *T. longimanus, Hipposideros lankadiva* (Widmaier and Kunz, 1993). In *Cynopterus sphinx* the cells are arranged in groups. Whereas, in *Rousettus leschenaulti* and *Pteropus giganteus* the cells are arrange in columns (Sapkal, 1978) and *P. giganteus giganteus* (Papadkar and Dhamani, 2012).

The zona fasciculata shows presence of polyhedral cells arranged in straight column and forms a group of 2-5 cells. The cords are arranged in a redial manner that runs towards the medulla. Similar pattern of arrangement in zona fasciculata is observed in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *P. giganteus* (Sapkal, 1978, Papadkar and Dhamani, 2012), *H. lankadiva* (Dhamani, 2004) and *T. longimanus* (Shende, 2009). The irregular short cords with one or two radially arranged merge cells observed in *R. leschenaulti* (Sapkal, 1978)

The zona reticularis with anastomosing cords of varying shape and size is present just below the zona fasciculata and adjacent to the medulla. Similar morphological observations are reported in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *P. giganteus* (Sapkal, 1977; Papadkar and Dhamani, 2012), *C. sphinx* and *H. lankadiva* (Dhamani, 2004). However in *R. leschenaulti* zona reticularis is not distinctly demarcated from zona fasciculata (Sapkal, 1977). In *T. melanopogon,* the reticulum occurs in the form of islets of cells (Lowry and Lall, 1986) but is absent in *T. longimanus* (Shende, 2009).

Medulla of *T. kachhensis* shows well developed distinct demarcation. This zone consists of numerous irregular stands or short cords of 4-8 cells and few groups of 6-10 cells which are separated by sinusoids and capillaries. Similar structure of medulla is reported in *M. lyra lyra* (Bhima Rao and Sarkar, 1975), *R. leschenaulti* and *P.*

giganteus (Sapkal, 1978; Papadkar and Dhamani, 2012) supporting present observations. Two types of medullary cells in *P. giganteus* and *R.leschenaulti* on the basis of staining with basic dyes were reported (Sapkal, 1978). However, in the present study adrenal gland is fixed in Bouin's fixative followed by double staining using haematoxylin-eosin, could not produce similar results. Therefore, the present study is unable to give an account of the two types of cells in medulla.
During sexually active period there is an increase in the size of adrenal gland; it is oval to circular during sexually quiescence period and becomes oblong to elliptical in shape during sexually active period. Histoarchitectural study of adrenal gland during sexually active period is advanced over that of adrenal gland of sexually quiescence period.

The cells of glomerulosa are elongated consists of group of 7-10 large polyhedral cells with large eccentrically placed nucleus. More lipid vacuoles are observed during sexually active period than those of sexually quiescence period. During sexually active period, the zona fasciculata is well developed than the zona glomerulosa. Lipid vacuoles in the cells of zona fasciculata are increased during sexually active phase than those found in the cells of sexually quiescence period. Similar structures are observed in *H. lankadiva* & *T. longimanus* (Sapkal, 1978) The cells of zona reticularis are polymorphic with few vacuolations. These lipid vacuoles are less than those found in the cells of zona fasciculata of the active phase but more than the cells of zona reticularis of sexually quiescence period.

The medulla is well developed, spherical or irregular in shaped and occupies largest area as compared to the medulla of sexually quiescent bat with granular and basophilic cytoplasm. Similar morphological findings are reported in *H. lankadiva* (Dhamani, 2004).

In the present study, during lactation and post lactation, the proportion of interrenal tissue was less, which depicts that the bat were more comfortable and less stressed at this stage. Active growth and comfortable environmental temperature during this period also added the effect. However, in the subcapsular, inner and central zones, the overall mean percentage of interrenal tissue was the highest during estrus and pregnancy. This increase might be due to higher demand of the interrenal hormones for the ovulation and formation of eggs. In this bat the uterus is bicornuate and the contralateral ovary shows the continuous folliculogenesis. Hydroxycorticosterone and desoxycorticosterone are reported to be essential for ovulation in bat. In pigeons, adrenal hypertrophy began at 108 hours before ovulation and disappeared after another 108 hour period. This also supported the role of interrenal hormones in ovulation.

During estrus the zona fasciculata is well developed and made up of polygonal cells with vesicular nuclei and vaculations are more pronounced due to lipid droplets. Thus zona fasciculata is more developed than two other zones. Such distinct features are also observed in *Taphozous melanopogon* (Lawry and Lall, 1987) during the non pregnant state. There is an increase in the size of adrenal gland during pregnancy, it is oval to elongate in shape.

Histoarchitecture of adrenal gland during early pregnancy is advanced over that of adrenal gland of estrus female bat. Adrenal gland is oval to elongated in shape. Cortex and medulla is well developed. Zona glomerulosa increase in size and measure about 120µ in diameter. This zone is wider than that of zona glomerulosa of estrus stage. Cells of glomerulosa are elongated with darkly stained nuclei. Cytoplasm is eosinophilic and Vacuolations are observed in cytoplasm.

Zona fasciculata decreases in size during pregnancy as compare to estrus period of reproductive cycle and measure about 113 µ in diameter. The cells are hypertrophied and nucleus is vesicular, round and darkly stained. The cytoplasm is eosinophilic and more vacuolated as compare to estrus period because of high lipid droplets.

During early pregnancy zona reticularis is also increase in size and measures 105 µ in diameter. The cells are hypertrophied. Cytoplasm is eosinophilic with darkly stained nuclei and more vacuolated as compare to estrus. Zona fasciculata merges with Zona reticularis without any clear demarcation.

Medulla is more developed during early pregnancy and measure about 580µ in diameter. Hypertrophied medullary cells are arranged in the acini encircling the blood spaces. Cell cytoplasm is lightly stained and granular with vaculation. Blood supply is also increased and blood vessels are observed encircling the cells. The hyper activity of adrenal gland during pregnancy in this species of female bat correlates with the findings of

Sonwane, 2010 in *Megaderma lyra lyra* and Nerkar, 2007 in *Taphozous longimanus.*

The adrenal gland in this species of female bat is oval to elongated in shape during late pregnancy. Cortex is reduced in size and medulla is extensively developed. Zona glomerulosa is made up of acinus like cells with round nucleus. Vacuolations are more pronounced in the cytoplasm. Zona glomerulosa measures about 105µ in diameter. Cell cytoplasm is lightly stained.

Zona fasciculata increases in diameter and measures about 130 µ. Zona fasciculata merge with zona glomerulosa above and zona reticularis below without any demarcation. It is in the form of cords which are not radially arranged and irregular in distribution. Cell cytoplasm is lightly stained and cytoplasmic vacuolation are more pronounced.

Zona reticularis is smaller cortical zone measuring about 95µ in diameter. Cells are large with faintly stained centrally placed nucleus and shows prominent nucleolus. Few lipid vacuoles are also seen in the cell.

Medulla is the largest zone measuring about 600µ in diameter. It is made up of group of a cells surrounded by blood capillaries. The compactly arranged group of cells observed during early pregnancy are now seen scattered during late pregnancy. Cell cytoplasm appears granular and vacuolated. The present observations are in conformity with observation reported on adrenal gland of *Megaderma lyra lyra* (Sonwane, 2010) and *Hipposideros lankadiva* (Seraphim, 2004).

Adrenal gland during lactation is oval to triangular in shape. During this stage the cortex and medulla is clearly demarcated. Zona glomerulosa is increases in size as compared to late pregnancy and measure about 136µ in diameter. A hypertrophied cell of zona glomerulosa shows granular cytoplasm and small number of lipid vacuoles as compared to the cells observed during pregnancy.

Zona fasciculata is also hypertrophied and measured about 174 µ in diameter. Cell cytoplasm is lightly stained with centrally placed nucleus and clear nucleolus. The cell cytoplasm shows large number lipid vacuoles.

Zona reticularis is smaller in diameter as compared to late pregnancy and measure about 80µ in diameter. These cells have deeply stained granular cytoplasm and darkly stained nucleus. Blood sinuses are also seen in between the network of cells. Lipid vacuoles are seen in the cytoplasm of the cells. Size of medulla decreases and measure about 510µ in diameter. Cytoplasm is deeply stained with vesicular nucleus. The nucleolus is eccentric in position.

The acini like cell cords are seen with presence of blood sinuses at the center (Chavhan et. al., 2011). The adrenal medulla in this species of female bat composed of two type of cells. One type of cell are enveloped by the membrane and found in cluster, and the second type is lightly stained. Two type of cell is also reported in many species of bats, *Megaderma lyra lyra* (Sonwane, 2010). *Hipposideros lankadiva* (Seraphim, 2004). *Rousettus leschenaulti, Pteropus giganteus* (Sapkal, 1978), *Taphozous longimanus, Cynopterus sphinx*.

The adrenal gland produced steroids such as cortisol and cortisone during embryonic and prenatal periods in bat *Megaderma lyra lyra* (Sonwane, 2010). The functional significance of higher proportion of cortex is not fully understood, but one possible explanation could be the increased need to regulate water and electrolyte balance in ducks. It is known that the adrenal cortex produces glucocorticoids and mineralocorticoids that aid in water and electrolyte balance; thus a larger cortex is the adaptation to its habitat.

The highest IC ratio of 2:1 attained during anestrous and estrus in this study can be correlated to the physiological status. Search in the literature has shown no morphometric data on the adrenal gland of any of the chiropteran bat species in India. These structural differences in the adrenal gland of the *T. kachhensis* during reproductive period seem to indicate some physiological relationship.

The hypertrophy of cortical cells suggests that steroid secretion and elaboration from them is involved with at least some aspect of male and female production.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:226-234
UGC Approved Journal No 48951

Original Article Open Access

Bird diversity of agro- forest ecosystem in and around Nagbhid, Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Deshmukh GD and Dhamani AA (2018) Bird diversity of agro- forest ecosystem in and around Nagbhid, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A12: 226-234.

ABSTRACT

The present study was undertaken to explore species diversity of birds, seasonal abundance of birds and their migratory pattern in and arrond the study area. The study site (20º33'N to 20º35'N and longitude 79 º39'2E to 79 º39'4E) spreads over an area of 20 Km^2 located near Nagbhid, taluka level town in Eastern part of Vidarbha of Maharashtra State. It comprises numerous ponds and lakes apart from large Ghodazari Lake. It presents unique geographical site having mountaneous dry deciduous tropical forest, dominated by teak *Tectona grandis* and bamboo *Dendrocalamus strictus*, interspersed with meadows and paddy cultivations. A toal of arround 120 species belonging to 50 families 17 orders were recorded during Jan, 2015 to Dec. 2017. The species recorded included 6 Breeding Migrant (BM), 32 Passage Migrant (PM) and 82 Residents (R). Among the orders, Passeriformes is the richest order in terms of avian species diversity, represented by 56 species while family Muscicapidae is found predominant. Wooly necked stork newly recorded during the present study is vulnerable (VU) species according to IUCN red data list. Present study will helps in designing conservation strategy as this aquatic ecosystem adversely affected by fishing and agricultural activity which leads to bio-accumulation of pesticide in the pond posing serious threat and hence require immediate attention.

Key Words – *Passeriformes, Migrant, Resident, Ghodazari, Muscicapidae*

INTRODUCTION

Birds are widespread in their occurrence, almost found everywhere in the world. Bird families and genera have broad geographical ranges, yet many individual species are specialized in their requirements and have narrow distributions. Birds are mobile and responsive to environmental changes. The variety of avian species in ecosystems reflects the well being of its habitat. Birds are likely to work better as biodiversity indicator taxa in terrestrial habitats than in either freshwater or marine habitats. Birds are the indicators of environment and are being used for conservation and

environmental impact assessment (Donald *et al.* 2001; Gregory, *et al*., 2003). The India checklist acknowledges a total of 1263 species of birds for India, constituting about 12% of the world avifauna (Pravin *et al.*, 2016) while Bird life International projected 1212 species of avifauna, out of which 995 are landbirds. (BirdLife, 2018) Bird communities have been studied fairly well both in temperate and tropical forests (Abdulali, 1981; Islam & Rahmani, 2004; Blake 2007; Pravin and Namir, 2009, 2015; Acharya, *et al*., 2010; Kasambe *et al*., 2016; Pravin, *et al.,* 2016). According to the Forest Research Institute, Maharashtra State comprises, 20 IBA sites (Important Bird Areas) have been identified, in which seven are wildlife sanctuaries; four are national parks, and nine non-protected areas. The forest area of the State is 6.38 million ha, constituting 20.75% of its geographical area. Reserved forest constitutes 76%, protected forest 14% and unclassified forest 10%. There are six national parks and 36 wildlife sanctuaries in the State, covering 4.68% of the State (ENVIS, 2018). There are six tiger reserves, namely Melghat, Pench, Sahyandri, Navegaon-Nagzira, Bor and Tadoba-Andhari Tiger Reserve (ENVIS, 2018). Abdulali (1981) listed 540 species of birds from Maharashtra. Two biomes are found in Maharashtra, the Indian Peninsula Tropical Moist Forest (Biome-10) in the Western Ghat region, and Indo-Malayan Tropical Dry Zone (Biome-11) in the remainder of the State. Past studies documented bird community of Maharashtra, mostly in Western Ghat, (Gole, 2000; Kumbhar and Ghatge, 2014; Kasambe, *et al*, 2015), Marathwada, (Balkhande, et al., 2012) and in Vidarbha by Chittampalli, 1993; Wagh, *et al*., 2015; Bayani & Dandekar, 2017). Most of the study pertaining to diversity of avifauna in this eastern part of the Vidarbha (Maharashtra) carried out in protected forests like Tadoba-Andhari Tiger Reserve, Nagzira Wildlife Sanctuary and Umred Karhandla. The study area had been in media during last decade due to man-wild conflict which resulted in the casualties inflicted by wild animals like tiger, leopard and wild boar on human life. Hence this study has been undertaken to explore rich avifauna of this unexplored habitat.

METHODOLOGY

Study area:

The study site (20º33'N to 20º35'N and longitude 79 º39'2E to 79 º39'4E) spreads over an area of 20 Km^2 located near Nagbhid, taluka level town in Eastern part of Vidarbha of Maharashtra State. It is situated in the newly approved Ghodazari Sanctuary.

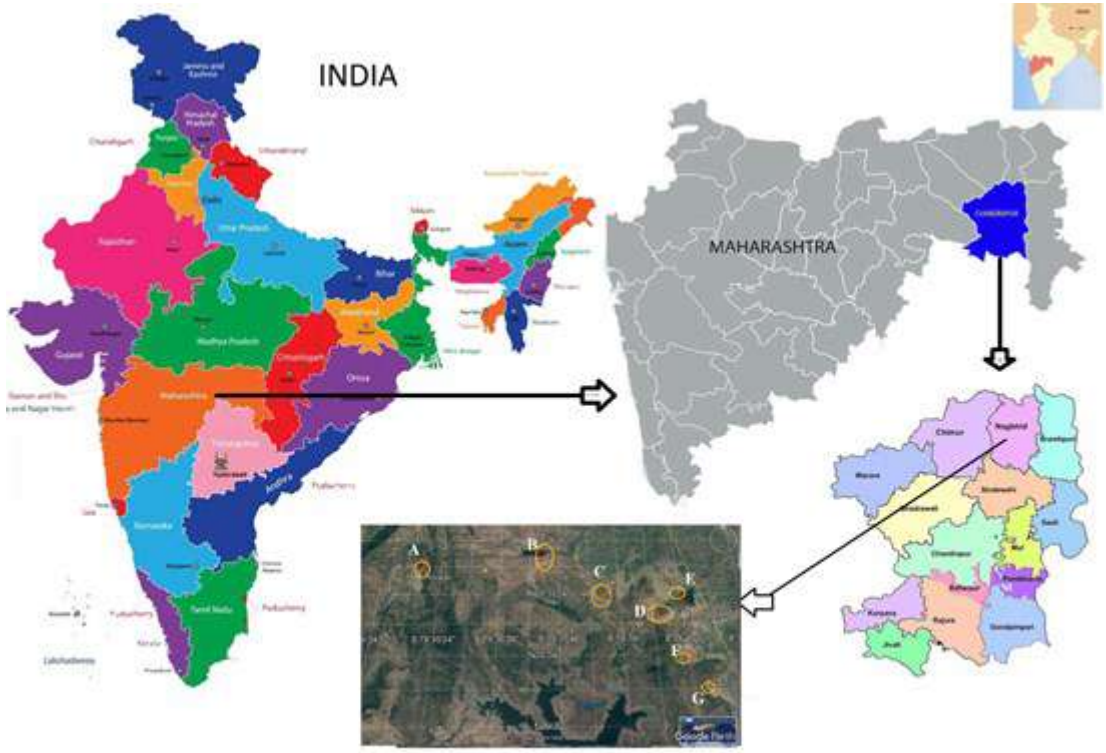

Fig. 1. Study site showing A.Korambi lake B. Kasarla Lake C. Dongargaon pond D. Pandav Lake E. Navkhala Pond , F. Dev Talav (Pond) and G. Tukum Pond

Study area comprises seven water reservoirs, Dev Talav (Lake), Pandav Lake, Navkhala Pond, Tukum Pond, Dongargaon Pond, Kasarla lake and Korambi Lake. It presents unique geographical site having mixed vegetations of tropical dry deciduous forest, dominated by teak *Tectona grandis,Terminalia arjuna, T. tormentosa,* and *Butea monosperma* interspersed with patches of tropical moist rainforest *Syzigium cumini, Terminalia chebula, Emblica officianalis* and bamboo *Dendrocalamus strictus*. Foothills have meadows with shrubs like *Lantana camara* and paddy cultivations interspersed with thorny shrub, *Acacia nilotica*, *Zizyphus jujuba* and *Azadirecta indica* as a predominant flora. This unique climatic condition of agro-forest ecosystem provides suitable feeding ground for avifauna.

Bird Sampling:

Preliminary bird survey of bird community was carried out during Jan, 2014 to Dec. 2016. The avian survey was conducted in 10 sq. km perimeter by monthly visit to the study area. Four sampling sites with radius of 500m had been randomly selected in the study area. (Table.1) according to point transect method for sampling of birds. (Bibby *et al.* 2000) According four point clusters Observation of birds was done by Olympus 118760 10x50 DPSI Wide-Angle Binocular and wherever possible photographed by digital camera Canon EOS 750D. The identification of birds was done as per the photographic guides to the birds of India (Ali and Ripley, 2001; Grimmett *et. al*., 2011). Qualitative data on threats to vegetation and birds were also gathered throughout the study period.

RESULT

In the present survey, total 120 species of avifauna, representing 17 orders and 50 families are recorded during the study period. Migratory status shows that 82 are residents (R), 32 Passage Migrants (PM) and 6 are Breeding Migrants (BM) (Fig. 2). Foraging guild of birds in the study area indicates dominance of insectivorous birds, followed by omnivorous, grainivorous, frugivorous, piscivorous and carnivorous birds while herbivorous and nectorivorous birds are very few. (Fig.3). Maximum abundance recorded from Order - Passeriformes with 47% of total avian species represented by 56 species belonging 25 families.

Maximum abundance noted from Fam-Muscicapidae respresented by 11 species followed by Fam-Accipitridae, Anatidae and Sturniidae represented by 6 species each. Conservation status of bird community of study area indicates that three birds, Black Headed Ibis (*Threskiornis melanocephalus*), Black Stork (*Ciconia nigra*) and Lesser Adjutant (*Leptoptilos javanicus)* are placed threatened catagory, while all other birds are Least Concern (LC) category as per IUCN list. Checklist of bird community in the study area is prepared on the basis bird field guides of Ali & Ripley, 2001; Grimmett, *et al*., (2011) and India check list by Pravin, *et al.*, (2016), eBird (2017) and Bird Life International (2018). (Appendix Table.1 & 2).

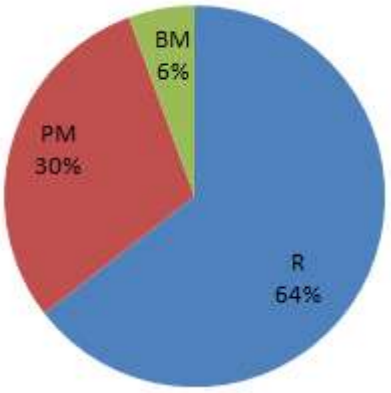

Fig. 2: Showing migratory status

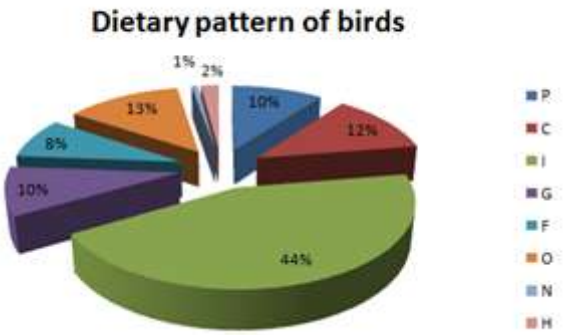

Fig. 3: Dietary pattern of Birds community

Table 1: List of Birds

SN	Common Name	Zoological Name	S	A	IUCN	FG	M
Ord – Ciconiformes							
Fam - Ciconidae							
1	Asian Openbill Stork	*Anastomus oscitans*	MN	++	LC	P,C	BM
2	Painted stork	*Mycteria leucocephalia*	MN	+	LC	P,C	BM
3	Wooly necked stork	*Ciconia episcopus*	MN	+	VU	C,I	PM
4	Black Stork	*Ciconia nigra*	WN	+	LC	P,I	PM
5	Lesser adjutant	*Leptoptilos javanicus*	WN	+	VU	P,I	PM
Ord – Peliconiformes							
Fam - Ardeidae							
6	Purple Heron	*Ardea pupurea*	WN	+	LC	P,I	PM
7	Indian Pond Heron	*Ardea grayii*	AL	+++	LC	P	R
8	Catle Egret	*Bubulcus ibis*	AL	++++	LC	I	R
9	Little Egret	*Egretta garxetta*	MN	+++	LC	P	R
10	Intermediate Egret	*Ardea intermedia*	MN	++	LC	P,I	PM
Fam - Threskiorniodae							
11	Black Headed Ibis	*Threskiornis melanocephalus*	MN	++	NT	P,C,I	BM
12	Glossy Ibis	*Plegadis falcinelus*	AL	+++	LC	P,C,I	BM
13	Little Cormorant	*Phalacrocorax niger*	AL	+++	LC	P	R
Ord – Accipitriformes							
Fam - Accipitridae							
14	Oriental Honey Buzzard	*Pernis ptilorhynchus*	WN	++	LC	I	BM
15	Shikra	*Accipiter badius*	AL	+++	LC	C	R
16	Black winged Kite	*Elanus caeruleus*	WN	+++	LC	C	R
17	Black Kite	*Milvus migrans*	AL	++	LC	C	R
18	Bramhiny Kite	*Haliaster indus*	AL	++	LC	C	R
19	White Eyed Buzzard	*Butastur teesa*	AL	+	LC	C	R
Ord – Gruiiformes							
Fam - Raliidae							
20	Waterhen	*Amaurornis phoenicurus*	AL	++	LC	O	R
21	Eurasian Coot	*Fulica atra*	WN	++	LC	O	PM
22	Common Moorhen	*Gallinula chloropus*	AL	++	LC	O	R
Ord – Gulliformes							
Fam - Phasianiidae							
23	Peafowl	*Pavo cristatus*	AL	+++	LC	G	R
24	Grey Francolin	*Francolin pondicerianus*	AL	++	LC	G	R
25	Lesser Whissling duck	*Dedrocigna javanica*	WN	+++	LC	O	R
26	Indian spot billed duck	*Anas poecillorhyncha*	AL	++++	LC	O	R
Ord – Apodiformes							
Fam - Apodidae							
27	House Swift	*Apus nipalensis*	AL	++	LC	I	R
28	Asian Palm Swift	*Cypsiurus balasiensis.*	AL	++	LC	I	R
Ord – Charadiiformes							
Fam - Charadriidae							
29	Yellow Wattled Lapwing	*Vanelius malabaricus*	WN	++	LC	I	R
30	Little Ringed Plover	*Charadrius dubios*	WN	++	LC	I	PM
Fam - Turnicidae							
31	Jungle Bush Quill	*Purdicula asciatica*	AL	+++	LC	I,G	R

