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Dr. Sujata H. Shende
Department of Botany, Shivramji Moghe mahavidhya Pandharkawada.

Abstract

Most of the population Pandharkawada depends on bore well for drinking purpose and present investigation was undertaking to study the fluoride in these resources the water samples of bore well in Green Park Colony area in Pandharkawada City were collected before and after rainy season fluoride and other parameter were analyzed by standard methods. Fluoride concentration was found to be ranging between 1.78mg/L- 1.98 mg/L before are after rainy season the result of study indicate that ground water quality in the study area is suitable for drinking respect to fluoride it being beyond permissible limit.

Key words – Fluorides, Groundwater Pandharkawada city.

Introduction

Fluoride is widely dispersed in nature and is a common constituent of most Soil,rockes,plants and animal it forms only Fluoride and no other oxidation stabs are found (Hem 1992). High Percentage of fluoride causes toxic effect concentration of fluoride between 0.6 to 1.0 mg / L in water. tooth decay and enhances brain development. In India drinking water standards have been suggested permissible limit of fluoride in drinking water 1.0 mg /L which is lower than the maximum loleance limit (1.5 mg /L) Fluoride ions have dual signification in water supplies.high percentage of fluoride causes dental fluorosis person concentration less than 0.6 ppm result in dental caries and dental (Mothing Rao & Venkateshwarulu 2000) .Hence it is essention to maintain fluoride