32	Rain Quill	*Cotunix coromandelica*	AL	++++	LC	I,G	R
Fam - Recurvirostridae							
33	Black Winged Stilt	*Himantopus himantopus*	WN	++	LC	P,C,I	PM
Ord – Anseriformes							
Fam - Anatidae							
34	Indian Runner Duck	*Anas platyrhynchos domesticus*	AL	+++	LC	O	R
35	Gadwall	*Mareca strepera*	WN	+++	LC	O	PM
36	Indian spot billed duck	*Anas poecillorhyncha*	AL	+++	LC	O	R
37	Mallard	*Anas platyrhhynchus*	WN	++	LC	H	R
38	Lesser Whissling duck	*Dedrocigna javanica*	WN	+++	LC	H	R
39	Northern pintail	*Anas acuta*	WN	++	LC	H	PM
Ord – Columbiformes							
Fam - Columbidae							
40	Laughing Dove	*Spilopelia senegalensis*	WN	+++	LC	G,F	R
41	Blue Rock Pigeon	*Columba livia*	AL	++++	LC	G,	R
42	Green Pigeon	*Treron phoenicopterus*	WN	++	LC	G,F	PM
Ord – Cuculiformes							
Fam - Cuculidae							
43	Asian Koel	*Eudynamis scolopaceous*	AL	+++	LC	F	R
44	Greater Coucal	*Centropus sinensis*	AL	+++	LC	C	R
Ord – Strigiformes							
Fam - Tytonidae							
45	Barn owl	*Tyto alba*	AL	+++	LC	C	R
Fam - Strigidae							
46	Mottled Wood Owl	*Stryx ocellata*	SM	+	LC	C	R
47	Spotted Owlet	*Athene brama*	AL	++	LC	C,I	R
48	Great Horned Owl	*Bubo bengalensis*	SM	+	LC	C,I	R
Ord – Caprimulgiformes							
Fam - Caprimulgidae							
49	Indian Nightjar	*Caprimulgus asiaticus*	AL	+++	LC	I	R
50	Jungle NightJar	*Caprimulgus indicus*	AL	++	LC	I	R
Ord – Bucerotiformes							
Fam - Upupidae							
51	Common Hoopee	*Upupa epops*	AL	+++	LC	I	R
Fam - Bucerotidae							
52	Indian Grey Hornbill	*Ocyceros birostris*	SM	++	LC	C,F	PM
Ord – Coraciiformes							
Fam - Coracidae							
53	Indian Roller	*Coracius bengalensis*	AL	+++	LC	I	R
Fam - Halcyonidae							
54	White Throated Kingfisher	*Halcyon smyrnensis*	AL	++	LC	C,I	R
Fam - Alcedonidae							
55	Common Kingfisher	*Alcedo atthis*	AL	+++	LC	P	R
56	Blue Eared Kingfisher	*Alcedo menintings*	AL	++	LC	P,I	R
57	Pied Kingfisher	*Cerule rudis*	AL	++	LC	P,I	R
Fam - Meropidae							
58	Green bee-eater	*Merops orientalis*	AL	++++	LC	I	PM
Ord – Psitaciformes							

Fam - Psittacidae							
59	Rose Ringed Parakeet	*Psitacula krameri*	AL	+++	LC	F	R
60	Plum Headed Parakeet	*Psitacula cyanocephala*	WN	+	LC	F	PM
Ord – Piciformes							
Fam - Megalamidae							
61	Coppersmith Barbet	*Psilopogon hematocephala*	AL	+	LC	F,I	R
Fam - Picidae							
62	Pygmy Brown Capped Woodpecker	*Dendrocopus nanus*	WN	+	LC	F,I	R
63	Lesser Goldenback	*Dinopium benghalense*	WN	+	LC	F,I	R
Ord – Passeriiformes							
Fam - Pittidae							
64	Indian Pitta	*Pitta brachyura*	WN	+	LC	I	PM
	Fam - Ploceidae						
65	Baya Weaver Bird	*Ploceus phillipinus*	AL	++	LC	G	R
	Fam - Dicruridae						
66	Black Drongo	*Dicrurus macrocerces*	AL	++++	LC	I	R
	Fam - Oriolidae						
67	Indian Golden Oriole	*Oriolus kundoo*	MN	++	LC	F	PM
	Fam - Monarchidae						
68	Asian Paradise Flycatcher	*Terpsiphone paradisi*	SM	+	LC	I	PM
69	Black Naped Monarch	*Hypothymis azurea*	SM	+	LC	I	BM
	Fam - Corvidae						
70	Indian Jungle crow	*Corvus culminatus*	AL	++	LC	O	R
71	House Crow	*Corvus splendens*	AL	+++	LC	O	R
72	Rufous Treepie	*Dendrocitta vagabunda*	WN	++	LC	I	R
	Fam - Hirudinidae						
73	Red Rumped Swallow	*Cercopis daurica*	SM	++	LC	I	R
	Fam - Paridae						
74	Great Tit	*Parus major*	AL	++	LC	O	R
	Fam - Alaudidae						
75	Oriental SkyLark	*Alauda gulgula*	AL	+++	LC	O	R
76	Ashy Crowned Sparrow Lark	*Erimopteryx griseus*	WN	++	LC	O	PM
	Fam - Timalidae						
77	Jungle Babbler	*Turdoides striata*	AL	++	LC	I	R
78	Common Babbler	*Turdoides caudata*	AL	+++	LC	I	R
	Fam - Pycnonotidae						
79	Red Vented Bulbul	*Pycnonotus cafer*	AL	+++	LC	O	R
	Fam - Cisticolidae						
80	Plain Prinia	*Prinia inornata*	WN	+++	LC	I	PM
81	Jungle Prinia	*Prinia sylvatica*	MN	+	LC	I	PM
82	Common Tailor Bird	*Orthrotomus sutorius*	AL	+++	LC	I,F	R
	Fam - Aegithinidae						
83	Common Iora	*Aegithina tiphia*	AL	++	LC	I	R
	Fam - Zosteropidae						
84	Oriental White Eye	*Zosterops palpebrosus*	SM	++	LC	O,N	PM
	Fam - Phyloscopidae						
85	Paddyfield Warbler	*Acrocephalus agricola*	SM	++++	LC	I	PM
86	Clamerous Reed Warbler	*Acrocephalus stentoreus*	SM	++	LC	I	PM

87	Common chiffchaff	*Phylloscopus collybita*	WN	++	LC	I	PM
	Fam - Turdidae						
88	Pied Thrush	*Geokichia citrina*	WN	+	LC	I,F	PM
	Fam - Laniidae						
89	Long Tailed Shrike	*Lanius schach*	SM	++	LC	I	PM
	Fam - Sturnidae						
90	Common Maina	*Acridotherus tristis*	AL	++++	LC	O	R
91	Pied Myna	*Gracupica contra*	AL	++	LC	O	R
92	Bramhany Myna	*SturnUS pagodarum*	SM	+++	LC	O	PM
93	Asian Glossy Sterling	*Aplonis panayensis*	WM	++	LC	O	PM
94	Common Sterling	*Sturnus vulgaris*	SM	++	LC	O	R
95	Rosy sterling	*Paster roseus*	WN	+	LC	I,F	PM
	Fam - Campephagidae						
96	Small Minivet	*Pericrocotus cinammomeus*	AL	+	LC	I	PM
	Fam - Motaciliidae						
97	Paddyfield Pipit	*Anthus rufulus*	WN	++++	LC	I	R
98	White browed Wagtail	*Motacila maderaspatensis*	AL	++	LC	I	R
99	Grey Wagtail	*Motacila cinerea*	AL	+++	LC	I	R
	Fam - Muscicapidae						
100	Indian Robin	*Copsychus fulicatus*	AL	+++	LC	I	R
101	Magpie Robin	*Copsychus saularis*	AL	++	LC	I	R
102	White Rumped Shama	*Copsychus malbaricus*	WN	+	LC	I	R
103	Red Breasted Flycatcher	*Ficedula parva*	SM	+	LC	I	PM
104	Taiga Flycatcher	*Ficedula albicilla*	WN	++	LC	I	PM
105	Tickell's Blue Flycatcher	*Cyornis tickellae*	WN				
106	Hodgson's Redstart	*Phoenicurus hodgsoni*	WN	+	LC	I	PM
107	Black Redstart	*Phoenicurus ochurus*					
108	Common Stone chat	*Saxicola torquatus*	WN	+++	LC	I	PM
109	Bush Chat	*Saxicola caprata*	WN	+++	LC	I	PM
110	Brown Rock Chat	*Oenanthe fusca*	AL	++++	LC	I	R
	Fam - Rhipiduridae						
111	White Browed Fantail Flycatcher	*Phipidura auriola*	WN	++	LC	I	PM
112	Asian Brown Flycatcher	*Muscicappa dauurica*	WN	++	LC	I	PM
	Fam - Estriididae						
113	Chestnut Munia	*Lonchura atricapila*	AL	+++	LC	G	R
114	Scaly Breasted Munia	*Lonchura punctulata*	AL	+++	LC	G,I	R
115	Straberry Finch/Red Munia	*Amandava amandava*	WN	+	LC	G,I	R
116	White Rumped Munia	*Lonchura striata*	WN	+	LC	G,I	R
117	Indian Silverbill	*Euodice malabarica*	WN	+	LC	G,I	R
	Fam - Passeridae						
118	House Sparrow	*Passer domesticus*	AL	++++	LC	G	R
119	Chestnut Shouldered Petronia	*Gymnoris xanthocolis*	WN	++	LC	G	R
	Fam - Nectarinidae						
120	Purple Sunbird	*Cinnyris asiaticus*	AL	+++	LC	N	R

Appendix Table 1. Bird species recorded in and arround DevTalav (Pond), Maharashtra,India Jan, 2014 to Dec. 2016. A = Abundance, FG = Foraging Guild, IUCN=Internation Union for Conservation of Nature, M = Migratory status, MN = Monsoon, AL = All Seasons, WN = Winter, LC = Least Concern, NT = Near Threatened, P = Piscivorous, C = Carnivorous, I = Insectivorous, O = Omnivorous, F = Frugivorous, N = Nectorivorous, G = Grainivorous, BM=Breeding Migrant, PM=Passage Migrant, R=Resident

Table 2. Percentage occurrence of families of bird community in study area

Sr. No.	Families	% Occurence
1.	Ciconidae	4.1
2.	Ardeidae	4.1
3.	Threskiorniodae	2.5
4.	Accipitridae	5.0
5.	Raliidae	2.5
6.	Phasianiidae	3.3
7.	Apodidae	1.6
8.	Charadriidae	1.6
9.	Turnicidae	1.6
10.	Recurvirostridae	0.8
11.	Columbidae	2.5
12.	Anatidae	5.0
13.	Cuculidae	1.6
14.	Tytonidae	0.8
15.	Strigidae	2.5
16.	Caprimulgidae	1.6
17.	Upupidae	0.8
18.	Bucerotidae	0.8
19.	Coracidae	0.8
20.	Halcyonidae	0.8
21.	Alcedonidae	2.5
22.	Meropidae	0.8
23.	Psittacidae	1.6
24.	Megalamidae	0.8
25.	Picidae	1.6
26.	Pittidae	0.8
27.	Ploceidae	0.8
28.	Dicruridae	0.8
29.	Oriolidae	0.8
30.	Monarchidae	1.6
31.	Corvidae	2.5
32.	Hirudinidae	0.8
33.	Paridae	0.8
34.	Alaudidae	1.6
35.	Timalidae	1.6
36.	Pycnonotidae	0.8
37.	Cisticolidae	2.5
38.	Aegithinidae	0.8
39.	Zosteropidae	0.8
40.	Phyloscopidae	1.6
41.	Turdidae	0.8
42.	Laniidae	0.8
43.	Sturnidae	5.0
44.	Campephagidae	0.8
45.	Motaciliidae	2.8
46.	Muscicapidae	9.1
47.	Rhipiduridae	1.6
48.	Estriididae	4.1
49.	Passeridae	1.6
50.	Nectarinidae	0.8

DISCUSSION

Avifauna of study area in and arround Nagbhid, within the proposed Ghodazari Wildlife Sanctuary in Bramhapuri Forest Division rangeof Maharashtra, remained unexplored till date. During present survey of study site, total 120 species of avifauna, representing 17 orders and 50 families are recorded during Jan, 2014 to Dec. 2016. Bayani and Dandekar (2017) recorded 255 species of avifauna from Tadoba-Andhari Tiger Reserve (TATR) forest in Maharashtra, which is located in the viscinity of study area.

Foraging guild of birds in the study area indicates dominance of insectivorous birds, followed by omnivorous, grainivorous, frugivorous, piscivorous and carnivorous birds while herbivorous and nectorivorous birds are very few which indicates that agro-forest ecosystem in the study area provide food for their sustenance. Ecosystem of local area impacted composition of bird community and their foraging guild (Gregory, *et al.,* 2003; Bhagwat, *et al*., 2008; Beaudrot, *et al*., 2016; Karanth, et al., 2016). Substantial number of rare bird species like Purple Heron, White-Eyed Buzzard, Mottled Wood Owl, Indian Pitta, Plum-Headed Parakeet, Black Stork, Wooly Necked Stork, Bar Headed Goose, Great Horned Owl, Black Naped Monarch and Asian Paradise Flycatcher, adds to the richness of avifauna. As such rare species are indicative of rich diversity of birds in this habitat hence need special conservation measures. (Prendergast, *et al*., 1993).

Birds like Rosy Sterling are winter visitors from their breeding ground in Europian countries (Nyagolov, *et al*,. 2003) found to perched on *Butea monosperma* tree during flowering in the month of March. *Anastomus oscitans*, *Threskiornis melanocephalus, Plegadis falcinelus, Pernis ptilorhynchus and Hypothymis azurea* are breeding migrants, migrated from their faraway nesting places. *Anastomus oscitans* and *Threskiornis melanocephalus* are monsoon breeding migrants arrive from their faraway nesting places in North-East India, Burma and Bangladesh to breeding places in the month of June-July and departs from breeding place in the month of December. (Wells et al., 1999; Ali and Ripley, 2001; Das, et al., 2014; Pramanik, *et al*., 2016). Black Stork, Wooly Necked Stork and Bar Headed Goose are newly recorded in study area within Ghodazari Sanctuary.

CONCLUSION

In the present study of avifauna, migratory birds are observed in few numbers as compared to resident birds. The substantial number of insectivorous and granivorous birds in the study area underline the significance of agroforest ecosystem. Anthropogenic activities like livestock grazing, fishing, uses of pesticides in agriculture and deforestation are posing threat to the bird diversity in the study area hence need conservation measures.

Acknowledgements

I acknowledge the help and expertise provided by our friends, Mr. Narendra Lohbare and Satish Charthal. Without their assistence this work would not have been possible.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:235-239
UGC Approved Journal No 48951

Original Article Open Access

Catfish fauna (Order- Siluriformes) Diversity of Pranhita River Sub basin at Sironcha, Gadchiroli District, Maharashtra, India

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Sayyad Nagma and Dhamani Amir (2018) Catfish fauna (Order-Siluriformes) Diversity of Pranhita River Sub basin at Sironcha, Gadchiroli District, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A12: 235-239.

ABSTRACT

Catfish investigation of Pranhita River sub basin at Sironcha Dist. Gadchiroli, Maharashtra was carried out during July 2015 to June 2017. Pranhita River is boon for peoples of Sironcha. The documented paper deals with the diversity, abundance and conservation (IUCN) status of catfish. Sampling sites were selected along 10km and visited fortnightly. A total of 15 species of order siluriformes belonging to 5 families and 9 genera were recorded during study period. In study Bagridae was dominant family with 8 species, from families like Sisoridae and Pangasiidae single species recorded from each. Abundance shows majority of catfish are common, few are uncommon and only one species was rare. 4 catfish species were under near threatened category while 11 were under least concern category according to IUCN red list status.

Keywords: Catfish, Siluriformes, Pranhita, River, Sironcha

INTRODUCTION

Pranhita River (19°35'24"N and 79°47'59"E) is major tributary of Godavari River system formed by the confluence of Wardha and Wainganga Rivers at Kouthala (village in Maharashtra) at an elevation of about 146m above mean sea level. Main tributaries of Pranhita River are Dina, Nagulvagu and Peddawagu. Total length of river is 113km. It form boundary between Gadchiroli district of Maharashtra state and Adilabad district of Telangana state. The river is very beneficial for peoples of Sironcha as it is ultimate source of water for drinking and irrigation, beside this it provide shelter to various endemic flora and fauna.

The study area for present documentation was Sironcha a pollution free area, located at southward region of Gadchiroli District of Maharashtra State, India. This Area is mostly surrounded by rivers such as Indravati (East), Godavari (South) and Pranhita (West).

In this town during summer temperature may riches up to 48^0 C and in winter it may falls up to 8^0 C. Area of Sironcha never faces the condition of drought because of Pranhita River sub basin.

Fishes are large group of vertebrates having enormous variation in shape, size, biology and habitat (Bobdey 2014). Catfish are group of fishes belongs to order siluriformes of teleostie fishes and are characterized by the grayish or silver colored roughly cylindrical body without scales and a large mouth with barbels, a character that give catfish their name (Fink & Fink 1981). They are present nearly all countries of world. There are about 3407 species of catfish in the World (Armbruster 2011). Indian water bodies provide shelter to 197 species of catfish (Jayaram 2009). Various researchers have studied the catfish diversity includes Patra (2011) studied the catfish diversity of karala River of West Bengal and recorded presence of 7 species. Kumbar and Lad (2014) reported 13 catfish species from Krishna River, Maharashtra. Lalronunga et al. (2014) recoded 37 species of catfish from rivers of Barak drainage of Mizoram. Gedekar and Tijare (2012) studied the fish diversity of Wainganga river of Gadchiroli district and documented 49 fish species out of which 9 species belong to order siluriformes. Shaikh (2014) reported 37 species of fish from Pranhita River at Sironcha out of which 8 species were of Catfish.

However, very little information available about catfish diversity of Pranhita River without separate account, therefore the aim current research is to provide separate record of catfish diversity, abundance, their Conservation (IUCN) status and to create awareness regarding their conservation.

MATERIAL AND METHODS

Data on Pranhita River catfish diversity were taken from 2 selected sampling sites along 10 km during study period of two years from July 2015 to June 2017. Fish samples were collected from local fishermen. Two sampling sites are Site-1 and Site-2. Site-1(Sironcha fishing Station) is located at Westward direction of Sironcha town near Vithhaleshwar temple. Site-2 (Nagram fishing Station) is on Southwestern direction of Sironcha near Nagram Village. It is 7 km from Sironcha town, after this station river travels little and combines with Godavari River near Kaleshwaram.

Fish samples were collected fortnightly and photographed by using Canon Eos 1300d DSLR camera after that immediately fishes brought to laboratory to preserve them in 10% formaldehyde solution for further investigation. Identification of fishes was based on standard taxonomic keys as described by Talwar and Jhingran (1991), Jayaram (1999), Day (1958) and Fish Base website was also referred (www.fishbase.org). Checklist of captured catfish their scientific name, common name, abundance as C(common) U (uncommon) O (occasional) and R(rare) and conservation (IUCN)status as NT (near threatened) and LC (least concern) is presented in Table 1.

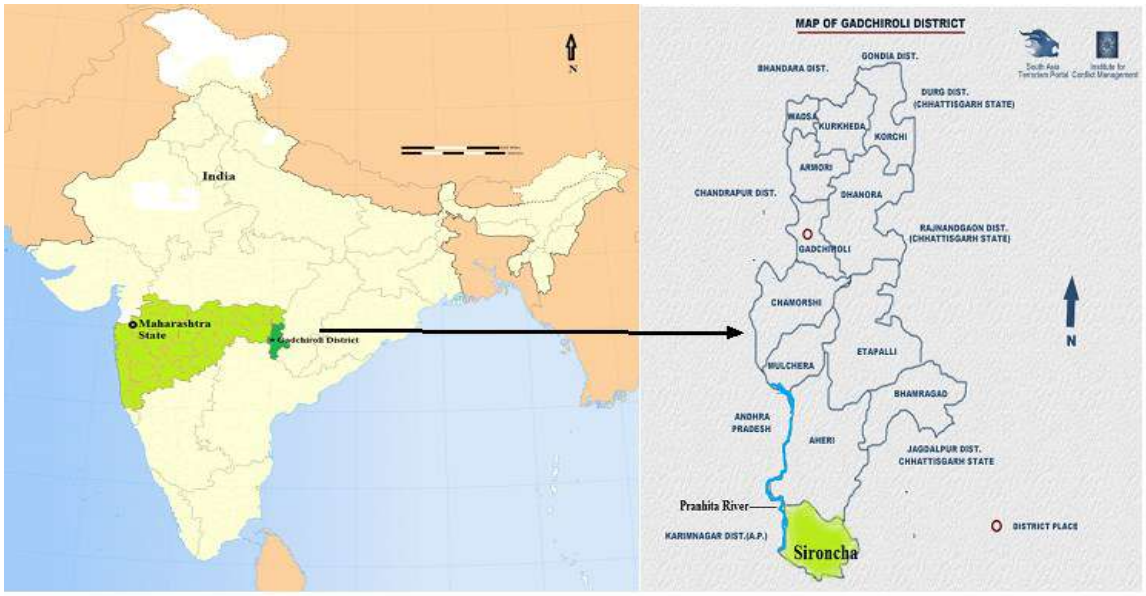

Fig 1: Location map of Pranhita River at Sironch

RESULTS AND DISCUSSION

In present catfish diversity investigation from Pranhita River sub basin at Sironcha during July 2015 to June 2017, 15 species were recorded belonging 5 families and 9 genera of order siluriformes (Table-1). Dominant family was Bagridae with 8 species belonging to genus Mystus, Rita and Sperata (Fig-2). Second dominant family was Siluridae with 3 species of genus Ompok and Wallago. From family schilbeidae 2 species were documented these were *Clupisoma garua* and Eutropichthys vacha, from families like Pangasiidae and Sisoridae only one species reported from each i.e., *Pangasius pangasius* and Bagarius and bagarius.

The conservation (IUCN) status of species belonging to family Siluridae and Sisoridae shows near threatened (NT) and remaining families like Bagridae, schilbeidae and Pangasiidae species are under least concern (LC) category. 15 photographs of captured catfish (Fig. 2: Image 1-15) were documented through this paper.

Abundance based upon catch frequency shows (Fig-3) majority (53.33%) of catfish were common (C) such as *Ompok bimaculatus*, *Wallago attu, Mystus vittatus, Mystus cavasius, Mystus tengara, Rita kuturnee, Sperata seenghala, Clupisoma garua*, etc. Uncommon (U) Species were Ompok pabda, *Mystus bleekeri*, *Sperata aor*, *Eutropiichthys vacha* and *Pangasius pangasius*. Bagarius

Table- 1: Showing families, scientific names, Common name, abundance and IUCN status of catfish.

Sr. No.	Family	Scientific Name	Common Name	Abundance	Conservation Status (IUCN)
1	Siluridae	*Ompok bimaculatus*(Bloch)	Butter Catfish	C	NT
2		*Ompok pabda* (Ham)	Pabdah Catfish	U	NT
3		*Wallago attu* (Bloch & Schneider)	Boal	C	NT
4	Bagridae	*Mystus vittatus* (Bloch)	Stripe Dwarf Catfish	C	LC
5		*Mystus cavasius* (Ham)	Gangetic Mystus	C	LC
6		*Mystus tengara* (Ham)	Tengara Catfish	C	LC
7		*Mystus bleekeri* (Day)	Day's Mystus	U	LC
8		*Rita gogra* (Sykes)	-	R	LC
9		*Rita kuturnee* (Sykes)	Deccan Rita	C	LC
10		*Sperata seenghala* (Sykes)	Seenghala	C	LC
11		*Sperata aor* (Ham)	-	U	LC
12	Schilbeidae	*Clupisoma garua* (Ham)	-	C	LC
13		*Eutropiichthys vacha* (Ham)	Bachawa	U	LC
14	Pangasiidae	*Pangasius pangasius* (Ham)	Pungas	U	LC
15	Sisoridae	*Bagarius bagarius* (Ham)	Goonch	O	NT

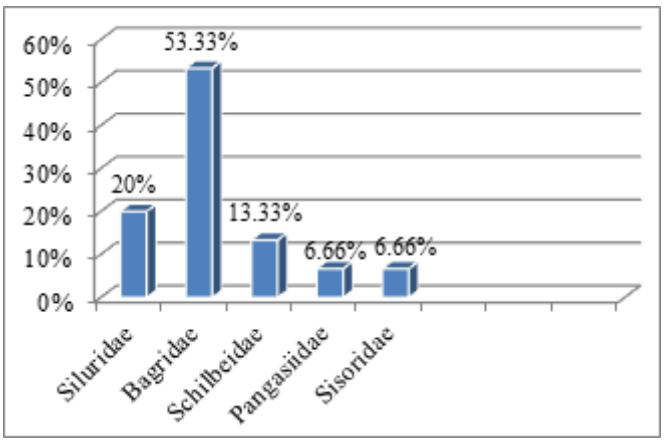

Fig. 2: Family wise Composition of Catfish fauna

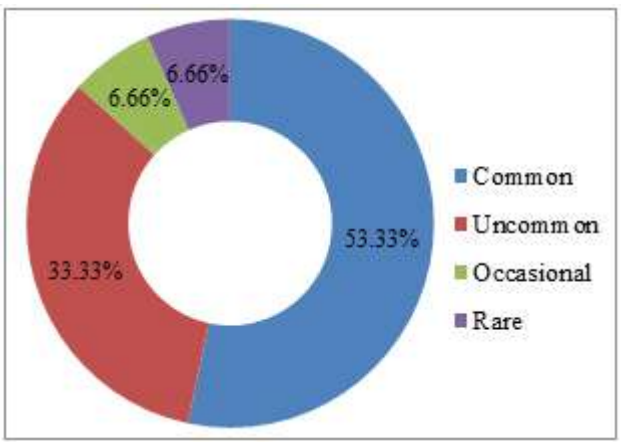

Fig. 3: Abundance of Catfish Fauna

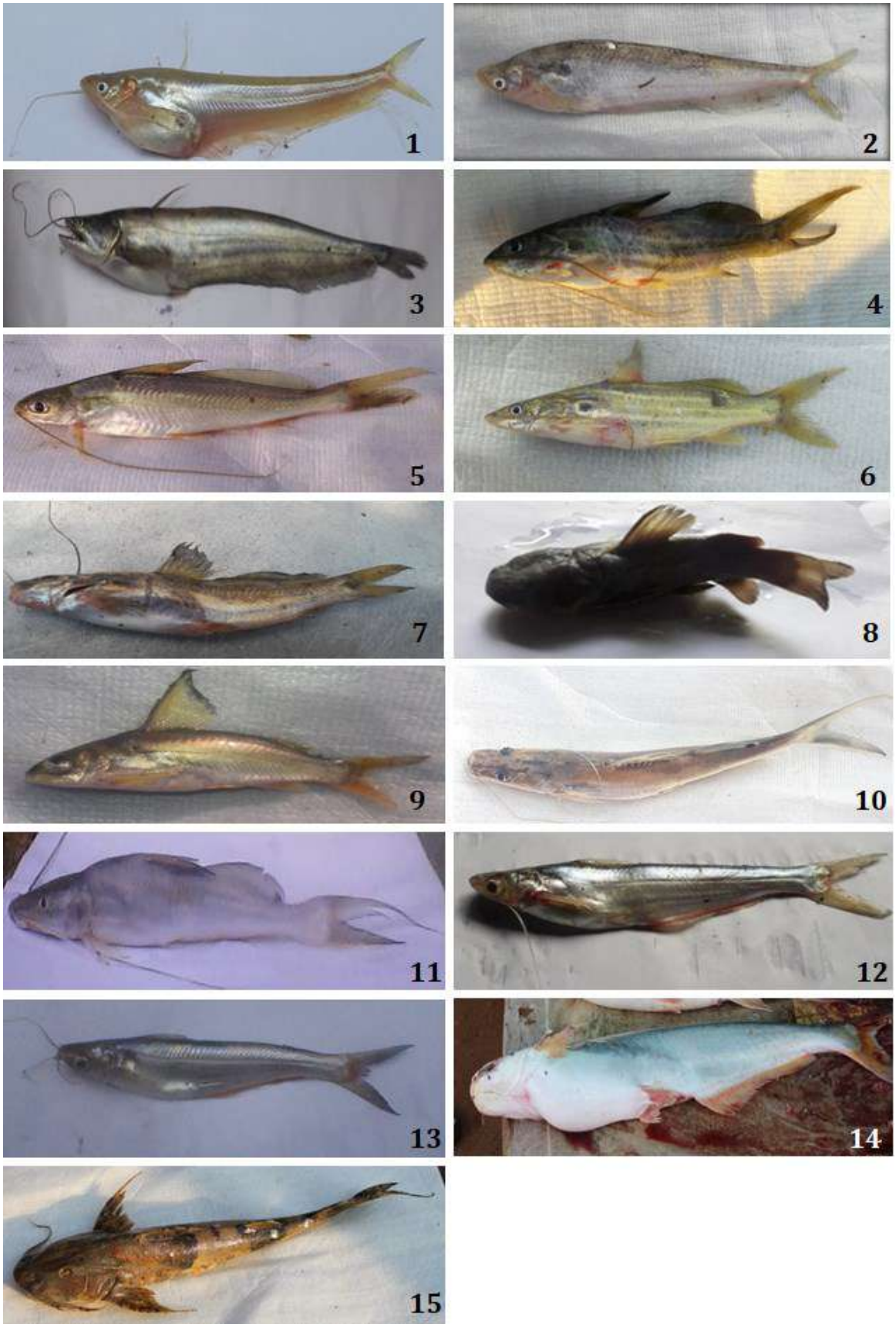

Fig. 2: 1: *Ompok bimaculatus,* 2: *Ompak pabda,* 3: *Wallago atta* 4: *Mystus vittatus* 5 : *Mystus Cavasius 6: Mystus tengara* 7: *Mystus bleekeri* 8: *Rita gogra* (preserved specimen) 9: *Rita kuturnee* 10: *Sperata seenghala* 11: *Sperata aor* 12: *Clupisoma garua* 13. *Eutropiichthys vacha* 14. *Pangasius pangasius* 15. *Bagarius bangarius.*

bagarius are occasionally captured (7 to 9 times) during study period by fishermen while rare fish *Rita gogra* was caught only once in two year. Most abundant fish which caught daily by fishermen in great number was *Rita kuturnee*. Both Rita species has great demand in local fish market of Sironcha because of its taste and it is preferred more after Labeo rohita by local villagers.

Pranhita river is unpolluted river as there were no industries in surrounding area and river never dry up in summer therefore it is suitable area for conservation of catfish, beside this fishermen of this area mainly use environment friendly fishing technique, do not use technique such as liming and dynamite fishing which cause decline of rare species but over exploitation may occur because of demand in local fish market which may cause loss of catfish fauna. Strict management measures and educating local people will help to conserve catfish.

Acknowledgement: First Author grateful to University Grant Commission (UGC) for providing fellowship through Maulana Azad National Fellowship (MANF-JRF) Scheme to carry out research.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018: 240-246
UGC Approved Journal No 48951

Original Article Open Access

Histomorphology of the larval hindgut of the Dragonfly *Bradinopyga geminata* (Rambur) (Odonata: Libellulidae)

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Chaudhari SS, Bakare SS & Dhamani AA (2018) Histomorphology Of The Larval Hindgut Of The Dragonfly *Bradinopyga geminata* (Rambur) (Odonata: Libellulidae), *Int. J. of. Life Sciences*, Special Issue, A12: 240-246.

ABSTRACT

The larval hindgut of dragonfly *Bradinopyga geminata* (Rambur), was studied with light microscopy. The hindgut of dragonfly differentiate into ileum and rectum. The ileum is narrow tubular at anterior and posteriorly it is dilated spherical bulb. Ileum is located between midgut and rectum of hindgut. Ileum occupies the 20% length of total length of the alimentary canal. In the transverse section of ileum measure 430µm- 450µm diameter approximately. The internal 6 longitudinal folds are of unequal height and project into the lumen irregularly. The epithelium of ileum is consists single layer cuboidal epithelium with finely granular cytoplasm. The inner surface of the epithelium covered with by a prominent cuticle. The rectum is usually enlarged sac like structure which posteriorly connects with anal tube, the modified structure of anterior rectum in dragonfly is branchial chamber. It occupies the one third length of the alimentary canal. Rectum of dragonfly larvae is almost 3 times bigger in diameter than that of ileum. The rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. Rectal basal pads encloses medulary fat cells from which pass trachea, number of gills projects into a rectal lumen and cover the maximum area of rectal lumen gills are underlying by thin cuticle.

Key words: Hindgut, Ileum, Rectum, Dragonfly, Larva

INTRODUCTION

The insect hindgut is located at posterior region of alimentary canal. Anteriorly it attached with midgut and posteriorly ends with anus. There is insufficient information available on the functional morphology and histological structure of hindgut of dragonfly *Bradinopyga geminata* (Rambur). However number of papers contributed significantly to certain aspects of the the hindgut, such as (Tillyard, 1917; Snodgrass,1954).

The hindgut isinternaly lined with cuticular layer which is thinner and permeable than foregut. The epithelium is thin, but the cells are more cuboid than in the foregut while those rectal pads are tall with clear cytoplasm, this includes pylorus, ileum and rectum (Chapman,1982; Tembhare and Wazalwar,2002). Both the foregut and hindgut have a cuticular lining, which is lacking in the midgut. The length of gut is roughly correlated with diet; insect feeding on a largely protein diet tends to have a shorter gut than those feeding largely on carbohydrates but this is not always true (Snodgrass,1935; Chapman,1982; Gullan and Cranston 2005). The anterior portion of the rectum has been modified into a branchial chamber with six gill folds it may be assumed that this part of the rectum is concerned with respiration as well as absorption of ions (Greven and Rudolph,1973). The ileum of anisopteran larvae reveals the existence of thick and thin epithelia. The thick epithelium apparently organised for ion transport. The cells are covered with a multilayered cuticula (Moens,1980). In terrestrial insects most of these ions and water are reabsorbed by the rectal papillae, because they live in fresh water, larvae of Odonata do not need to reabsorb water; however, the retention of salts is very important in osmoregulation. In these larvae the rectum is filled mainly with water, and thus reabsorption takes place in the first segment of the hindgut, the ileum (Moens, 1984). Dragonflies possess tracheal gills which are located in the rectum. Using stereological methods, we estimated the morphometric diffusing capacity for oxygen across the gill epithelium, i.e., from rectal water to the gill tracheoles, in the larvae of *Aeshna cyanea*. (Wichard and Komnick,1974; Kohnert *et al,* 2004). In Zygoptera, there are 3, in Anisoptera up to about 500 epithelial pads in the rectum which show fine structural features of transporting epithelia. Rectal ventilation provides contact of these epithelia to the external medium from where the ions are absorbed (**Neill** 1960; Hughes and Mill 1966; Mill and Pickard,1972; Komnick,1978; Miller,1994). The rectal chloride epithelia of the larval dragonfly *Aeshna cyanea*. These epithelia function in active ion absorption and maintain a high concentration gradient between the haemolymph and the fresh-water environment. (Schmitz and Komnick,1976; Kukulies and Komnick,1983). The larval rectum of dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. All of them are more or less causally connected with rectal ventilation (Wichard and Komnick,1974; Pickard and Mill,1974; Mill and Pickard,1975; Komnick, 1982). The present work is therefore, proposed to understand functional morphology and histological structure of larval hindgut of dragonfly *Bradinopyga geminata* (Rambur).

METHODOLOGY

The dragonfly larvae collected from water tank with the help of nylon insect collecting net. The dissection was carried out in saline water under stereoscopic binocular microscope (ZEISS). Larval alimentary canal was exoposed and separate out the hindgut (ileum and rectum) form rest of the alimentary canal for further processing of morphological and histological demonstration. The hindgut especially ileum and rectum were fixed in Alcoholic Bouin's fixative for histological demonstration for a period of 12-18 hrs. The Bouin's fixed tissues were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax at 60-62 ^{0}C. The sections of 4 - 6 µm thick were cut on Rotary microtome prepare slide and proceeded for histological H-E staining techniques. The slides were observed under microscope in 10x, 45x and 100x magnification and photographed.

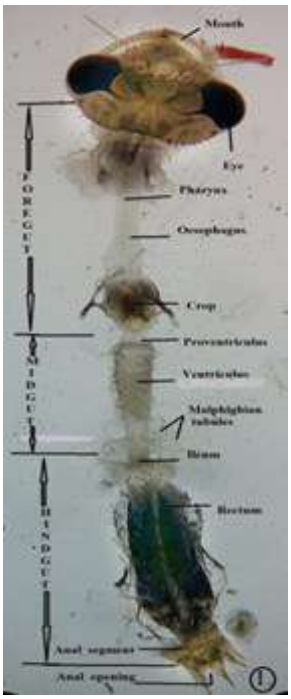
RESULTS AND DISCUSSION

The wide variation in the insect alimentary structure has led to some inconsistencies in nomenclature. The terminology used in this paper follows that of Tillyard (1917) and Tembhare and Wazalwar(2002).

The alimentary canal of dragonfly *Bradinopyga geminata* (Rambur), consists of foregut divided into three parts pharynx, oesophagus and crop, midgut divided into two proventriculus and ventriculus and lastly hindgut divided into two anteriorly ileum and posteriorly rectum (Fig.1). In this paper our focus is on the morphology and histological structure of the hindgut these regions are considered separately as follows. Thelarval alimentary canal length approximately15 mm and total length of larva is near about 16-17mm.

Ileum: The ileum is tubular cylindrical structured organ lies behind malphighian tubules, anteriorly its narrow tube-like structure and posteriorly its dilated spherical bulb in shape (Fig.1). Ileum is S-shape,

because of dorsally it attached at middle near the malphighian tubules. The ileum measures approximately 3mm in length it occupies the one fifth length of total length of the alimentary canal. In the transverse section of light microscopy ileum shows lateral diameter approximately 430µm - 450µm because of ileum is laterally compressed and dorsoventral diameter approximately 650 µm - 700 µm. The 6 longitudinal folds of ileal epithelium are of unequal height and project into the lumen irregularly (fig.2). The longitudinal muscle was weakly developed and the circular muscle are well developed, circular muscle in size approximately 3 µm (fig.3). The epithelium of ileum is consisting of a single layer of cuboidal epithelial cells are syncytial with finely granular cytoplasm, cell size approximately 4µm – 5 µm. Cell show deeply stained small nuclei without prominent nucleoli the nucleus size is 1 µm. The lumen surface of the epithelial cells is coated by a prominent cuticle (Fig.2,3&4).



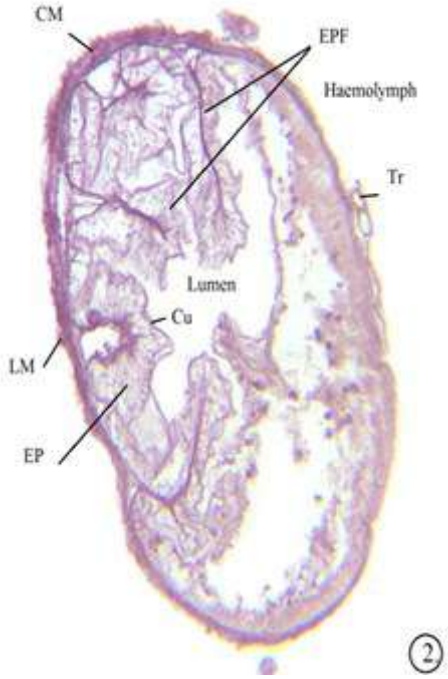
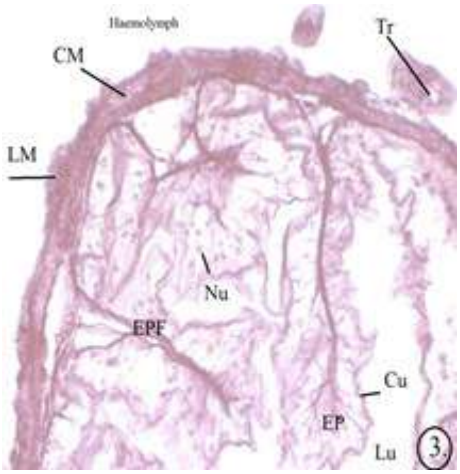


Fig.1- Whole mount of alimentary canal of larval dragonfly *Bradinopyga geminate* (Rambur), shows mouth, foregut, midgut, hindgut and anus.

Fig.2-**Magnificatiion 100xCM-** Circular muscle**, EPF-** Epithelial fold **,LM-** Longitudinal muscle**,EP-** Epithelial cells**,Cu-**Cuticle,**Tr-** Trachea, **Lu-**Lumen and **He-** Haemolymph.



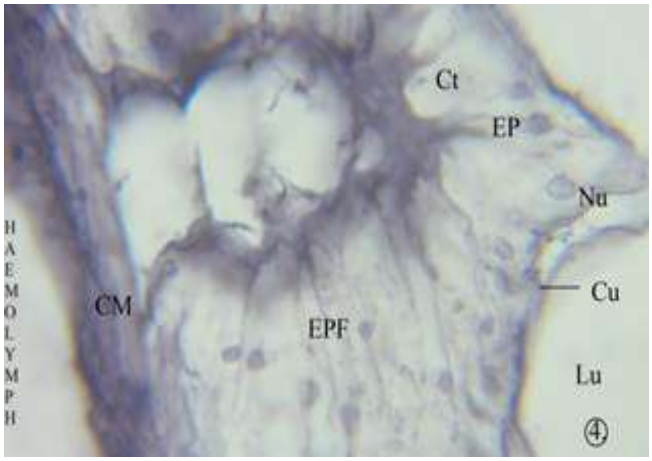


Fig.3- Magnificatiion 400xCM-Circular muscle, **LM-**Longitudinal muscle**, EPF-**Epithelial fold,**Nu-** Nucleus, **Cu-** Cuticle, **Ep-**Epithelial cells**, Lu-**Lumen**,Tr-**Trachea.

Fig.4- Magnificatiion 1000x CM-Circular muscle, **EPF-**Epithelial fold, **Ct-**Finely granular cytoplasm **EP-**Epithelial cells**, Nu-**Nucleus, **Cu-**Cuticle**, Lu-**Lumen.

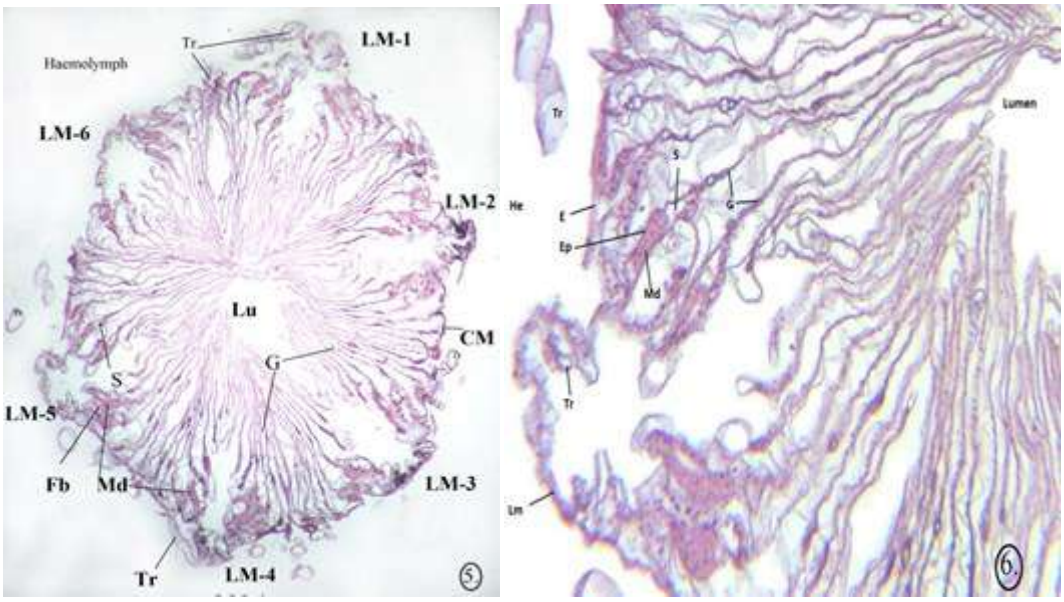


Fig.5-Magnificatiion 50x LM-6 Bands of lomgitudinal muscle**, Tr-**Trachea, **G-**Gill lamellae **CM-**Circular muscle,**Md-** Medullary cell **Fb-** Fat body cell **S-** sinus **Lu-**Lumen**, He-** Haemolymph.

Fig.6-Magnificatiion400x Tr-Trachea**, He-**Haemolymph**, E-**Epithelial lining of branchial chamber**,Ep-**Rectal pad Epithelium**, Md-** Medullary cell**, Lm-**Longitudinal muscle, **G-**Gill lamellae, **S-** sinus**,** lumen.

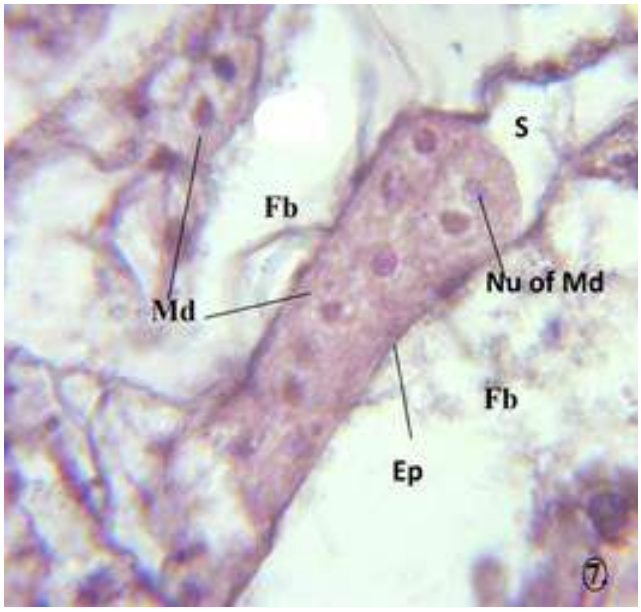


Fig.7-Magnificatiion1000x Md-medullary cells**,Fb-**Fat body cell,**Ep-**Rectal pad Epithelium**, Nu-**Nucleus**, S-** sinus

Rectum: The rectum is usually enlarged sac like structure which posteriorly connects with anal tube, the modified structure of anterior rectum in dragonfly is branchial chamber (Fig1). The rectum measures approximately 5 mm in length it occupies the one third length of the alimentary canal. In the transverse section rectum show lateral diameter approximately 1200µm - 1500µm because of rectum is laterally compressed and dorsoventral diameter approximately 1800µm-2000 µm. Rectum of dragonfly larvae is almost 3 times bigger in diameter than that of ileum, because the rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. At the base of gill lamellae there are 6 pairs of basal pads with epithelial cells. Basal pad epithelial cell size approximately 3µm - 4µm and the nucleus size is about 1µm (Fig.7).

Rectal basal pads enclose medullary fat cells from which pass trachea (Fig.5 &6), number of gills projects into a rectal lumen and cover the maximum area of rectal lumen gills are underlying by thin cuticle (Fig.5 &6). The longitudinal muscle was well developed and having 6 rows cover entire length of rectum, because of these hexagonal structure shows and the circular muscle are also well developed (Fig.5). The pumping action of rectum during rectal respiration and jet-propulsionis for offence and defense mechanism toattack on pray or instantly go away from predator. There is a tremendous tracheal network covered the rectum and enter into a basal pad cell for rectal respiration by the help of rectal gills protruded in the lumen of the rectum 100 and above in number (Fig.5).

Alimentary canal of insects consists of three primary regions; foregut (stomodaeum), midgut (mesenteron), hindgut (proctodaeum). The gut epithelium is one cell-layer thick throughout the length of the canal. The gut is supported in body by muscle anteriorly and posteriorly, but elsewhere only by connective tissue and especially by tracheae which, in insects forms an important element of connective tissue. Both the foregut and hindgut have a cuticular lining, which is lacking in the midgut.The length of gut is roughly correlated with diet; insect feeding on a largely protein diet tend to have a shorter gut than those feeding largely on carbohydrates but this not always true(Snodgrass,1935; Cranston and Gullan, 2005).

In the hindgut of aphidophagous ladybird, *Adalia bipunctata*, the epithelium was cubical in the ileum and rectum and was squamous in the rectal canal. The ileum presented six longitudinal folds and a thin circular muscle layer (Borges**,** 2015).

The alimentary tract in the nymphs of dragonflies, mentioning the adaptation of the foregut for chewing and the hindgut for respiration and jet-propulsion. The dragonfly ileum turns upward in the fifth segment, close in front of the transverse muscle of the abdomen and expands into an oval sac. The intestine then continues as the huge respiratory chamber which arises by a narrow extremity from the upper end of the ileal sac and reaches to the end of the eighth abdominal segment. The respiratory chamber is commonly regarded as an enlarged anterior part of the rectum, but the narrow, cylindrical following part of the intestine has in itself the typical features of the rectum of other insects (Snodgrass, 1954).

The anterior hindgut is called the ileum, the generally narrower middle portion is the colon, and the expanded posterior section is the rectum. In many terrestrial insects the rectum is the only site of water and solute desorption from the excreta, the ileum makes some contribution to osmoregulation. The resorptive role of the rectum is indicated by its anatomy (Cranston and Gullan, 2005). In dragonfly *Bradinopyga geminate* the hindgut divided into ileum and rectum.

The numerous trachea found penetrating the thickened epithelium can be explained as necessary because respiration is more difficult in these regions owing to the thickness of the walls, hence the many tracheoles penetrating the thickened epithelium. A comparison with the rectal respiration areas of Anisoptera larvae seems to strengthen this view. In the rectal gills of these larvae, the minute branches of the tracheae are separated from the water of the rectum by a very thin epithelium. This seems to show that respiration takes place most actively through a thin epithelium (Cullen, 1918).

In Anisoptera, a pair of chitinous parts, which in life are constantly moving in a horizontal plane to and fro from each other in the process of respiration. The rectal tracheal-gills situated in the rectum probably function in constantly renew the water (Calvert, 1893).

In dragonfly long straight region of quite even diameter throughout most of segment seven the ileum, a decided bulbous enlargement throughout most of the eighth segment probably the pouched region of Carroll, a short constricted section entering segment nine and a very much thickened cylindrical region running through the ninth segment and most of the tenth. This enlargement has three broad folds along its whole length and between them thin, darkly pigmented areas; one of these folds is mid-dorsal, the other two latero-ventral, the anterior part of the rectum, the hind part of segment ten is occupied by a small vestibule leading to the anus, the walls of which are well supplied with tracheae (Whedon, 1918).

The relative importance of the role that respiratory, excretory, and cutaneous processes play is not as clear in aquatic insects, although three hypothesis may be considered. These hypotheses are (1) ion exchange across the cuticular respiratory surface is the dominant response to changes in extracellular acid-base balance in water breathing insects, (2) ventilatory control of hemolymph CO_2 is used for pH regulation by air breathing species, as observed in locusts and (3) differences in responses to pH changes in water and air breathing aquatic insects are only quantitative, not qualitative (Cooper,1994).

Well-developed rectal pads, it may be doubted whether the function of these pads is also absorption. It should, however, be remembered that the need for water conservation depends not only on the habit of the insect but also on the quantity of water in its food and the percentage of water in the body In aquatic forms, the pad is confined to the dorsal and lateral walls of the ileum and extends up to the junction of the ileum and rectum. The cells are tall, show long striations and rich tracheation (Bahadur, 1963).

The rich tracheal supply of the rectum of *Uropetala carovei* associated with the removal of dissolved oxygen from the medium, suggested that this tissue might be a suitable one for maintenance *in-vitro,* enabling the transport functions to be studied underideal experimental conditions. The present paper gives an account of experiments designed to establish that the rectum of *Uropetala carovei* was capable of active transport of ions under *in vitro* conditions (Green, 1978). The rectal lumen of anisopterous and zygopterous larvae is also ventilated through the anus fine structural and electrolyte histochemical results which suggest that the epithelial pads of zygopterous larvae are rectal chloride epithelia analogous to the rectal chloride epithelia of anisopterous larvae and probably osmoregulatory rather than respiratory in function (Komnick, 1974).

In anisopteran larvae light microscope, study epithelium lining the lumen of the ileum is composed of a thin and highly folded layer, and a thicker layer containing enlarged cells. Both epithelia are coated with a thin cuticula. The cells in the thin epithelium are nearly cuboidal, approximately 12 µm high, with a clear homogenous cytoplasm. Flattened nuclei which contain mosaic like dispersed chromatine occur in the basal parts of these cells. Most nuclei have two nucleoli. The thicker epithelium is composed of cylindrical cells which are spheric and spotted and occur more apically in the cell. An intensively stained zone is present just beneath the cuticula and in the basal part of these cells (Moens, 1980).

The tracheal gills in *Aeshna cyanea* are designed such that the tracheolar supply of the respiratory epithelia corresponds with the surface area of the gill lamellae. This is consistent with the hypothesis that rectal gills alone can satisfy the metabolic demand of the larvae. It would be of great interest whether the morphology of the branchial chamber can respond to hyperoxic or hypoxic environments during larval development. The ends of the gill lamellae from some approaches becomes clear, because the outer surface is relatively large and tracheoles appear to be particularly rare, diffusion distances could be extreme and these regions could have a special effect on the calculated diffusing capacity (Kohnert *et al*, 2004).

In *Libellula* gills the fat-body cells form an epithelium like stratum underneath the chloride epithelium. This stratum rests on a thin lining epithelium at the opposite side of the gill lamella (Komnick, 1982).

The respiratory epithelium of the rectal tracheal gills of *Aeshna cyanea* larvae is of cellular construction throughout the entire length. It contains numerous tracheoles surrounded by a thin cytoplasmic sheath of tracheoblasts and extracellularly located in deep invaginations of the epithelial cells. The tracheoles run nearly parallel to each other in radial orientation, they are, however, irregularly distributed with respect to their distance from each other and from the cuticle. This arrangement of the tracheoles presumably provides optimum conditions for oxygen uptake (Komnick,1974).

In most hymenopterans ileal lumen was lined by a cuticle that extended throughout its entire length and included the rectum. Transverse sections showed that the epithelium consisted of 4-6 folds that bulged into the lumen (Santos and Serrao,2006). In dragonfly *Bradinopyga geminata* the ileum transverse section shows 6 epithelial folds bulged in lumen.

In the larva of Palm Weevil, *Rhynchophorus phoenicis* the hindgut divided into ileum and rectum which terminates in the anus **(**Omotoso and Adedire,2010).The rectal sac is formed by enlargement of the rectal portion of the hindgut in *Bombyx mori*, it consists of epithelium, connective tissue, and many rectal pads, have a cortex and medulla(Izzetoglu and Ober, 2011).

In *Platynotus belli* epithelium of ileum consists of cuboidal cells supported by a prominent basement membrane. The nuclei of the epithelial cells are small & round. The intima is thin, chitinous and is provided with small spines. rectum is divided in to anterior rectum and posterior rectum. The epithelium of rectum bears broad folds and is made up of large cuboidal cells with large round nuclei. Internally, the epithelium is lined by thick intima and externally by isolated few longitudinal and well developed circular muscles. The longitudinal muscles are thrown into six external lengthwise bands give somewhat hexagonal appearance to the rectum (Sarwade and Bhawane,2013).

CONCLUSION

The alimentary canal of dragonfly *Bradinopyga geminata* (Rambur), consists of foregut, midgut and lastly hindgut divided into two ileum and rectum. The larval alimentary canal length approximately 15 mm and total length of larva is near about 16 - 17 mm. The ileum and rectum occupies the one fifth and one third length respectively of the alimentary canal. The rectum

is usually enlarged sac like structure because the rectum of larval dragonflies serves various functions such as swimming by jet-propulsion, breathing, storage of lipid and glycogen, and osmoregulatory salt uptake. The longitudinal muscle are well developed and having 6 rows cover entire length, circular muscles are also well develop for pumping of rectum.

Acknowledgments

Authors are thankful to CHLR, Department of Zoology, N.H. College Bramhapuri, Maharashtra, for providing necessary facilities in the progress of work.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:247-253
UGC Approved Journal No 48951

Original Article Open Access

Diversity of Zooplanktons in Janala Lake, Mul, Maharashtra (India)

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Manuscript details:

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Borkar KR and Deshmukh GD (2018) Diversity of Zooplanktons in Janala Lake, Mul, Maharashtra (India), *Int. J. of. Life Sciences*, Special Issue, A12: 247-253.

ABSTRACT

Zooplanktons play a very crucial role in the trophic dynamics and energy transfer in aquatic ecosystem. Their abundance increases in eutrophic water. They are also sensitive to pollution and many species are recognized as indicators of pollution. It is an integral component of an aquatic ecosystem. The study site Janala Lake is located near Mul, situated between 20^0,07·N and 79^0 ,67· E. Water samples were collected once in month from the selected sampling sites of Janala lake to analyze the diversity of zooplanktons for the period of 24 months i.e. from January 2011 to December 2012. Zooplankton belonged to Rotifera, cladocera, copepod and ostracoda and both the lakes, the two years average showed the following sequence of their abundance. Janala Lake = Rotifera > Copepoda > Cladocera > Ostracoda. In the present investigation, total zooplankton was recorded maximum during summer and minimum during monsoon.

Key words: Zooplankton, Rotifera, Copepoda, Cladocera, Ostracoda

INTRODUCTION

Fresh water ecology emphasizes mainly the study of relationship between organisms and the fresh water environment. Study of all aspects (physical, chemical, geological and biological) of fresh water is termed as Limnology (George, 1997). Lakes are characterized by distinct biotic and abiotic environment. Lakes maintain ecological balance of flora and fauna and their interrelationship regulate surrounding climate and recharge ground water, but unfortunately, they are dying. The lakes are getting polluted due to inflow of domestic effluents, apart from pollution, resulting from washing of clothes, Vehicles, Cattle, immersion of Idols during certain festivals etc. All these activities are deteriorating the quality of the water in the lake resulting in the accumulation of the toxic chemicals and other sludge leading to ecological imbalance.

Zooplanktons play a very crucial role in the trophic dynamics and energy transfer in aquatic ecosystem. Their abundance increases in eutrophic water. They are also sensitive to pollution and many species are recognized as indicators of pollution. It is an integral component of an aquatic ecosystem. Investigation on seasonal change in zooplankton diversity has been undertaken by various workers (Ganapati, 1943; George, 1997; Edmondson, 1995; Dhanpati, et al, 2000; Narasimha *et al*, 2001; Padmanabhan and Bolagali, 2008; Jorge et al, 2009; Dahegaonkar et al., 2012; Parveen and Mola, 2013; Vasant et al, 2013; Khalokar, 2014 ; Kadam et al. 2014, Pradhan, 2014; Dekate and Baviskar, 2016, Kar and Kar, 2016).

Present study site, Janala lake is about 8 km away from the town and is surrounded by forest and little away from human habitation and therefore is still oligotrophic in nature. Therefore input of organic load due to domestic pollutants is less. Although many reports are available on the limnological profiles of lentic ecosystems from other district in Maharashtra, no attempts have been made to record the zooplankton diversity.

MATERIAL AND METHODS

The study site Janala Lake is located near Mul. Mul town is in the Chandrapur district of eastern part of Maharashtra and is situated between 20^0,07,N and 79^0 ,67, E. It is constructed by the minor irrigation department of the Maharashtra state and is about 8 km from Mul town near Janala Village, with a area of 26.62 hectare. Water samples were collected in polythene bottles (two litters capacity) once in month from the selected sampling sites of Janala lake to analyze the diversity of zooplanktons for the period of 24 months i.e. from January 2011 to December 2012.

For qualitative analysis, the samples were collected with the help of plankton net. Sweeps were made in all directions in the littoral zones. For the collection from open water, net was thrown to some distance from peripheral zone to the centre avoiding the macrophytes and solid floating material. Collected plankton was transferred to enamel tray, inside of the net was carefully washed so as to collect any sticking plankters. Zooplankton was preserved in 4% formalin and were observed and photographed by the Labomed make Digi 2 Pro camera attached to trinocular microscope. Detailed taxanomical identification was carried out by using the keys from Edmondson (1959); A.P.H.A. (1991), Tonapi, (1980) and Dhanapathi, (2000).

RESULTS AND DISCUSSION

The relative data of Zooplanktons of Janala lake is given in the table 1 and 2 selected zooplantons. The Zooplanktons comprises Rotifera, Cladocera, Ostracoda and Copepoda. Total 2331 ind/ltr Zooplankters were recorded in 2011 and 2222 ind/ltr during 2012. Rotifers were recorded as 1552 ind/ltr during 2011 and as 1504 ind/ltr during 2012. It contributed 66.5% during 2011 and 67.6% during 2012 of the total zooplankton. Fig. 1 shows graphical representation of zooplanktons during summer, monsoon and winter seasons in study period.

Fig.1 to Fig. 3 shows graphical represention of seasonal abundance of different groups of zooplankton. The zooplankters were recorded with maximum of 1179 ind/ltr during the summer of 2011 and minimum of 509 ind/ltr during the monsoon season of 2012. Rotifera was recorded with maximum of 812 ind/ltr during summer season of 2011 and minimum of 343 ind/ltr during the winter season of 2012. It contributed maximum of 52% in summer, followed in monsoon by 25% and in winter by 23% during 2011. During 2012 they were again dominant during summer by contributing 53% followed by monsoon 24% and 23% in winter. Cladocera recorded maximum of 162 ind/ltr during summer season of the year 2011 and minimum 71 ind/ltr during monsoon season of 2012. It contributed maximum of 48% in summer followed in winter with 28% and 24% in monsoon. During 2012, they were again dominant during summer by contributing 49% followed by winter with 28% and 23% in monsoon. Ostracoda recorded maximum of 29 ind/ltr during summer season of 2011 and minimum of 12 ind/ltr during monsson season of 2011 and 2012. Seasonally, Ostracoda was dominant in summer with 47% followed by 33% in winter and 20% in monsoon. During 2012, they were again dominant during summer by contributing 45% followed by winter 33% and 22% in monsoon. Seasonally, Copepoda was recorded maximum of 176 ind/ltr during the summer season of 2011 and minimum of 59 ind/ltr during monsoon season of 2012. It has shown highest percentage i.e. 46% in summer followed by 36% in winter and 18% during monsoon of 2011. During 2012, it has recorded maximum with 47% during summer, followed by 36% in winter and 17% in monsoon. Fig. 4. Shows, some of the zooplanktons in Janala lake.

Table 1 : Monthly Variations in Zooplankton in JANALA Lake during 2011

	Zooplankton / Month	J	F	M	A	M	J	J	A	S	O	N	D	Tot
A	**ROTIFERA**													
	Family: Brachionidae													
1	*Brachionus diversicornis*	5	3	11	8	8	7	3	1	1	0	5	10	62
2	*B. calyciflorus*	31	55	89	100	75	72	64	48	35	22	15	1	607
3	*B. falcatus*	82	85	99	115	83	80	35	12	5	28	55	68	747
4	*Keratella tropica*	11	17	6	0	0	0	0	0	0	0	0	0	34
	Family: Trichocercidae													
5	*Trichocerca longiseta*	0	3	8	20	24	6	3	3	4	9	7	0	87
	Family: Asplanchnidae													
6	*Asplanchna spp.*	0	1	2	0	0	1	2	0	0	0	6	3	15
	TOTAL	129	164	215	243	190	166	107	64	45	59	88	82	1552
B	**CLADOCERA**													
	Family: Sididae													
7	*Diaphanosoma sarsi*	0	0	0	0	3	2	0	0	0	0	0	0	5
	Family : Daphnidae													
8	*Ceriodaphnia cornuta*	0	0	3	2	2	0	0	0	0	0	0	0	7
9	*C. quadrangula*	0	0	0	1	0	0	0	0	1	1	0	0	3
	Family: Moinidae													
10	*Moina micrura*	0	0	0	0	10	7	1	0	0	0	3	0	21
	Family: Bosminidae													
11	*Bosmina longirostris*	25	29	30	40	39	29	20	10	6	20	18	24	290
	Family: Chydoridae													
12	*Chydorus sphaericus*	0	1	2	0	0	1	3	0	0	0	0	1	8
	TOTAL	25	30	35	43	54	39	24	10	7	21	21	25	334
	OSTRACODA													
1	*Cypris sp.*	9	13	2	3	11	8	2	2	0	0	1	10	61
	COPEPODA													
1	*Mesocyclop sp.*	12	10	5	0	0	0	0	0	5	5	3	7	47
2	*Eucyclop sp.*	7	11	21	13	7	3	2	0	0	3	3	2	72
3	*Cyclop sp.*	14	0	0	0	4	0	1	0	1	9	15	19	63

Table 2 : Monthly Variations in Zooplankton in JANALA Lake during 2012

	Zooplankton / Month	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Total**
A	**ROTIFERA**													
	Family: Brachionidae													
1	*Brachionus diversicornis*	4	2	10	7	7	6	2	1	1	0	4	9	53
2	*B. calyciflorus*	30	54	88	99	74	71	63	46	34	20	14	0	593
3	*B. falcatus*	81	84	98	113	82	79	34	11	4	28	54	67	735
4	*Keratella tropica*	10	16	5	0	0	0	0	0	0	0	0	0	31
	Family: Trichocercidae													
5	*Trichocerca longiseta*	0	2	7	19	23	6	2	2	3	9	6	0	79
	Family: Asplanchnidae													
6	*Asplanchna sp.*	0	1	2	0	1	1	1	0	0	0	5	2	13
	TOTAL	125	159	210	238	187	163	102	60	42	57	83	78	1504
B	**CLADOCERA**													
	Family: Sididae													
7	*Diaphanosoma sarsi*	0	0	0	0	2	1	0	0	0	0	1	0	4
	Family : Daphnidae													
8	*Ceriodaphnia cornuta*	0	0	2	3	2	0	0	0	1	0	1	1	10
	C. quadrangula	0	0	0	1	2	0	0	0	0	1	1	0	5
	Family: Moinidae													
9	*Moina micrura*	0	0	0	0	9	6	0	0	0	0	2	0	17
	Family: Bosminidae													
10	*Bosmina longirostris*	24	27	29	39	38	28	19	9	5	18	16	22	274
	Family: Chydoridae													
11	*Chydorus sphaericus*	0	1	1	0	0	0	2	0	0	0	0	1	5
	TOTAL	24	28	32	43	53	35	21	9	6	19	21	24	315
	OSTRACODA													
1	*Cypris sp.*	8	12	1	2	10	8	2	2	0	0	1	9	55
	COPEPODA													
1	*Mesocyclop sp.*	11	9	4	0	0	0	0	0	4	4	2	6	40
2	*Eucyclop sp.*	6	10	20	12	6	2	1	0	0	2	2	1	62
3	*Cyclop sp.*	13	0	0	0	3	0	0	0	0	8	14	18	56
4	*Diaptomus sp*.	2	7	8	19	67	25	17	3	7	20	9	6	190
	TOTAL	32	26	32	31	76	27	18	3	11	34	27	31	348

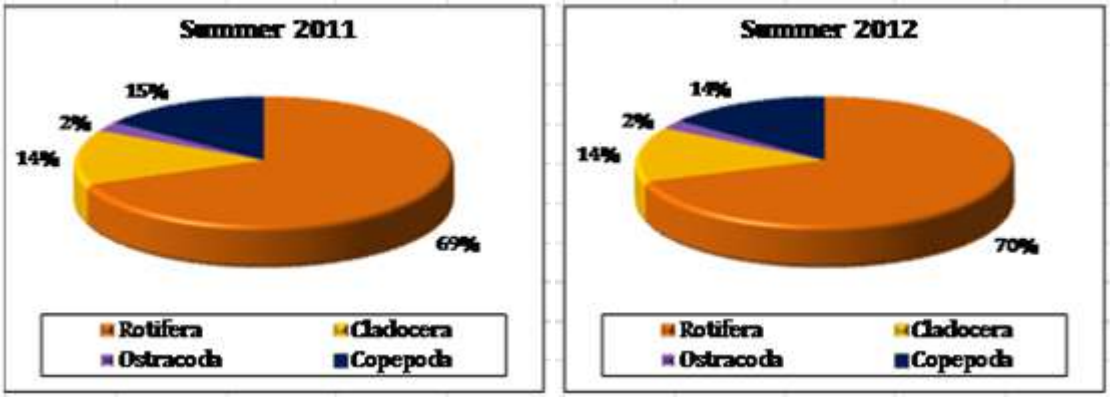

Fig. 1 Seasonal Distribution of Zooplankton during Summer in Janala Lake

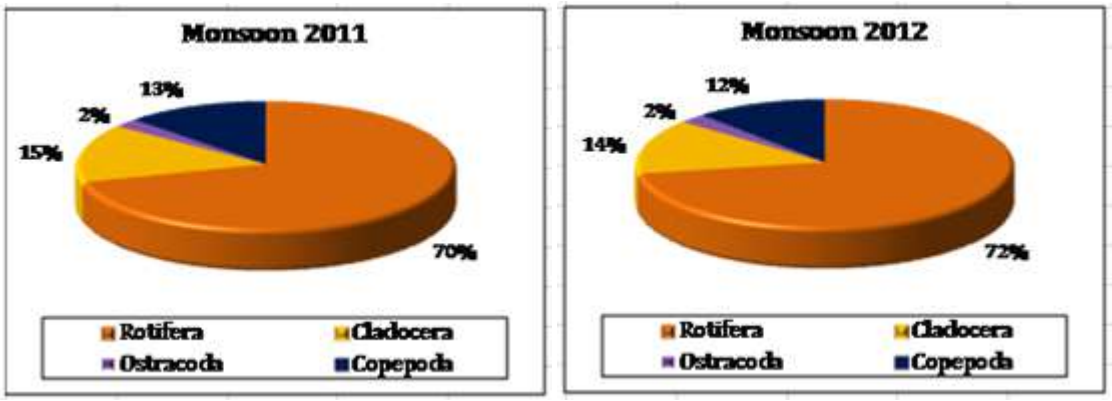

Fig. 2: Seasonal Distribution of Zooplankton during Mansoon in Janala Lake

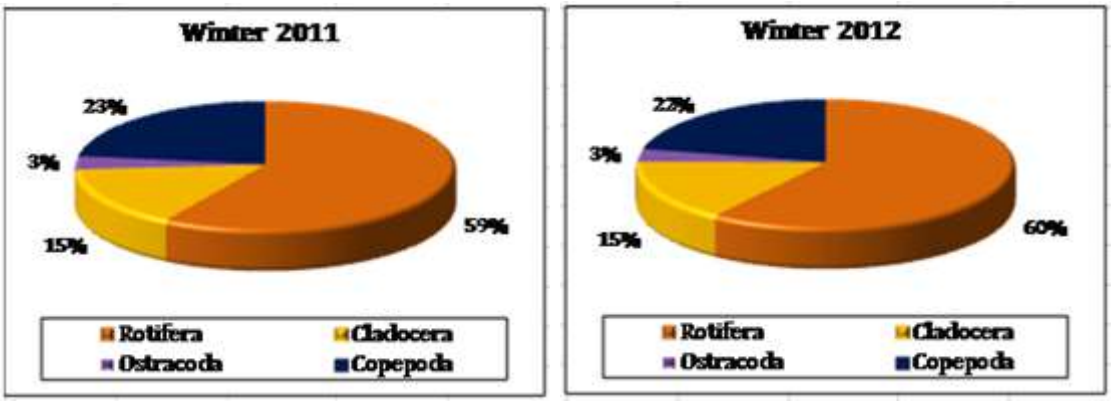

Fig. 3: Seasonal Distribution of Zooplankton during Winter in Janala Lake

DISCUSSION

Zooplankton diversity is one of the most important ecological parameters in water quality assessment. The zooplankton study has been a fascinating subject for a long time. Water bodies rich in phytoplankton are also rich in zooplankton diversity and biomass. Vijaykumar (1999) stated that in an aquatic ecosystem, zooplanktons play an important role not only in converting plant food into animal food but also provide an important food source for other higher organisms including fish. The zooplankton consisted of Rotifers, Cladoceran, Copepods and Ostracods in Janala lake. The quantitative relationship amongst different groups of zooplankton in Janala lake it was Rotifera > Copepoda > Cladocera >Ostracoda during both the years of study. Seasonal fluctuations of zooplanktons in Janala lake during the study period shows that, rotiferans dominated the plankton population under study, while the others groups were encountered with the moderate numbers.

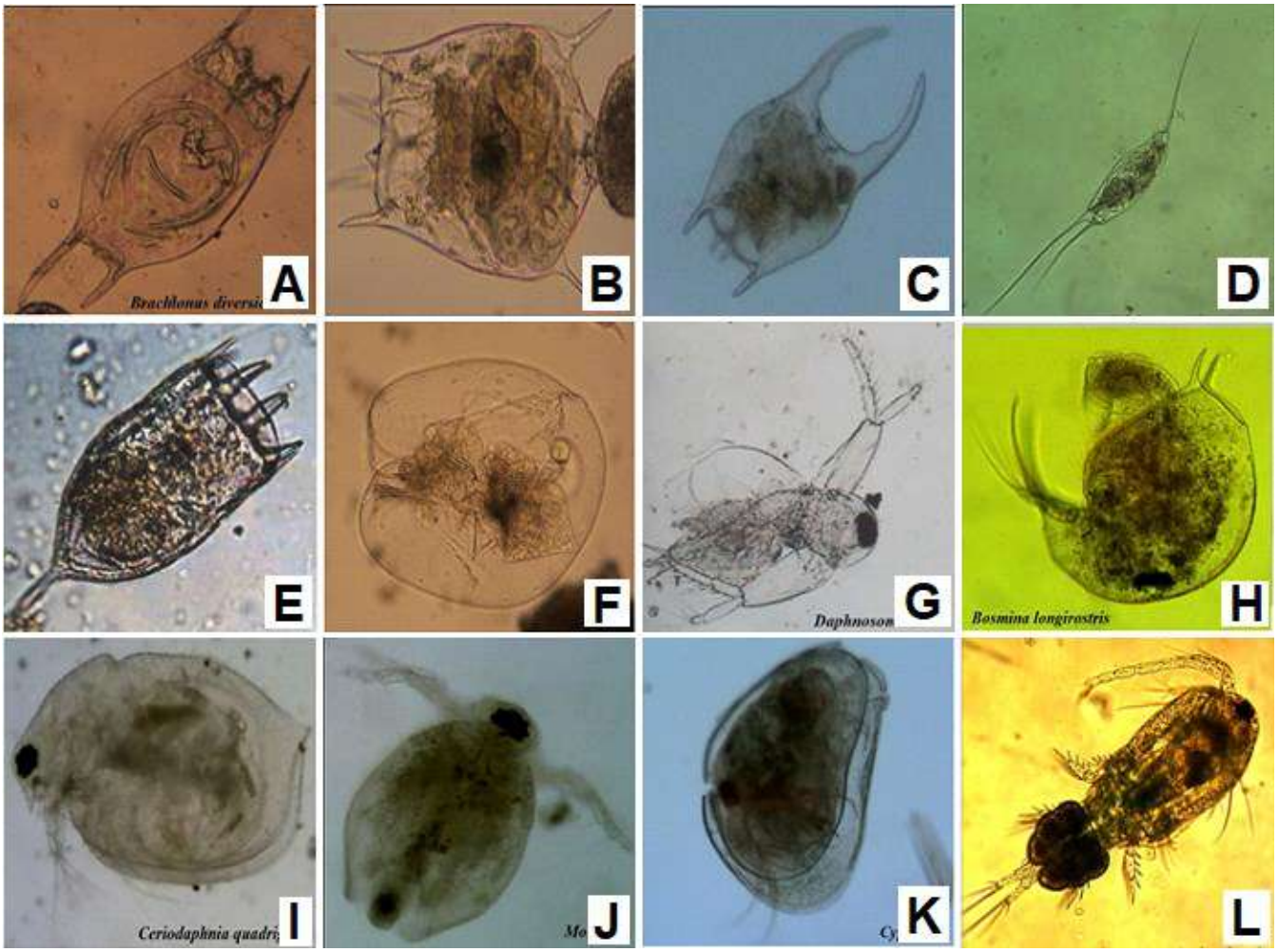

Fig. 4: Zooplankton Species Janala Lake : A: *Brachionus diversicornis,* **B:** *Brachionus calyciflorus,* **C:** *B. falcatus,* **D:** *Trichocerca longiseta,* **E:** *Keratella tropica,* **F:** *Asplanchna sp.,* **G:** *Diaphanosoma sarsi,* **H:** *Diaphanosoma sarsi,* **I:** *Bosmina longirostris,* **J:** *Ceriodaphni quadrangular,* **K:** *Moina sp,.* **L:** *Cypris sp.,* **M:** *Cyclop* Female

High density of Rotifers during summer might be due to high temperature which is suitable for their growth, reproduction and development and availability of nutrients due to bacterial decomposition. But high density of Rotifers during monsoon season may be attributed to high temperature and availability of rich nutrients during June-July months, while low density of zooplanktons during winter season coincides with substantial decrease in temperature in the lakes during winter season. In the present investigation, *Brachionus sp.* is very common in both the two lakes under study. Jorge et. al. (2009) and Parveen and Mola (2013) reported enormous growth of Rotifers in lakes and reservoirs indicating eutrophic conditions. In the present study also Mul lake shows the higher numbers of Rotifers throughout the study period indicating its eutrophic nature. Dahegaonkar, *et al.*, (2012) noticed that the Rotifers are very common in Indian waters and their occurrence in eutrophic water bodies. Bhandarkar et. al. (2008) reported the seven Brachionus species and mainly *B. falcatus* was most dominant species in Kalikar pond, Bramhapuri.

Summer maxima of ostracoda in Janala lake may be attributed to higher water temperature, decrease in water level, and increased availability of its food. Similar result was reported by Padmanabhan *et. al*. (2008) in Dalvoi lake, Mysore. Ostracode play an important role in transferring the energy from producers to the consumers and they occupy an intermediate position in aquatic food web by being live food for fishes. The diversity, abundance and seasonal fluctuations of ostracods have direct link with water quality (Padmanabhan and Belagali, 2008; Parveen and Mola, 2013).

The copepod diversity was represented by four species and found more in number during summer in Janala lake, during both the years, 2011 and 2012 respectively. However, minimum number was recorded during monsoon season in Mul lake and Janala lake in both the years. Padmanabhan and Belagali, 2008, observed that the Cyclops are sensitive to pollution and increased with an increase in nutrients and is in agreement with our observation.

In the present investigation, total zooplankton was recorded maximum during summer and minimum during monsoon.

CONCLUSIONS

In the present investigation, Janala lake were investigated for the limnological profiles, for two years i.e. from January 2011 to December 2012. Zooplankton belonged to Rotifera, cladocera, copepod and ostracoda and both the lakes, the two year average showed the following sequence of their abundance. Janala Lake = Rotifera > Copepoda > Cladocera > Ostracoda. In the present investigation, total zooplankton was recorded maximum during summer and minimum during monsoon.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:254-258
UGC Approved Journal No 48951

Original Article Open Access

Algal blooms and its impact on status of lendra pond at brahmapuri, dist. Chandrapur. (MS), India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Murkute VB and Chavan AW (2018) Algal blooms and its impact on status of lendra pond at brahmapuri, dist. Chandrapur. (MS), India, *Int. J. of. Life Sciences*, Special Issue, A12: 254-258.

ABSTRACT

An algal bloom is a rapid increase or accumulation in population of algae in aquatic ecosystem and it is recognized by discoloration in water from their pigments. Harmful algal blooms are a rapid uncontrolled growth of algae in aquatic environment. Excess proliferation of phytoplankton species such as Dinoflagellates, Diatoms and Cyanobacteria are referred as harmful algal blooms. Harmful algal blooms represent natural phenomena which poses serious problems to human health, environmental sustainability and aquatic life due to the production of toxic or accumulated biomass. In the present study also, the Lendra pond is suffering from such Harmful Algal Blooms which have already creates its harmful effect on aquatic fauna and indirectly affecting the human health. The sustainability of pond is also in danger. Mass fish kill was reported from Lendra pond which is the result of Harmful Algal Blooms. It is very important to sustain the life of such water bodies to perish our environment and ultimately the human health, too.

Keywords: Algal blooms, Eutrophication, Fish kill.

INTRODUCTION

Recently the eutrophication has become a priority given the increasing pressure on aquatic ecosystem are being subjected from anthropogenic activities. But eutrophication is also be a cultural and characterized by two prime factors, viz., nutrient loading and harmful algal blooms (HAB). In the mentioned pond, named as Lendra, the toxic Cyanobacteria (also represent HAB) is quiet common. Harmful algal blooms represent the natural phenomena caused by a mass proliferation of phytoplankton in water body. The main group of organism generating HAB's is Diatoms, Dinoflagellates and Cyanobacteria. In fresh water body Cyanobacteria and Diatoms are common. All phytoplankton photosynthesize and their growth depends upon different factors such as sunlight, CO2, availability of nutrients i.e. like Nitrogen and Phosphorus. Additional factors influencing their life are water temperature, pH, climate changes, salinity,

water column stability and anthropogenic modifications of aquatic environment including nutrient over enrichment called eutrophication. Eutrophication can results in visible algal blooms which cause an increase in turbidity of water, can give bad odour to the water body. During bloom, algae produce nocive toxins that can render water unsafe and cause fish mortality and also affect the human health directly or indirectly. In the present study, we are going to report the intensification of Harmful Algal Blooms and its effects on fish health so as to proper remedies should be taken to sustain the life of water body which is used for various purposes, like fishery, recreation, human health and agricultural use.

METHODOLOGY

Study Area: Lendra pond is one of the unique aquatic ecosystems in many respects at Brahmapuri. It is 100yrs. old pond having area of 6.86hctr. It is situated near Railway station. Water of this pond was used for Agriculture, Fishing and Sociocultural practices.

Plate 1: The satellite view of Lendra Pond

Study was carried out from June 2005 to May 2007. Algal blooms were collected at random and brought to the laboratory, preserved in 4% formalin and observed under binocular microscope (model Digi 2 pro labomed) attached to the computer, photographed and identified by using pertinent literature (Edmondson, 1970; APHA,1975; Tonapi, 1980; Plaskit, 1997).

RESULTS AND DISCUSSION

The phytoplankton species found in the bloom are as follows:

Microcystis spp.; Anabaena spp.; Anacystis spp.; Oscillatoria spp.; Lyngbya spp.; Nitzchia spp,; Diatoma spp. were abundantly present in the water body blooms. Other phytoplankters were also found in the blooms (Table 1). Pond water is Green in colour and having very bad odour. Water is very turbid and the Transparency is found from 22.83 to 25.67 cm in average. Phosphate and Nitrate level is also reported very high here.

Fish kill:

In the present investigation, very poor fish fauna was recorded from the Lendra pond. Mass of fish kill occurs in this pond due to heavy load of algal blooms, which decaying results into depletion of Dissolved Oxygen and releasing of toxins by algal blooms. During the onset of summer, when water becomes less in the pond basin, several numbers of dead fishes were seen on the basin of pond. Various toxins produced by the Harmful Algal blooms affects the aquatic fauna of the Lendra pond. In the pond, most of the fishes were frequently killed by HAB poisoning. Fish mortality results into the economic loss of fish market.

Plate 2: Heavy encroachment of pond periphery by macrophytes.

Plate 3: Fish killed by causing damage to gill and operculum**.**

Plate 4: Pond water become greenish in colour due to algal blooms

Table 1:

SN	Phytoplankton	Quantity	SN	Phytoplankton	Quantity
1.	*Microcystis spp.*	+++++	21.	*Zygnema spp.*	+++
2.	*Anabaena spp.*	++++	22.	*Closterium spp.*	+++
3.	*Spirulina spp.*	+++	23.	*Cosmarium spp.*	++
4.	*Anacystis spp.*	+++++	24.	*Voucheria spp.*	++
5.	*Oscillatoria spp.*	+++++	25.	*Scenedesmus spp.*	++
6.	*Lyngbya spp.*	++++	26.	*Chlorocloster spp.*	++
7.	*Agmenellum spp.*	++	27.	*Coelastrum spp.*	++
8.	*Nitzchia spp.*	+++	28.	*Pandorina spp.*	++
9.	*Navicula spp.*	++	29.	*Clamydomonas spp.*	++
10.	*Diatoma spp.*	+++++	30.	*Tetraedon spp.*	++
11.	*Fragillaria spp.*	++	31.	*Phacotus spp.*	+++
12.	*Gyrisigma spp.*	++	32.	*Eudorina spp.*	++
13.	*Synedra spp.*	++	33.	*Palmella spp.*	+++
14.	*Gomphonema spp.*	++	34.	*Euglena spp.*	++
15.	*Volvox spp.*	+++	35.	*Phacus spp.*	++
16.	*Pediastrum spp.*	+++			
17.	*Chlorella spp.*	++			
18.	*Ulothrix spp.*	++			
19.	*Oedogonium spp.*	++			
20.	*Spirogyra spp.*	+++			

DISCUSSION

Eutrophication is considered a major problem for water environments and high levels of phosphate and nitrate inputs from different sources are principal causes of degradation of pond ecosystem. It is a process whereby water receives excess nutrients that stimulate blooms of algae.

In Lendra pond, nutrients are come from many sources like urban and rural wastewater, fertilizers applied to the agricultural fields and rice mill present neighbor to the pond. Inorganic nitrogen and phosphorus compounds and carbon are involved in eutrophication process and human activities can accelerate the nutrient rate entering into the ecosystem. Algal blooms especially Cyanobacteria are able to exploit anthropogenic modification of aquatic environment as evidenced by their higher affinity for nitrogen and phosphorus compared to other photosynthetic organism (Chorus *et al*., 1999).

Cultivated and uncultivated paddy fields are present nearby the pond which could be the source of fertilizer runoff. The land use pattern around the pond may be direct effect on water quality and aquatic vegetation of pond (Akasaka *et al*., 2010). Higher nitrate and phosphate values specify the highly polluted status of Lendra pond. Such consequent water pollution causes visible harmful Cyanobacteria blooms, surface scum, benthic macrophytes aggregation and floating plants mats. The bloom and floating plant mats decay takes place which leads to the depletion of dissolved oxygen in water and release of toxin which loses water clarity and disruption of food web. The algal biomass accretion not only discolor the water but also produce harmful effect and cause reduction in biodiversity due to shedding of benthos and fish kill (Jappesen *et al*., 2012, Peperzak, 2005).

In the present study, spontaneous fish kill takes place in the pond most frequently during onset of summer season may be due to fish may ingest the algal rich water which enter in the system during respiration and it induces the production of reactive oxygen species with resulting increase of oxidative stress and fish death (Shaharaki *et al*., 2013). At the same time, algal cell themselves able to produce reactive oxygen species as causative factor leading to fish mortality underlaying the important role of oxidizing compounds in bloom toxicity (Tang *et al*., 2009).

Table 2: Toxins produced by Cyanobacteria and their primary Targets.

SN	Toxin classification	Toxins	Most common Cyanobacteria genera producing toxin	Target organ	Reference
1.	Hepatotoxin	Microcystins	Mycrocystis Anabaena Oscillatoria	Liver	Boopathi *et al*.,2014; Jochimsen *et al*., 1998 Pearson *et al*.,2010
2.	Cytotoxin	Cylindrospermospin	Anabaena, Oscillatoria, Lyngbya	Liver	Pearson *et al*.,2010 Humpage *et al*. 2003
3.	Neurotoxin	Anatoxin	Anabaena, Oscillatoria	Nervous system	Astrachan *et al*. 1980; Bumke *et al*. 1999
		Saxitoxin	Anabaena, Lyngbya	Nervous system	Pearson *et al*., 2010; Van-Apeldoorn *et al*. 2007; Strichart *et al*. 1984
		B-Methylamino L-Alanin	Microcystis, Anabaena	Nervous system	Holtcamp *et al*. 2012; Jiang *et al*. 2014
4.	Dermatoxin	Lypopoly-Sachharides	Microcystis, Anacystis, Oscillatoria, Anabaena	Skin	Torokne *et al*. 2001; Blahova *et al*. 2013
		Lyngbyatoxins	Lyngbya	Skin	Osborne *et al*. 2001; Arthur *et al*. 2008
		Aplysiatoxins	Lyngbya, Oscillatoria	Skin	Churro *et al*.2012

Many fishes in the Lendra pond show the damage to the operculum and gills, may be causes by the spines of the Diatoms. Indeed, when gills are damaged by spinous diatoms, the epithelium shows lesions and produces excessive mucus that lead to asphyxiations (Kent *et al*.1995, Yang 1992). If such fishes are used as a food by human being, it may cause damage to the intestine of human. When toxins are released in water during HAB, their toxic effects on human are believe to occur through different routes. Consumption of contaminated fish food, inhalation through wind dispersed dried algal material, ingestion of water or scum, direct contact with skin or conjunctiva. Total seven number of harmful algae events are reported by India during 1980 to 2015 to the Harmful Algae Dataset HAEDAT (Isabella *et al*. 2016). Toxins produced by Cyanobacteria are given in the Table 2.

Regular monitoring of the Lendra pond status is mandatory so as to control the hazards of toxic algae in order to protect health of fish and fishery, human and to minimize ecosystem and economic losses caused by blooms. However, monitoring and management programmes may alleviate future HAB's episode if prevention method would have adopted. In this regard the reduction in nutrient load in water bodies may help to prevent certain types of HAB's are observed when sewage or waste discharged input in the pond are deeply monitored.

The aim of the study, that report the present status of the pond to the municipal corporation to take different approaches used to rehabilitate the pond to a clear water state by adopting the different methods to remove algae from water.

CONCLUSION

It is evident that, Lendra pond is essential for the community from utilitarian point of view and still it is grossly neglected, and totally unused now. The pond water was used for domestic purpose; therefore, phosphates from detergents could be strong reason for induction of algal blooms. Heavy emergence of Microcystis spp. in Lendra pond may be due to Nitrogen enrichment and high turnover rate of dissolved phosphorus. Emergence of Oscillatoria spp., Anabaena spp., Anacystis spp. produces various toxins which very toxic to fish fauna, directly results in mass fish kill in pond. The said pond is under threat. The study has revealed the need for conservation and the scale of restoration to be undertaken to sustain the Lendra pond.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018: 259-262
UGC Approved Journal No 48951

Original Article Open Access

Honey bees, diseases in loss of social immunity by changing climate

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gubbawar Shruti D (2018) Honey diseases in loss of social immunity by climate., *Int. J. of. Life Sciences*, Spe A12: 259-262.

ABSTRACT

Insect social life is generally associated with increased exposure to pathogens and the risk of diseases transmission, due to factor such as high population density, frequent physical contact and reduced genetic variability. Honey bees are attacked by numerous parasite and pathogens towards which they present a variety of individual and group level defense behaviors that reduce colony level parasite and disease loads are termed social immunity. Large-scale losses of honey bee colonies represent a poorly understood problem of global importance. Both biotic and abiotic factors are involved in this phenomenon that is often associated with high loads of parasites and pathogens. A stronger impact of pathogens in honey bees exposed to neonicotinoid insecticides has been reported, but the causal link between insecticide exposure and the possible immune alteration of honey bees remains elusive. Here, we demonstrate that the neonicotinoid insecticide clothianidin negatively modulates NF-κB immune signaling in insects. Viruses and other pathogens can spread rapidly in social insect colonies from close contacts among nestmates, food sharing and periods of confinement. There is a relationship between the effectiveness of social and individual immunity and the nutritional state of the colony. Parasitic Varroa mites undermine the relationship because they reduce nutrient levels, suppress individual immune function and transmit viruses. The maintenance of the immune system can be costly, and a lack of dietary protein can increase the susceptibility of organisms to disease. All species of honey bees has shown great adaptive potential, as it is found almost everywhere in the world and in highly diverse climates. the precise impact of potential environmental changes on honey bees as a result of climate change, there is a large body of data at our disposal indicating that environmental changes have a direct influence on honey bee development.

Keywords- Honey bees, social immunity, diseases, climate, Parasites

INTRODUCTION

Honey bees and other social insects comprise more than half of the insect biomass in the world making them one of the most ecologically successful insect groups. Contributing to this success is the coordination of activities among members of a colony. Essential tasks such as thermoregulation, brood rearing and resource gathering are efficiently executed due to the architecture and organization of the nest and spatial proximity among individuals. However, crowded conditions, warm tem- peratures, high concentrations of resources and periods of confinement in the nest are ideal for pathogen invasion and transmission that can lead to epidemics. The risk of disease outbreaks is mitigated by specialized group behaviors (social immunity) and immune systems in individuals. Honey bees are important pollinators in undisturbed ecosystems but are essential for the production of numerous high-value crops. Over the past decades, the health of honey bees has been in steady decline especially with arrival of parasitic Varroa mites. There has been considerable effort to identify parasites and pathogens that threaten the health and survival of honey bee colonies. Viruses have received much attention due to the significant loss of colonies especially over winter from Varroa mite and virus associations Greater attention also has been given to nutritional needs of colonies and how improvements in this area might reduce colony losses. This review will focus on the role of nutrition in immune response to viral pathogens. We briefly describe the connections between nutrition and individual immunity and speculate on the possible changing nutritional requirements of colonies throughout the year.

These changes might revolve around trade-offs between colony growth and immune defense. Within this framework, we include the effects of parasitism by Varroa because when the mite is present, optimal nutrition alone might not be sufficient to keep virus levels low. Honey bee viruses More than 20 viruses have been identified to infect honey bees worldwide. The most common are: Deformed wing virus (DWV), Black queen cell virus (BQCV), and Israeli acute paralysis virus (IAPV). Viruses infect all developmental stages and castes. Though always present in colonies, viruses often persist as covert asymptomatic infections. However, if colonies are under stress, virus levels can increase causing reduced worker longevity and brood survival and colony loss in winter or early spring Viruses such BQCV also can cause colony death.

Bee diseases and parasites*:* Numerous predators, parasites (mites) and pathogens (protozoa, bacteria and viruses) prey upon the honey bee.

Mites: The honey bee tracheal mite, *Acarapis woodi*, is a parasite of *Apis mellifera* and *Apis cerana*. It lodges itself in the trachea of worker bees, where it breeds, and eventually suffocaotes them. Although it was a pest in the20th Century, the tracheal mite is now no longer a major problem for world apiculture. *Tropilaelaps* spp. is a parasitic mite of *Apis dorsata* honey bees in tropical Asia. The introduction of *Apis mellifera* into the distribution range of *Apis dorsata* has provided the *Tropilaelaps* mite with a new host. A recent study based on molecular markers has identified at least four *Tropilaelaps* species in Asia, although *T. clareae* is the only one that is parasitic to *Apis mellifera*. *Tropilaelaps* are brood parasites, feeding on the haemo lymph of the bee brood and breeding there. A proliferation of these parasites can kill honey bee colonies and encourage the emergence of other pathogens. The mite is so reliant on brood that it dies after more than seven days without it.

Protozoa: *Nosema apis* is a microsporidian that attacks the midgut wall of adult honey bees. The disease can develop with no visible symptoms or manifest itself as a weakening of the colony, possibly ending in death. Colony infestation is latent. The disease tends to emerge mainly in early spring following long, wet winters: during winter, honey bees are prevented from going outside and drop their excrement inside the hive, forming a source of contagion for other bees. After this, the disease spreads rapidly.

Bacteria: The bacteria pathogenic to honey bees attack the brood a disease that has been known since ancient times, is caused by *Bacillus larvae*. This serious highly contagious disease occurs across the globe. A supply of pollen from outside the nest is usually all colonies need to overcome the disease, although heavy losses have been reported in the past.

Viruses: Eighteen different viruses have been identified in honey bees of the *Apis* genus. Some of these viruses are highly anecdotal, while others are latent and can be extremely prolific among the bees in our hives without causing any noticeable signs. For reasons as yet unknown, these viruses can become highly pathogenic

Viruses commonly detected in honey bee colonies.

Virus	Transmission	Lifestage infected	Symptoms]
Acute bee paralysis virus (ABPV)	Horizontal primarily through feeding, Varroa parasitism	Brood and adults	Paralysis, trembling, inability to fly, darkening and loss of hair on thorax and abdomen
Black queen cell virus (BQCV)	Horizontal primarily through feeding, Varroa parasitism, possible vertical transmission through eggs	Brood and adults	Dead queen larvae or prepupae sealed in queen cells with dark brown to black walls
Chronic bee paralysis virus	Horizontal primarily through feeding and contact, possible transovarial	Adults	Trembling inability to fly, bloated abdomens, black hairless bees
Deformed wing virus	Horizontal primarily through feeding, venereal, transovarial, transspermal, Varroa parasitism	Brood and adults	Deformed wings in emergent bees, premature aging of adults
Israeli acute paralysis virus (IAPV)	Horizontal primarily through feeding, transovarial, venereal, transpermal, Varroa parasitism	Brood and adults	Similar to ABPV. Also, reduced mitochondrial function, and possible disturbance in energy-related host processes.
Kashmir bee virus (KBV)	Horizontal primarily through feeding, transovarial, Varroa parasitism	Brood and adults	Weakening of colonies but no clear field symptoms

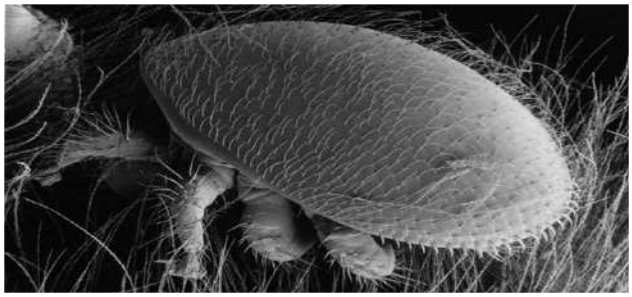

Fig- ***Varroa destructor*** **mite**

to honey bees causing trembling and paralysis that are observable at the colony entrance. This is the case with chronic paralysis virus (CPV) and acute paralysis virus (APV). It is not yet known how these viruses act to kill bees. No treatment exists to control such viruses, which can weaken or kill the colony.

Honey bee immune system:

The risk of disease outbreaks is reduced in colonies of honey bees and other social insects by group-level behaviors ('social immunity') and individual immunity. Together these provide multiple levels of disease prevention and responses to challenges from pathogens and parasites.

Social immunity: The collective defense against parasites and pathogens that emerges from the behavioral cooperation among individuals in colonies is 'social immunity'. With social immunity, many individuals do small tasks that collectively have a colony-wide impact on reducing the spread of parasites and pathogens. For example, workers remove adults that die in the colony and brood that are diseased or parasitized (hygienic behavior). Adults that die outside the nest also contribute to social immunity if they have high pathogen loads. Thermoregulatory behaviors also are a type of social immunity particularly when worker bees generate a behavioral 'social fever' against heat-sensitive pathogens such as chalkbrood fungus

Impact of climate change on honey bees:

Climate change can impact on honey bees at different levels. It can have a direct influence on honey bee behavior and physiology. It can alter the quality of the floral environment and increase or reduce colony harvesting capacity and development. It can define new honey bee distribution ranges and give rise to new competitive relationships among species and races, as well as among their parasites and pathogens.

Beekeepers will also be obliged to change their apiculture methods. They will favour moving their hives to new foraging areas and importing foreign races to test their value in the new environments. Bees adjust their behavior to weather conditions. They do not go out when it rains and, in extremely hot weather, they gather water to keep the colony cool. In contrast, the Asian species have remained in Asia, which might indicate lesser adaptability to different environments and fragility in the face of climate change. *Apis mellifera* seems to have more adaptive potential than its Asian cousins, which have low yields and have been subject to little transhumance. Humans, with whom *Apis mellifera* has co-evolved for several centuries, will certainly be decisive in helping honey bees to survive in hostile environments and in preserving the biodiversity of these species. Beekeeping is an essential pollination and production support tool in this respect. Other pathogens or haplotypes have more limited distribution ranges, such as *Tropilaelaps*, which to date has been found only in Asia. Climate change will lead to movements of honey bees of different species and races, bringing them into contact with pathogens with which they have never co-evolved, as has occurred with *Varroa destructor* and *Apis mellifera.* However, researchers agree that the bees' environment and stress, both of which are influenced by climate change, have been decisive factors in this heavy mortality. There appear to be strong interactions between diseases, pesticides, environment and climate. Climate change has an action on each of these factors. To understand the effect of climate change on the evolution of honey bee populations, each of these factors will need to be taken into account.

CONCLUSION

Phenomenon include pesticide use, new diseases, stress and a combination of these factors. As a result, climate change will shift the balance between the honey bee, its plant environment and its diseases. The honey bee has shown a great capacity to colonies widely diverse environments and its genetic variability should enable it to adapt to such climate change. However, the fear is that climate-induced stress will in future compound the various factors already endangering the species in certain regions of the world.

If humans modify the honey bee's environment, they also have a duty to take conservation measures to prevent the loss of this rich genetic diversity of bees. The composition of nutrients obtained from food influences microbial communities in the gut. The communities could affect immune function by providing essential nutrients, inducing host immune responses or reducing the growth of pathogens. While there is evidence for these benefits in other organisms, the role of microbial communities as extensions of social and individual immune systems has only begun to be explored in honey bees. Though improved nutrition can optimize colony growth and immune responses to virus, Varroa parasitism might undermine any benefits that nutrition might offer.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:263-266
UGC Approved Journal No 48951

Original Article Open Access

Zooplankton diversity in Balsamudra lake of Pauni, Dist. Bhandara, Maharashtra, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Meshram UG, Dahare RB and Dhamani AA (2018) Zooplankton diversity in Balsamudra lake of Pauni, Dist. Bhandara, Maharashtra, *Int. J. of. Life Sciences*, Special Issue, A12: 263-266.

ABSTRACT

Zooplanktons are cosmopolitan in nature and inhabit all freshwater habitats of the world, including polluted, industrial and municipal waste waters. Zooplankton forms an important link in the food chain, food webs, energy flow and cycling of matter. The present investigation made an attempt to study the zooplanktons in Balsamudra lake during the year 2013-2014. Quantitative study of plankton was done by Sedgwick-Rafter cell method. In the present investigation, Rotifera is dominant followed by Protozoa, Cladocera, Copepoda and Ostracoda.

Keywords: Water, Zooplanktons, diversity, Balsamudra Lake, Pauni.

INTRODUCTION

Hensen in 1887 coined the term 'plankton' for all organisms which float in water and do not execute individual movements of any importance..Zooplankton play an integral role and serve as bioindicators and is a well suited tool for understanding pollution status of water (Rajagopal et.al. 2010). Different environmental factors that determine the characters of water have great importance upon the growth and abundance of zooplankton. Zooplanktons are cosmopolitan in nature and inhabit all freshwater habitats of the world, including polluted, industrial and municipal waste waters. Zooplanktons form an important link in the food chain, food webs, energy flow and cycling of matter. Zooplanktons do not form an integral part of the lentic community but also contribute significantly to the biological productivity of the fresh water ecosystem (Wetzel and Likens, 1979). A number of studies has been carried out on the condition of ecology and freshwater bodies in various parts of India (Smitha *et al*, 2007) but in some parts of Vidarbha region (M.S), the ecological studies of freshwater bodies especially zooplankton studies is very scanty(Gadekar, 2014). The present investigation made an attempt to study the zooplanktons in Balsamudra Lake during the year 2013-2014.

METHODOLOGY

Balsamudra lake is located at the South-east side of Pauni, is at 20⁰47 ˡ31ˡˡ N. latitude and 79⁰38ˡ7ˡˡ E. longitude. It receives water from the surrounding catchment area during the monsoon period as well as from municipal drainage. The area of Balsamudra is spread over 4.92 hector. The depth of water is 10 to 12 feet during monsoon and 5 to 6 feet during summer season.

Fig 1. Map of Pauni town

Fig 2. Location map and the sampling stations in Balsamudra Lake

Sample for planktonic study were collected monthly. The samples were collected in the morning hours between 8.30 to10.30 a.m. 50 Lt of water sample was filtrated through the plankton net made of bolting silk number 25 with mesh size 64 lime. The collected samples were allowed to settle down by adding Lugol's iodine. Normally, sedimentation requires 24 hrs. After which supernatant was removed and concentrate was made up to 50 ml depending the number of plankton and preserved in 5% formalin for further studies. For the quantitative study, the concentrated sample was shaken and immediately one drop of sample was taken on a clear micro side with the help of a standard dropper, the whole drop was then carefully covered with the cover glass and observed. Quantitative study of plankton was done by Sedgwick-Rafter cell method. Plankton identification up to genera and whenever possible up to species level was classified according to keys given Edmondson (1959), Sehgal (1983), Adoni (1985) and APHA (2005) and standard analysis was undertaken as per Zar (2005).

RESULTS AND DISCUSSION

The Zooplanktons in group are represented by Protozoa, Rotifera, Cladocera, Copepoda and Ostracoda. The data is tabulated in Tables 1. In the present investigation during the year 2013-14 Rotifera is dominant followed by Protozoa, Cladocera, Copepoda and Ostracoda. In Rotifera 14 species were recorded among which *Brachionus calyriflorus* (107 no./lit) is dominant followed by *Cephallodella gibba* (74 no./lit), *Monostyla bulla* (49 no./lit), *Brachionus falcatus* (48 no./lit), *Keratella tropica* (32 no./lit), *Keratella serrulata* (25 no./lit), , *Trichocera similes* (23 no./lit), *Trichocera sp.*(23 no./lit), *Keratella cochleris*(22 no./lit), *Brachionus caudata* (18 no./lit), *Lecane luna* (17 no./lit), *Monostyla clasterocera* (16 no./lit), *Lepadella patella* (16 no./lit), *Monostyla species*(15 no./lit),. Sharma *et al*. (2011) reported 49 species in Pichhola lake, Jaipur, Rajasthan. Harney *et al*. (2013), reported 30 species in Kanhala pond and 24 species in Pindavani pond, Bhadrawati. maximum Rotifera during winter may be due to favorable temperature and availability of abundant food. In Protozoa 11 species were recorded among which *Amoeba proteus*(35 no./lit) is dominant followed by *Actinophrys sol.* (26 no./lit), *Difflugia lebes* (18 no./lit), *Centrophyxis aculeata* (15 no./lit), *Difflugia corona*(15 no./lit), *Arcella vulgaris* (13 no./lit), *Bursaria truncatella* (12 no./lit), *Paramecium caudatum* (9 no./lit),

Table No.1: Total number of Zooplankton from Balsamudralake During 2013-14

Sr. No.	Components	Summer				Monsoon				Winter				Min	Max
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan		
1	Protozoa	15	11	10	15	14	3	0	10	21	31	23	22	**0**	**31**
2	Rotifera	28	30	29	32	28	13	14	33	66	64	97	92	**13**	**97**
3	Cladocera	27	22	26	21	9	20	15	12	61	56	36	32	**9**	**61**
4	Copepoda	8	4	3	6	7	5	5	6	6	10	13	13	**3**	**13**
5	Ostracoda	17	25	28	21	12	6	4	3	40	40	41	42	**3**	**42**
	Total	**95**	**92**	**96**	**95**	**70**	**47**	**38**	**64**	**194**	**201**	**210**	**201**	**38**	**210**

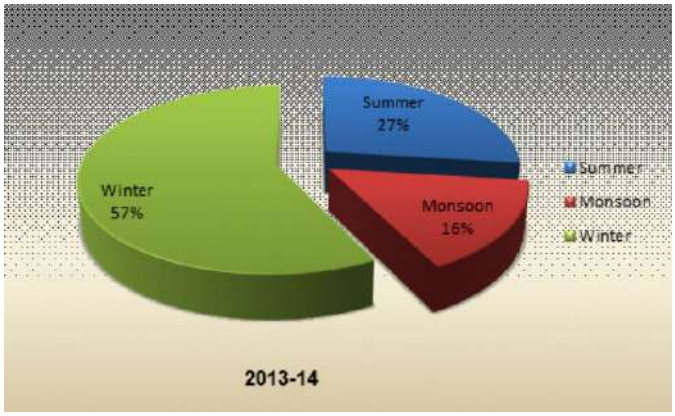

Fig 3: Distribution of Zooplanktons in Balsamudra Lake during the study period.

Spathidium sp. (9 no./lit), *Vorticella* (9 no./lit), *Paramecium bursaria* (6 no./lit). Harney *et al*., (2013), recorded 41 species of protozoa in Kanhala pond and 39 species in Pindavani pond, Bhadrawati, district Chandrapur, Maharashtra. Bera, Dutta *et al*. (2014), recorded 4 species at Kangsabati Reservoir, West Bengal. , the dilution of water caused by monsoon rain explains low Protozoan count in monsoon. The maximum population during winter may be due to favorable temperature and availability of abundant food in the form of bacteria and suspended detritus.

In Cladocera 8 species were recorded among which *Bosmina longirostris* (107 no./lit) is dominant followed by *Moina dubia* (57 no./lit), *Ceradaphnia* (46 no./lit), *Chydorus sphacricus* (26 no./lit), *Daphnia laevis* (22 no./lit), *Alona sp.* (19 no./lit), *Simocephalus vetalus* (13 no./lit), *Macrothrix rosea* (10 no./lit). Kedar (2002) recorded 9 species of Cladocera in Rishi lake, Karanja (Lad) of Washim district. Pawar and Phulle (2005) reported 20 species of Cladocera in Petwadaj dam, district Nanded, Maharashtra. , maximum Cladocera was recorded during winter season and minimum during monsoon season. In Copepoda 5 species were recorded among which *Cyclops sp.* (27 no./lit) is dominant followed by *Diaptomus forbesi* (18 no./lit), *Naupli* (15 no./lit), *Phyllodiaptomus female* (14 no./lit), *Microcyclops varicans* (12 no./lit). Sharma *et al*. (2011), recorded 11 species in Pichhola lake, Jaipur, Rajasthan.

In Ostracoda 3 species were recorded among which *Cypris species* (122 no./lit) is dominant followed by *Stenocypris malcomsonil* (97 no./lit), *Centrocypris* (70 no./lit). Harney *et al*. (2013), recorded 2 species in Kanhala pond Pindavani pond and Malhara pond, Bhadrawati, district Chandrapur, Maharashtra. Bera, Dutta *et al*. (2014), reported 2 species at Kangsabati Reservoir, West Bengal. In present study, maximum Ostracoda was recorded during winter season and minimum during monsoon season.

CONCLUSION

Zooplanktonic population of the Balsamudra lake reveals the eutrophic condition which is an account of activities such as domestic waste disposal in the form of sewage and solid wastes, disposal of wastes materials, dumping of dead animals, human wastes etc.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:267-270
UGC Approved Journal No 48951

Original Article Open Access

Application of bentonites as an agent for the purification of solid matter contaminated waters

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Jha AK (2018) Application of bentonites as an agent for the purification of solid matter contaminated waters, *Int. J. of. Life Sciences*, Special Issue, A12: 267-270.

ABSTRACT

In this study possibilities of the application of optimum conditions of the adsorption and coagulation treatment with bentonites was explored and optimized and effects of the treatment were focused on, water contaminated with dispersions of different organic polymers, water contaminated with food particles and waste water characterized by emulgated and fine dispersed solids. The turbidity and SS of the treated wastewater were reduced with the increase of bentonite dosage, and reached minimum at the same dosage which was about 250mg/L. The pH 6 as the optimum value was under consideration not only the treated efficiency but also the cost of the treatment

Keywords: Bentonites, water contamination, organic polymers, water purification

INTRODUCTION

Bentonite is a clay rock of which the active mineral is montmorillonite (Jahn, 1977). The activation of bentonite does not change, in principle, the natural characters of this rock, and, therefore, the application of activated bentonites lowers or eliminates the need of the addition of chemical electrolytes into the waste water which can be observed from the environmental point of view as secondary contaminators (Liu and Zheng, 2008; Duan *et al.*, 2010). Activated bentonites destabilize different dispersion systems, which are decomposed by creation of flakes as impurities are being adsorbed on particles of bentonites (Gunister *et al.*, 2006). Bentonite particles saturated with impurities are relatively heavy, they sediment quickly & hence can be used, on the type of impurities, they are liquidated or exploted in different low-waste technologies.

METHODOLOGY

Sewage water samples were collected from Nagpur city at locations Paungaon, Mahalgaon and Parshad along Nag River and Pili River area, for a period of one year, December 2015 to January 2016.

In the laboratory, the sewage samples were preserved in the refrigerator at 4°C and examined when needed so that the potential for volatilization or biodegradation of the samples can be minimised. Collection and preservation of samples for the pH, COD, iron, and turbidity were done according to the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, and WEF 2005).

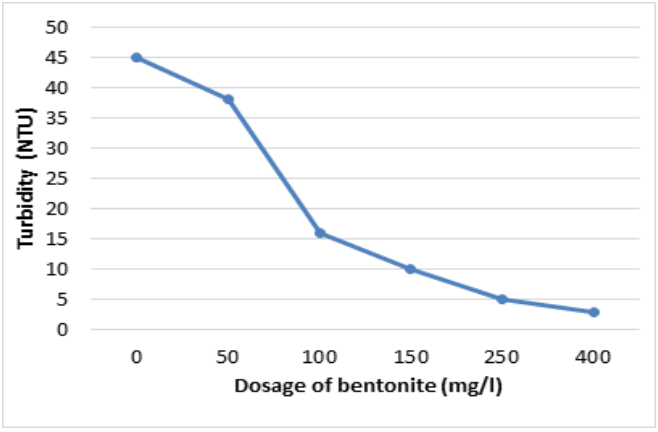
Activated bentonites are supplied in bags or bulky as a dry powder with the maximum grain size 0.3 mm up to 3.0 mm. They stored under the roof on pallets or transported into bins. Before being applied activated bentonites are mixed in a propeller mixture with water during 0.5 until 2.0 hours. The resulting slurry contains various Bentonite dosages of, 50g, 100g, 150g, 250g and 400g of dry matter per one liter. Such slurry can be pumped into the purification station.

Waste water, from which bigger foreign matters are removed in the traditional way, is pumped into the mixing basin where the predetermined amount of specially activated bentonites is added during the continuous mixing. After about 60 seconds, pH of the mixture can be regulated if necessary. For this purpose, hydrated lime or a mineral acids are added. After another one minute mixing, the slurry is transferred into a sediment basin. Bentonites saturated with impurities gets sediments, while purified water flows over the upper edge of the sedimentary device.

RESULTS AND DISCUSSION

Effect of the Bentonite Dosage:

As shown in Figure 1a and 1b, the turbidity and SS of the treated wastewater were reduced with the increase of bentonite dosage, and reached minimum at the same dosage which was about 250mg/L. It is because that the SS of the treated water was devoted by fines concentration. The light beam will be scattered and reflected by the small particles. This is exactly corresponds with the determination theory of turbidity.



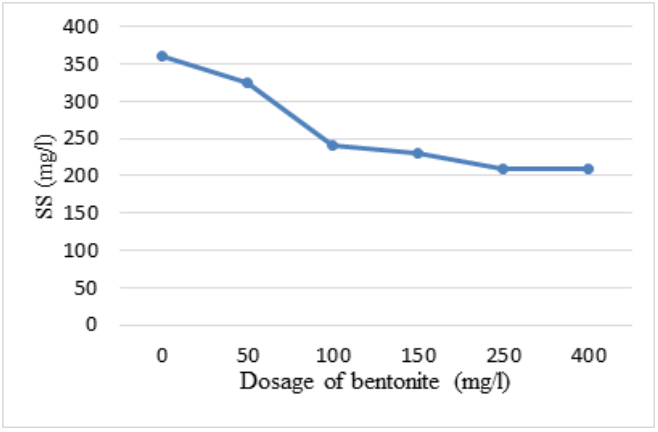
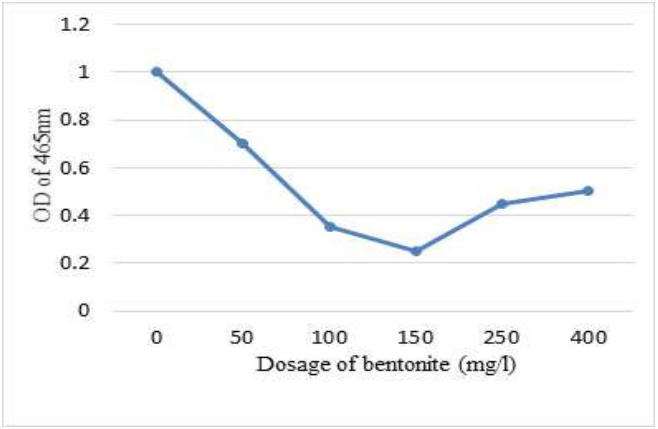


Figure 1 (a) Effect of the bentonite dosage on Turbidity **(b).** Effect of the bentonite dosage on Suspended Solid (S.S.)



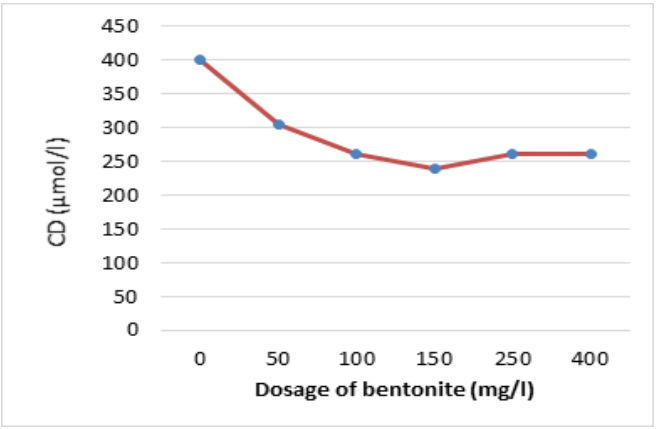


Figure 1 (c). Effect of the bentonite dosage on Optical density (O.D.). (**d).** Effect of the bentonite dosage on Cationic Demand (C.D.)

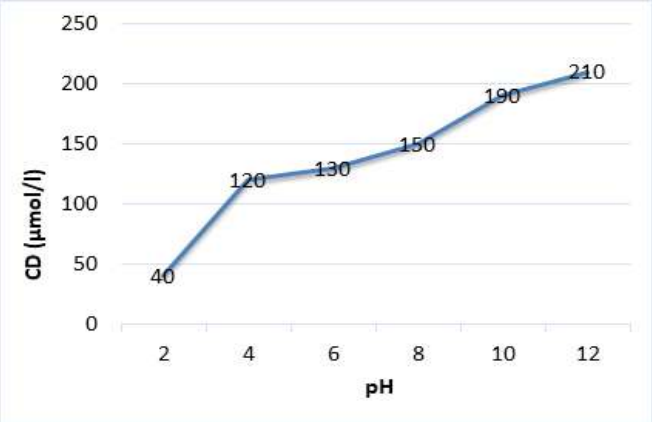
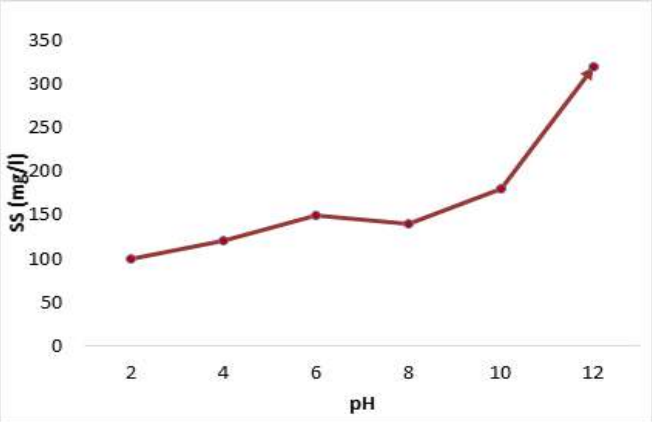


Figure 2 (a). Effect of pH on Cationic Demand (C.D.)



(b). Effect of pH on Suspended Solid (S.S.)

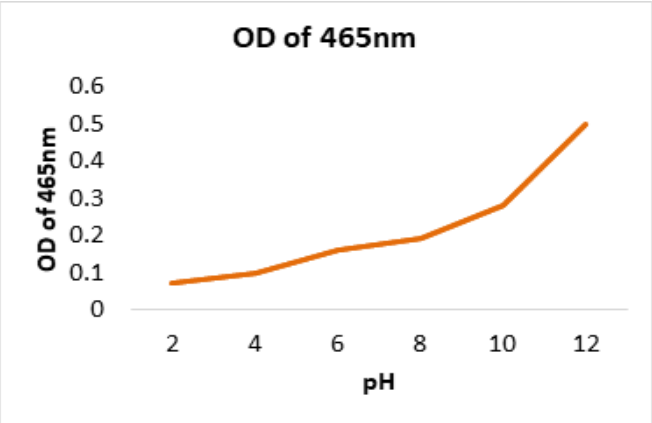
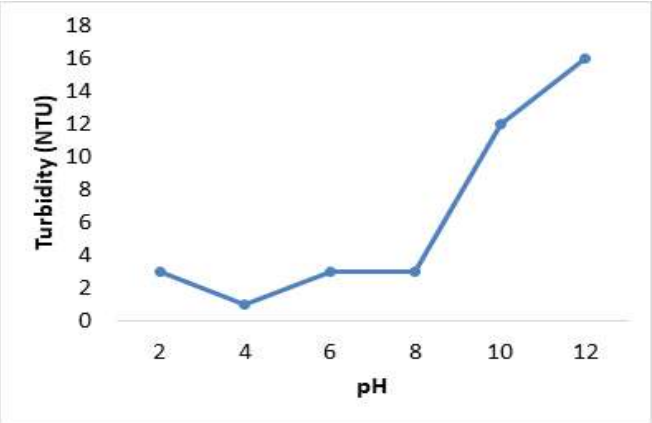


Figure 2 (c). Effect of pH on Optical density (O.D.).



(d). Effect of pH on Turbidity.

So the turbidity of water in this study was linear with the content of SS. The CD and 465 nm OD of the of the treated water were decreased with increase of bentonite dosage and reached the minimum at about 150mg/L dosage, but increased at the dosage of 250mg/L, then drop again while the dosage reached at 400mg/L (Figure 1c and 1d). This was most likely caused by removal of CD and colour substance due to two ways: adsorption and coagulation mechanism.

Figure 1 further showed the removal of the four indices were significant only when the dosage from 50 mg/L to 150 mg/L. So, the 150 mg/L was the optimal bentonite dosage.

Effect of the initial pH

Figure 2a, 2b, 2c, 2d showed the effect of initial pH on the bentonite treatment. As a whole, all of CD, SS, 465nm OD and turbidity of the treated water were increased with the initial pH value increased. The colour and CD of the treated water increased slightly with increased of pH, the acidic property water becomes stronger, the CD and colour of treated water will become smaller. It is different that the colour of the treated water altered little with pH from 2 to 6 to that of raw material puling effluent.

The colour of raw material puling effluent will change very significantly because the acidic precipitation of lignin that contributes to the colour of these effluents. The SS of the treated water increased slightly with pH from 2 to 6, and from 8 to 12, but decreased at pH 8. The turbidity decreased with pH from 2 to 4, and then increased from pH 4 to 8, after pH 8, with the increase of pH value, the turbidity increased significantly. It was maybe due to good dispersion of bentonite at alkaline environment. The dispersion of absorbed water and expanded bentonite in water caused the increasing of turbidity. The above mentioned factors should be under consideration choice of pH value. Except for the SS of treated water, the other three indexes are good enough for the treatment at pH 6. Choose the pH 6 as the optimum value was under consideration not only the treated efficiency but also the cost of the treatment.

Table 1 : Dosage of Bentonite

Sr. No.	Type of waste water	Dosage of bentonite / mg/L	Neutralization
1	slightly oil polluted water	50-300	With hydrated lime to pH 3-5
2	Strongly oil polluted water	100-400	Usually not necessary
3	Waters with dispersions of organic polymers	100-200	Usually not necessary
4	Water from cattle breeding	100-250	Usually not necessary or with hydrated lime to pH 6
5	Waters from food industry	50-250	Alternatively H_2SO_4 or Hydrated lime
6	Didested sludges from biological sewage plants mechanical dewatering	100-400	Usually not necessary

Average specific consumption of specially activated bentonites and eventually other supporting matter is presented in table 1. It has to be notified, that the exact consumption of bentonites must be determined experimentally depending on the type of waste water.

The best result of the application of bentonites for water treatment is reached in water with pH between 5-8. This is consistent with finding of Inglezakis *et al*., (2007) and Aziz *et al.*, (2007). Therefore, the regulation of water acidity is recommended. Alkaline waste waters, therefore, need the neutralization with the acid.

The specially activated bentonite, being blended with water in the recombined ratio, show the acidic reaction with pH 3.5 after the bentonite slurry is blended into waste waters, the pH of the mixture is lowered proportionally. If then the acidity of mixture fall bellow pH 5, hydrated lime is to be added in order to maintain the acidity of the slurry between pH 5-8 again.

Through the studies of influence factors we draw up the optimal conditioned for water treatment. The optimal condition were bentonite dosage 150mg/L and pH value 6.4.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:271-274
UGC Approved Journal No 48951

Original Article Open Access

Rotifers as an indicator of water quality

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Kulkarni Rajlaxmi Ranrag and Zade SB (2018) Rotifers as an indicator of water quality, *Int. J. of. Life Sciences*, Special Issue, A12: 271-274.

ABSTRACT

Rotifers are the connecting link between primary producers and consumers in aquatic food web. Ramala Lake is a historical impoundment situated in the heart of Chandrapur city. In present investigation zooplankton composition of water indicate more rotarian diversity in comparison to other groups. Rotifers stood first not only quantitatively but qualitatively also. The group was represented by 21 species of 12 genera.

Key words – Ramala Lake, zooplankton, rotifer, biodiversity, bioindicator

INTRODUCTION

Zooplankton is ecologically and economically important heterogenous group of tiny aquatic microorganisms. These are either herbivorous feeding on phytoplankton or carnivorous feeding on other zooplankton. They themselves fed upon by fish. The rotifiers constitute a dominant component of freshwater zooplankton and serve as a food for young ones and an adult of commercially important culturable fishes (Sharma 1991). These are considered as important bioindicator in depicting trophic status of water quality in ecosystem (Sladeck, 1983; Berzins and Pejler, 1987.) Rotifera is one of the fascinating groups of zooplankton in an aquatic ecosystem. Rotifera, also called as wheel animalcules, is one of the major phyla consisting about 1500 species. These are microscopic animals having flattened and ciliated ventral surface. Rotifer occurs almost universally in freshwater habitat. Rotifer diversity refers to varieties of species within their community. Rotifers are also known as the Rotatoria, originating in fresh water. They are harmless microorganisms with transparent bodies displaying a variety of forms with an amazing alacrity in movement and behavior; play a major role in trophic structure of an ecosystem by their numerical abundance. Eutrophic water bodies have rich rotifer diversity. Rotifers are the smallest animals and occur worldwide in primarily freshwater habitats. They are important in fresh water ecosystem as they occur in all biotypes. About 95% of the rotifers

are encountered in fresh water, while 5% are from brackish or marine water and most are free living. Like the other zooplankton, rotifers also form a link in the aquatic food chain. They have a rapid turnover and high metabolic rates and feed on detritus. Rotifers are extensively cultured as fish food.

The present study deals with rotifer species diversity and richness of Rotifers in Ramala lake of Chandrapur city, Maharashtra. Ramala lake is perennial rainfed water body constructed in Chandrapur city by Gond Raja in fifteenth century along the north east side of city wall. Lake is situated at 79˚18′ E longitude and 19˚18′ N latitude and about 232 meters above MSL.

METHODOLOGY

The zooplankton samples were collected early in the morning from the three sampling sites on monthly basis for two years i.e. from November 2005 to October 2007. The water was filtered through plankton net, made of bolting silk cloth and concentrate was collected in glass bottle, fixed in 4% formalin and specimens were identified according to the key from Edmondson (1959), Battish (1992).

RESULTS AND DISCUSSION

Zooplankton is an important index of secondary production and natural source of food for higher organisms including fishes. Zooplankton plays a key role in transferring energy from one trophic level to other in aquatic habitats. They are also used as biological indicators of water body.

The abundance of zooplankton has been governed by cumulative effects of physico-chemical variables, (Ahemed and Alizera,1992). Generally the species included in the zooplankton belongs to Protoza, Rotifera, Cladocera, Copepoda and Ostracoda. Zooplankton from Ramala Lake comprised of above said major five groups. Maximum density of zooplankton was found in summer. During present study, 43 species of zooplankton were identified.

The rotifers invariably constitute a dominant component of freshwater zooplankton and contribute significantly to their dynamics and production. (Sharma,1991). These organisms are regarded as valuable bioindicator to depict the trophic status of water quality. (Pejler, 1987) Rotifer species have been identified as indicators of water pollution (Arora, 1962). Several species of Brachionus are recorded from highly polluted fresh water lake. Hussainsagar, Hyderabad by Malathi et al. (1998). Varma and Datta (1987) reported eutrophication of water bodies on basis of Brachionus species. Rotifers play an important role as grazers, suspension feeders and predators within the zooplankton community. The difference in the periodicity and population density of different rotifers species can be analyzed by considering the nutritional ecology and biotic interactions. Rotifer species exhibit marked differences in their tolerance and adaptability to changes in physiochemical and biological parameters. Such changes are dramatic and sudden in case of urban ecosystem.

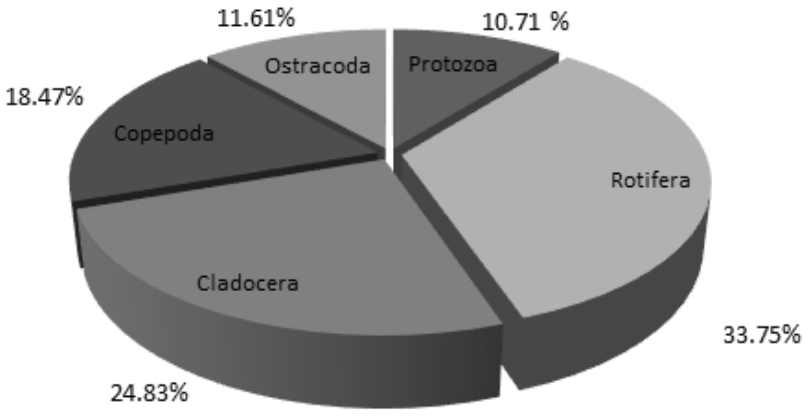

Fig. 1: Percentage composition of Zooplankton

Table 1: Rotifer species recorded from Ramala lake during study period November 2005 to October 2007.

Sr. No.	Species	Station I	Station II	Station III
1.	Brachionus calyciflorus	+	+	+
2.	Brachionus bidenta	+	+	+
3.	Brachionus falcatus*	-	+	+
4.	Brachionus forficula	-	+	+
5.	Brachionus durgae	+	+	+
6.	Brachionus quadridentatus	+	+	+
7.	Brachionus calyciflorus var.Melhin	+	+	+
8.	Keratella tropica	+	-	+
9.	Rotaria rotatoria*	+	-	-
10.	Lecane papuana	+	-	+
11.	Lucane bulla*	+	+	-
12.	Lecane styrax	-	-	+
13.	Testudinella sp.	-	-	+
14.	Filinia longiseta	+	-	-
15.	Asplanchna intermedia	+	-	-
16.	Trichocera Porcelhs	+	-	-
17.	Trichocera kostei	+	-	-
18.	Plantionus patulus	-	-	+
19.	Platuas quadricornis	+	+	-
20.	Philodina sp.	+	+	+
21.	Polyarthra indica	+	+	+

(+) = Present; (-) = Absent, (*) = Pollution indicator species

Water samples from three different stations were collected qualitative and quantitative analysis of zooplankton for the period of two years. During the present study, Rotifers stand first in order abundance, contributing 33.76% of total zooplankton. The rotifer population of the lake ranged between 01 units/L at station II in the month of February 2007 and 48 units/L at station II in the month of March 2006.The observation of zooplankton composition of water body indicates more Rotarian diversity followed by Cladocera, Copepoda and Ostracoda. Rotifers dominated the zooplankton population. Rotifers were not only numerically abundant but also showed maximum diversity.

Rotifers are microscopic soft bodied fresh water invertebrates. Their distribution and ecology have interesting evolutionary implication (Reid and Wood, 1976). Rotifers have been used to indicate trophic status of water body. Since long time the rotifers have been used as bioindicators of water quality, because of their diversity and cosmopolitan distribution. The group Rotifera was presented by 21 species of 12 genera. The lake represented highest number of species of genus Brachionus. 6 species pf this genus were recorded during the period of investigation. *Brachionus calyciflorus* and *Brachionus bidenta* dominated the lake at all the stations. *Brachionus falcatus* found only at station II.

The genus *Lecane* was represented by three species whereas genus *Trichocera* was represented by two species. Remaining 10 genera were represented by one species each. Rotifers were found to be maximum at the three stations. The group was found to be most dominant group at all the station. Maximum density of rotifers was found in summer followed by winter and monsson Welch (1952). Tandon and Sigh (1972) have shown a direct relationship between rotifer population and water temperature.

Brachionus sp. were found to be dominant throughout the study period. Six different species of Brachionus were found among which *Brachionus calyciferous* and *Brachionus bidentata* were found to be very common. Pollution indicator species like *Rotaria, Lepadella, Brachionus falcatus* were found abundant at station I and station II. Edmondson (1965) and Baker (1979)

observed the high rotifer population in winter and attributed to favorable temperature and availability of abundant food. In the present investigation, the species of rotifers found other that *Brachionus* and *Keratella* are *Lecane sp., Trichocera, Filina, Testudinella, Asplanchna Rotaria, Plantionus, Platyas, Cephalodella* etc. Tijare and Thosar (2007) have recorded nine species of *Brachionus* and 20 species of Rotifers from three lakes of Gadchiroli district. Similar observations with 7 species of *Brachionus* also made by Somani and Pejawar (2003) from Masuunda lake, Thane (M.S.).

According to Reid and wood (1976) rotifers never follow any predictable population pattern in fresh water impoundment. Deshmukh (2001) reported 28 species of Rotifera from Chhatri Lake of Amaravati with maxima in summer, which corroborate with the present investigation.

CONCLUSION

Rotifers are chiefly fresh water forms and presence of these microorganisms in abundance is related to the suitable conditions for their survival. Since long time, the rotifers have been used as bioindicator of water quality because of their diversity and cosmopolitan distribution. Rotifers such as B. forficula and Filinia logiseta are considered as indicator of eutrophy. But according to quantitative analysis of rotifers in present investigation which is not exceeding 200/1, the water body is oligotrohpic. Eutrophic water bodies have rich rotifer diversity

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:275-278
UGC Approved Journal No 48951

Original Article Open Access

Cladocera diversity of three water bodies of Bhadrawati, dist- Chandrapur (M.S.), India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Harney NV, Dhamani AA and Andrew RJ (2018) Cladocera diversity of three water bodies of Bhadrawati, dist- Chandrapur (M.S.), India, *Int. J. of. Life Sciences*, Special Issue, A12: 275-278.

ABSTRACT

Cladocera is a primarily-freshwater monophyletic group, an important component of the microcrustacean zooplankton. They inhabit most types of continental fresh and saline water habitats, occurring more abundantly in both temporary and permanent stagnant waters. The present paper describes the biodiversity of Cladocera fauna of Kanhala, Pindavani and Malhara pond, located near the Bhadrawati town of Chandrapur district. Qualitative and quantitative analysis of Cladocera community was undertaken on monthly basis from October 2005 to September 2007. A total of 12, 8 and 10cladocera species were identified during the period of Oct. 2005 to Sep. 2006, while a total of 10, 11 and 9cladoceraspecies were identified from Oct. 2006 to Sep 2007 in Kanhala, Pindavani and Malhara pond respectively. In the present investigation, Cladocera was maximum during the winter season and minimum during the monsoon season in all the ponds.

Key words- Kanhala, Pindavani and Malhara pond, Cladocera diversity, Seasonal variation.

INTRODUCTION

The Cladocera component of zooplankton plays an important role in the benthic trophodynamics. Most of the Cladocerans are primary consumers and feed on microscopic algae and fine particulate matter in the detritus thus influencing the cycling of matter and energy in benthos. Cladocera is an important component of zooplankton and form the most dominant groups of fish food organisms. The present investigation has been undertaken to study the statistical qualitative and quantitative analysis of cladoceracommunity at the Kanhala, Pindavani and Malhara pond located near Bhadrawati town of Chandrapur district.

MATERIAL AND METHODS

The three ponds selected for study viz. Kanhala, Pindavani and Malhara pond. They are principal freshwater bodies located in the rural area, around the vicinity of Bhadrawati town, located in the Chandrapur district of Maharashtra State, India. It is situtated at about 211 m above MSL and at 20°06[1] 35.67[11]N latitude and 79°07[1]7.33[11] E longitude.

Samples for plankton were collected monthly in the morning hours between 8.30 to 10.30 a.m. About 50 Lt. of water sample was filtrated through the plankton net made up of bolting silk number 25 with mesh size 64 lime. The collected samples were allowed to settle down by adding Lugol's iodine. Normally, sedimentation requires 24 hrs. After which supernatant was removed and concentrate was made up to 50 ml depending the number of plankton and preserved in 5% Formalin for further studies.

The quantitative study of rotifers was done by Sedgwick – Rafter cell method, the concentrated sample was shaken and immediately one drop of sample was taken on a clear micro side with the help of a standard dropper, the whole drop was then carefully covered with the cover glass and observed. Identification up to genera and whenever possible up to species level was classified according to keys given by Prescott (1954), Edmondson (1959), Sehgal (1983), Adoni (1985) and APHA (1985).

RESULTS AND DISCUSSION

A total of 12, 8 and 10 cladocera species were identified during the period of Oct. 2005 to Sep. 2006, while a total of 10, 11 and 9 cladocera species were identified from Oct. 2006 to Sep 2007 in Kanhala, Pindavani and Malhara pond respectively.

Table 1 : Seasonal variation of Zooplankton in Kanhala Pond During year 2005-06

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	29.750	±	5.309	25.750	±	12.477	24.000	±	4.062

Table 2 : Seasonal variation of Zooplankton in Kanhala Pond During year 2006-07

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	27.250	±	10.848	23.750	±	14.703	20.250	±	3.562

Table 3 : Seasonal variation of Zooplankton in Pindavani Pond During year 2005-06

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	30.250	±	13.718	22.000	±	3.937	15.750	±	4.815

Table 4. : Seasonal variation of Zooplankton in Pindavani Pond During year 2006-07

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	24.500	±	5.766	18.750	±	0.433	18.250	±	3.961

Table 5 : Seasonal variation of Zooplankton in Malhara Pond During year 2005-06

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	13.500	±	1.118	12.250	±	1.479	7.250	±	1.299

Table 6 : Seasonal variation of Zooplankton in Malhara Pond During year 2006-07

Sr. No.	Components	Winter			Summer			Monsoon		
		Mean		S.D.	Mean		S.D.	Mean		S.D.
1	Cladocera	15.000	±	0.707	14.500	±	1.658	11.750	±	3.767

In Kanhala pond during 2005-06,12 species were recorded among which *Bosminalongirostris* (124 no./lit) is dominent followed by Moinadubia (52 no./lit), *Moinabranchiata* (34 no./lit), *Chydorussphaericus* (21 no./lit) ,*Simocehalusvetulus* (21 no./lit), *Sidacrystallina* (14 no./lit) and *Kurzialatissima* (13 no./lit.), *Macrothroixlaticornis* (10 no./lit), *Alonella nana* (10 no./lit), *Macrothrixrosea* (8 no./lit), *Dunbevediacrassa* (6 no./lit) and *Pleuroxusprocurvus* (5 no./lit) and during 2006-07, 10 species were recorded among which *Bosminalongirostris* (109 no./lit) is dominent followed by *Moinadubia* (35 no./lit), *Moina branchiate* (28 no./lit.), *Chydorussphaerius* (27 no./lit), *Simocephalusvetulus* (25 no./lit) and *Sidacrystalline* (21 no./lit), *Kurzialatissima* (14 no./lit), *Alona nana* (10 no./lit), *Macrothrixrosea* (8 no./lit) and *Pleuroxusprocurvus* (8 no./lit).

In Pindavani pond during 2005-06,9 species recorded among which *Bosminalongirostris* (142 no./lit) was dominent followed by *Chydorussphaericus* (27 no./lit), *Alonella nana* (26 no./lit), *Sida crystalline* (25 no./lit) and *Moina branchiate* (19 no./lit), *Kurzialatissima* (10 no./lit), *Macrothixrosea* (9 no./lit), *Pleuroxusprocurvu* (8 no./lit) and *Macrothrixlaticornic* (6 no./lit) and 2006-07, 11 species were recorded among which *Bosminalongirostris* (103 no./lit) is dominant followed by *Chydoroussphaericus* (30 no./lit), *Alonella nana* (23 no./lit), *Sidacrystalina* (20 no./lit), *Moinabrachiata* (17 no./lit), *Kurzialatissima* (13 no./lit), *Pleuroxusprocurvus* (8 no./lit), *Macrothrixrosea* (7 no./lit), *Dunbevediacrassa* (6 no./lit) and *Macrothrixlaticornis* (5 no./lit).

In Malhara pond during 2005-06,10 species were recorded among which *Bosminalongirostris*(36 no./lit) is dominent followed by *Sida crystalline* (18 no./lit), *Moinabranchiata* (18 no./lit), *Miona dubia*(18 no./lit), *Macrothrixrosea* (13 no./lit), *Chydorussphaericus*(10 no./lit), *Alonella nana* (8 no./lit), *Macrothrixlaticornis* (5 no./lit), *Pleuroxusprocurvus* (4 no./lit) and *Dunbevedia crassa* (4 no./lit) and during 2006-07, 9 species were recorded among which *Bosminalongirostris* (48 no./lit) is dominent followed by *Sidacrystallina* (30 no./lit), *Moinadubia* (24 no. /lit), *Moina branchiate* (18 no./lit), *Macrothrixrosea* (15 no./lit), *Alonella nana* (12 no./lit), *Macrothrixlaticornis* (8 no./lit), *Chydorussphaericus* (6 no./lit) and *Pleuroxuprocurvus* (4 no./lit).

The Cladocera component of zooplankton plays an important role in the benthic trophodynamics. Most of the Cladocerans are primary consumers and feed on microscopic algae and fine particulate matter in the detritus thus influencing the cycling of matter and energy in benthos.

In Cladocera, a total of 12 species are recorded at all the sampling sites of the three ponds under study. In Kanhala pond, Cladocera is represented by 12 species (2005-06) and 10 species (2006-07), in Pindavani pond, Cladocera is represnted by 9 species (2005-06) and 11 species (2006-07) and in Malhara pond, Cladocera is represented by 10 species (2005-06) and nine species (2006-07).Kamble and Meshram (2005) reported two species of Cladocera of Khattijapur tank, Achalpur, Amravati district of Maharashtra. Pawar and Pulle (2005) reported 20 species of Cladocera in Petwadaj dam, Nanded, Maharashtra. Sahoo and Jameson (2006) reported three species of Cladocera in cattle waste fed fish pond of Thoothukudi, Tamilnadu. Patil *et al.,* (2008) reported nine species of Cladocera in Rishi lake and 8 species in Yedshi lake of Karanja (Lad), Maharashtra.
Among the different species of Cladocera species in Kanhala pond, *Bosminalongirostris*was dominant followed by *Moinadubia, Chydorusspaericus, Simocephalousvetulus*and*Sidacrystalina.* In Pindavani pond, *Bosminalongirostrias* was dominant followed by *Chydorus*sp.*, Alonell nana, Sidacrystalina*and*Moina branchiate* and in Malhara pond *Bosminalongirostrias* was dominent followed by *Sidacrystalina, Moinabranciata, Moinadubia*and*Macrothrixrosea.*

In the present investigation, Cladocera was maximum during the winter season and minimum during the monsoon season in all the ponds.Rajan*et al.,* (2007) observed minimum cladocerans population in premonsoon and monsoon and maximum in post monsoon in three polluted water bodies of Virudhnagar district, Tamilnadu. The maximum population of Cladocerans in winter can be attributed to favorable temperature and avaibality of abundant food in the form of bacteria, nonplanktons and suspended detritus. In contrast, Choube (1997) found high density of cladoceran in the month of June and low in the month of December. In the present investigation, maximum Cladocerans in winter is linked to favorable temperature and avaibality of abundant food.

Conflicts of interest: The authors stated that no conflicts of interest.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:279-282
UGC Approved Journal No 48951

Original Article Open Access

Ichthyofaunal diversity and its conservation in Purkabodi lake near Lakhani dist. Bhandara (MS)

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Tichkule GC and Bakare SS (2018) Ichthyofaunal diversity and its conservation in Purkabodi lake near Lakhani dist. Bhandara (MS), *Int. J. of. Life Sciences*, Special Issue, A12: 279-282.

ABSTRACT

The Fishes are rich in protein, healthy and delicious food for man. They occupy all three levels in aquatic ecosystem such as primary, secondary and tertiary consumers of food web. The present investigation was aim to observe the ichthyofaunal diversity in Purkabodi lake, near Lakhani. It is 24km towards East from district headquarter Bhandara and situated on both side of NH-6 India. The wild life sanctuary Nagzira and Koka are about 30to 40km from Lakhani. The study was carried out for a year from October 2013 to September 2014. Literally there is no report on the fish diversity in this lake. During the study, total 23 species were identified belonging to 7 order and 13 families.

Key Words: Ichthyofauna, diversity, conservation, Lakhani.

INTRODUCTION

Fishes are invariable living components of water bodies, they are good indicators of the ecological health of the water they inhibit. The understanding of fish faunal diversity is a major aspect for the exploitation of fresh water reservoirs and the sustainable as well as economical management (Battul *et al*.2007). Biological production in any aquatic body gives direct correlation with its physic-chemical status which can be used as trophic status and fisheries resources potential. Lakes in India support rich variety of fish species, which in turn, support the commercial exploitation of the fisheries potential (Krishna and Piska 2006). According to Pawar *et al*. (2006) the thorough knowledge of fishery resources, their availability and distribution in a particular water body is essential for proper utilization of its fishery resources. Workers like Day (1878), Misra(1962), Motwani and Saigal(1974), Jain (1998), Rathod *et al*.,(2008) and Paliwal *et al*.,(2013) have made valuable contribution in the study of ichthyofauna.

Study site: Purkabodi Lake ($20^0$59'38" N, $79^0$47'35" E) was constructed as a part of irrigation project by government of Maharashtra and situated in the periphery of 5 to 10 km of Lakhani.

METHODOLOGY

To study the ichthyofaunal diversity of Purkabodi lake, the specimens were collected from local fishermen during the time of fishing. Collected samples were brought to laboratory washed, cleaned, observed and identified up to species by following the literature of Day (1878), Talwar and Jhingran (1991), Jayaram (1999), and Vishwanath *et al* (2011). Samples are preserved in 10% formaldehyde. Fishes were identified following their general body form, morphometric and meristic characteristics using above literature.

RESULT AND DISCUSSION

In the present investigation 23 species of fishes were recorded. The data was tabulated in following table.1.

These 23 species were belonging to 7 orders and 13 families. The order cypriniformes was dominant with 8 species followed by siluriformes with 6 species, perciformes 3, ophiocephaliformes 3 and each one of clupiformes, synbranchiformes and beloniformes. Similar observations were earlier made by Sakhare and Joshi (2002) in Palas-Nilegaon reservoir of Osmanabad district, Maharashtra. They reported 28 fish species. Shedge (2007) reported 24 species of fish in Nira river of Pune dist. of Maharashtra. Paliwal *et al*., (2013) recorded 35 species in Itiadoh reservoir. Londhe and Sathe (2015), and Thakre *et al*,. (2016) also reported similar results. Paritha Bhanu and Deepak (2015) concluded that mainly human interference in lakes and rivers were responsible for the less distribution of fishes. Pollution and intense hot climatic conditions affected the growth and distribution of fishes. Certain adaptations are developed in fish species due to pollution.

Fig. 1: Fishfauna in Purkabodi Lake A. *Tillapia* B. *Clarius batrachus* **C**. *Catla catla* **D.** *Labeo rohita* **E,** *Mystus cavasius* **F.** *Labeo calbasu* **G.** *Ophiocephalus punctatus* **H**. *Heteropneustes fossilis* **I**. *Lepidocephalus guntea* **J.** *Puntius sophor* **K.** *Ompok pabda* **L.** *Notopterus.*

Table: 1. Ichthyofaunal diversity in Purkabodi lake during 2013-14

SN	Order	Family	Scientific Name
1	Cypriniformes	Cyprinidae	*Catla catla*
2	Cypriniformes	Cyprinidae	*Labeo rohita*
3	Cypriniformes	Cyprinidae	*Labeo calbasu*
4	Cypriniformes	Cyprinidae	*Cirrhinus mrigala*
5	Cypriniformes	Cyprinidae	*Cyprinus carpio*
6	Cypriniformes	Cyprinidae	*Rasbora rasbora*
7	Cypriniformes	Cyprinidae	*Puntius sophor*
8	Cypriniformes	cobitidae	*Lepidocephalus guntea*
9	Siluriformes	Bagridae	*Mystus vitatus*
10	Siluriformes	Bagridae	*Mystus seenghala*
11	Siluriformes	Siluridae	*Ompok pabda*
12	Siluriformes	Siluridae	*Wallago attu*
13	Siluriformes	Heteropneustidae	*Heteropneustus fossilis*
14	Siluriformes	Clariidae	*Clarias batracus*
15	Perciformes	Nandidae	*Nandus nandus*
16	Perciformes	Cichlidae	*Tilapia mossambica*
17	Perciformes	Anabantidae	*Anabus testudineus*
18	Clupiformes	Notopteridae	*Notopterus chitala*
19	Ophiocephaliformes	Channidae	*Ophiocephalus punctatus*
20	Ophiocephaliformes	Channidae	*Ophiocephalus striatus*
21	Ophiocephaliformes	Channidae	*Ophiocephalus murulius*
22	Synbranchiformes	Mastacembelidae	*Mustacembelus armatus*
23	Beloniformes	Belonidae	*Xenentodon cancila*

Fish fauna having less adaptive capability was going on the way of scrub down and fishes having more adaptive capability are more in quantity and show the dominancy. Agricultural runoff containing harmful chemicals, pesticide and insecticides mix into the lake and harm the fish fauna. Fish species were important indicators of ecological health. The abundance and health of fish showed the health of water bodies (Hamzah, 2007). Present study helps to study and conserve the diversity of fish fauna. To conserve the diversity the fishery authorities should investigate and practice the proper exploitation and management of this fishery resources according to ecological principals. Scientific methods of fishing and practice should help to conserve the valuable biodiversity and the health of water body.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:283-285
UGC Approved Journal No 48951

Original Article Open Access

Analysing human trait from population of Nagbhid, based on Hardy- Weinberg's Principle

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Rudey RJ (2018) Analysing human trait from population of Nagbhid, based on Hardy- Weinberg's Principle, *Int. J. of. Life Sciences*, Special Issue, A12: 283-285.

ABSTRACT

Hardy-Weinberg states that in random mating population an equilibrium is established between allelic frequency and remains unaltered from one generation to the next regardless of their dominant and recessive relationship if mutation, migration, selection, genetic drift does not act on it. In present study total sample of 289 individuals, aging from 18 to 79 years were surveyed from Nagbhid area. Those were the students of the college, their parents, relatives and other people from Nagbhid area to study the morphological trait of length of index finger in order to discourse Hardy- Weinberg law. As this is sex influenced character, males and females were analyzed separately to see whether it follows Hardy-Weinberg Principle or not. In the sample of 119 males, 80 showed dominant character and 39 males were recessive. In the sample of 170 females 136 were found recessive. And it was observed that both the sexes show very high degree of deviation than expected by application of Chi- Square test. Hence it was established that certain forces like nonrandom mating, mutation migration selection have great effect.

Keywords: Hardy-Weinberg principle, Allelic frequency, genetic drift, mutation, Chi Square test.

INTRODUCTION

Evolution can be defined as changes in gene frequency within the population. The mathematical model that explains change in gene frequency in population i.e. evolution was provided by Godfrey Hardy and Wilhelm Weinberg in 1908. They specified a relationship between genotype frequency and allele frequency in their principle which states that 'in random mating population equilibrium between frequencies of allele is established in one generation and remain unchanged in successive generations regardless of their dominant and recessive relationship if factors like mutation, migration, selection, genetic drift do not act on it'. The mathematical model of Hardy and Weinberg is based on some assumptions that there should be large population size, random mating, isolation, no gene mutation, segregation of alleles according to

Mendel's law of segregation, no selection pressure and there should be normal meiosis. Model can be easily applied to two allele system. When population show deviation from Hardy and Weinberg's principle means population may be randomly mating (Wakeley *et al*., 2016).

In present study one morphological character length of index finger compared to ring fingr was analyzed. According to Phelps (1952) relative index finger length is sex influenced trait in man, According to Mcdonald *et al*. (2007) short length of index finger compared to ring finger is dominant trait in male and short length of index finger is recessive trait in female. Present study is focused on distribution of genes and gene frequency of Nagbhid population and how it leads to evolution.

Aim of the present study is to see whether the population of Nagbhid follows Hardy-Weinberg's principle or not.

METHODOLOGY

Present study is based on survey method. (Kohli *et al*.2015) Data is collected by analyzing the length of index finger which is genetically transmitted, autosomal, sex influenced trait of students from Rashtrapita Mahatma Gandhi (R.M.G.) college Nagbhid, Chandrapur, Maharashtra, India, their parents, relatives and other people from Nagbhid. Data was collected in the month of August 2017. Permission of the Principal of R.M.G. college was taken and conscent of the parents, relatives and people surveyed was also taken.

Nagbhid is a place located at longitude of 79.67 and latitude of 20.57 Data include 289 adults of age ranging from 18 – 79 years. Out of these 289 individuals males are 119 and females are 170 (Joshi 2008). Data was collected by direct measurements of fingers from mid point of each proximal crease to tip of the finger (L. Gillam *et al*.2008) by using a scale. Also outline of hands was drawn on paper. Horizontal line was drawn from index finger to ring finger and length of index finger was confirmed.

RESULTS AND DISCUSSION

In present study sample is collected from students of R.M.G. College Nagbhid, their parents, relatives and other people of Nagbhid. Data of 289 individuals included 119 males and 170 females.

Females: out of 170 females 34 showed long index finger which indicates presence of dominant gene for length of finger in them. And 136 females showed short length of index finger compared to ring finger which indicates presence of recessive allele for length of finger in them.

In present study for same genetically transmitted, sex influenced, morphological character 119 males were analyzed. Out of 119 males 80 males showed short length of index finger which indicates that these males have dominant alleles for length of finger and 39 males showed long index finger which indicates presence of recessive alleles for length of finger.

To find out in observed and expected allele frequency values, 'P' value and Chi square test was applied (Engels 2009). Hardy-Weinbergs Principle in Algebric terms: - P2+2pq+q2=1 for genotype frequency.

P + q=1 for gene frequency.
P ---- Gene frequency for dominant allele.
P2 ---- Genotype frequency for dominant allele.
q ----- Gene frequency of recessive allele.
q 2----- Genotype frequency of recessive alleles.
Pq ----- genotype frequency of heterozygous.

Table 1: The distribution of morphological character among female and male individuals during survey.

Sr.No.	Sex	Dominant trait long index finger	Recessive trait short index finger
1	Female	34	136
2	Male	80	39

Table 2: The gene frequencies and genotype frequencies of female & male individuals, (N= 170 & N=119).

Sr.No.	Sex	Trait	P	q	p2	2pq	q 2
1	Female	Length of index finger	0.11	0.89	0.12	0.20	0.8
2	Male	Length of index finger	0.43	0.57	0.18	0.4	0.8

For females :

The data collected included 170 females. Out of them 34 had a long index finger and 136 females showed short index finger compared to ring finger. Their gene frequencies and genotype frequencies were calcutated.

Allele frequencies were calculated by using formulae : Frequency of p = p2 + ½(2pq) and frequency of q = 1 – p

Expected number of individuals were calculated and it was found that observed values differ from expected values. Chi square was applied and calculations were done to find out whether the values are significant or not.

The data collected included 119 males out of which 80 males showed dominant trait i.e. they showed short length of index finger and 39 males showed long index finger compared to ring finger meaning presence of recessive trait for length of index finger. Their gene frequencies and gene frequencies were found out.

Chi square method:-

$X^2 = \sum(Xo - Xe)^2 / Xe$

X^2 =96.2 For females

X^2 = 3.83 For males

From above values it is concluded that, there is a great significant difference between observed and expected values under Hardy- Weinberg law. Population of Nagbhid is showing great deviation from hypothesis and makes to conclude that population is not in equilibrium for genes of length of index finger.

CONCLUSION

Among the data collected from students of R.M.G. college Nagbhid district Chandrapur Maharashtra, India, their parents, relatives and other people of Nagbhid , it was found after application of Chi square method that there is a great degree of deviation than expected. The degree of deviation is so high that, it cannot be only due to chance only but certainly due to some forces like mutation, selection, migration, genetic drift etc. have great effect on the population.

Acknowledgements:

Author is thankful to Principal R.M.G. arts and science college Nagbhid for permission to collect data from students of college. Author is also thankful to Ms. Sharwari Panse, Ms. Aroona Ingole and Rima Mendhe for their great help during collection of data.

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International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:286-288
UGC Approved Journal No 48951

Original Article Open Access

Diversity of Benthic fauna in freshwater lakes of Pombhurna Tehsil of Chandrapur District, MS, India

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Pimpalshende Atul K and Sitre Shashikant R (2018) Diversity of Benthic fauna in freshwater lakes of Pombhurna Tehsil of Chandrapur District, MS, India, *Int. J. of. Life Sciences*, Special Issue, A12: 286-288.

ABSTRACT

The present study was carried out on Satara Bhosale and Satara Tukum lake of Pombhurna tehsil in Chandrapur district(M.S.) to analyze the benthic fauna present in it. 25 different benthic macrovertebrate forms belonging to Annelida, Nematoda, Arthropoda and Mollusca were found in lakes. 17 species observed in Satara Tukum lake and 24 species observed in Satara Bhosale lake. The diversity of species of Satara Tukum Lake is less than Satara Bhosale lake indicating that Satara Bhosale lake harbors more forms and is quite rich in biodiversity as compared to Satara Tukum.

Keywords- Benthic fauna, Satara Bhosale, Satara Tukum, diversity.

INTRODUCTION

The structure of benthic macro invertebrate communities provides precise an local information on recent events (Marques *et al.*, 2003). The benthic macrofauna resides on or inside the deposit of bottom soil and feeds on organic debris. They play very unique role through recirculation of nutrients in aquatic environment of ponds and lakes by accelerating the breakdown of decaying organic matter into simpler inorganic forms (Idown and Ugwumba, 2005). They also serve as food source for many form of fishes.

Literature Survey reveals that several studies were reported with respect to aquatic benthic diversity and water, sediment with physic-chemical status of the aquatic ecosystem (Wang *et al.* 2010, Jana and Manna 1995, Quasin *et al.* 2009, Garg *et al.* 2009). Literature review clearly shows that an in adequate information on the benthic form of water bodies of pombhurna tehsil of chandrapur district of Maharashtra state. From this point and view observations on the two freshwater lakes Satara Bhosale and Satara Tukum in relation to their habitat was studied and presented in this research paper.

METHODOLOGY

The benthic fauna sample were collected for qualitative estimation from both the lakes. The collection of mud sample was done with the help of scoop. The collected sample was further sieved with the help of copper sieve having mesh. Macro benthic invertebrates obtained after sieving were preserved in 4% formalin for further laboratory studies and identification. Benthic forms were observed under the dissecting microscope and classified into different species.

Study area

A) Satara Bhosale Lake- Satara Bhosale village is 16 km away from pombhurna tehsil and 27 km away from chandrapur. The lake is about 194 m above mean sea level and is at 19°89'56.63' N latitude and 79°62'98.79' E longitude (Fig.1).

Fig.2: Satellite image of Satara Bhosale lake

Fig.2: Satellite image of Satara Tukum lake

Satara Bhosale Lake receives the water from the surrounding catchment areas during the monsoon period. The area of Satara Bhosale is spread over 36 acres . The water depth of Satara Bhosale is 18 feet during monsoon and 6 feet during summer season. The water of this lake is primarily used for Cloth washing, bathing, fishing activities, agriculture, animals drinking and other domestic purposes.

B) Satara Tukum Lake: Satara Tukum village is 15 km away from pombhurna and 25 km away from chandrapur. The lake is freshwater in origin and about 194 m above mean sea level and is at 19°89'54.63' N latitude and 79°62'94.79' E longitude (Fig. 2). Satara Tukum receives the water from the surrounding catchment areas during the monsoon period. The area of Satara Tukum is spread over 34 acres. The water depth of Satara Tukum is 17 feet during the monsoon and 7 feet during the summer season. The water of this lake is primarily used for Cloth washing, bathing, fishing activities, agriculture and animal drinking and other domestic purposes.

RESULT AND DISCUSSION

During the study period total 25 species of macro benthic invertebrates belonging to four different phylum like molluscas, annelids, arthropoda and nematoda were recorded.

Table 1: Benthic forms recorded in Satara Bhosale and Satara Tukum lake during 2016-17

SN	CLASS/ PHYLUM	SPECIES	S.B	S.T.
1	Nematoda	*Diplogaster factor*	-	+
2	Nematoda	*Rhabditis sp.*	+	+
3	Nematoda	*Paradoxorhabitis sp.*	+	+
4	Annelida	*Pheretims posthuma*	+	+
5	Annelida	*Hirudinaria granulose*	-	+
6	Oligocheta/ Annelida	*Aeolosoma sp.*	+	+
7	Oligocheta/Annelida	*Tubifex sp.*	+	+
8	Oligocheta/Annelida	*Pterobdella sp.*	+	+
9	Oligocheta/Annelida	*Chaetogaster*	-	+
10	Celeoptera	*Dinecutus sp.*	-	+
11	Crustacea/Arthropoda	*Cancer*	+	+
12	Diptera/Arthropoda	*Mosquito larva*	+	+
13	Odonata/Arthropoda	*Dragon-fly*	+	+
14	Hydrachnidia/Arthropoda	*Water mite*	+	+
15	Hydrophilidae/Arthropoda	*Cybister sp.*	-	+
16	Diptera/Arthopoda	*Chironomous larva*	-	+
17	Hemiptera/Arthopoda	*Belostoma sp.*	+	+
18	Hemiptera/Arthopoda	*Nepa sp.*	+	+
19	Hemiptera/Arthopoda	*Ranatra sp*	+	+
20	Gastropda/Mollusca	*Lymnaea sp.*	-	+
21	Gastropda/Mollusca	*Pila globosa*	+	+
22	Mollusca	*Planorbis exustus*	-	+
23	Mollusca	*Vivipera bengalensis*	+	+
24	Mollusca	*Indonia coerulea*	+	+
25	Mollusca	*Melania scabra*	+	+

In Satara Bhosale lake 17 and Satara Tukum lake 25 species were recorded. Macro benthic fauna as bio-indicator of water quality in Kishore sagar lake, Kota(Rajasthan) India. International Environment committee 13th conference paper (wuhan).

Aknowledgement

The authors are thankful to Dr. L.S. Ladke, Principal, N.S. Science and Arts college, Bhadrawati for permitting and providing necessary laboratory facilities for conducting this research work in CHLR in Zoology affiliated to Gondwana University, Gadchiroli.

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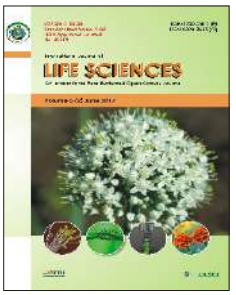

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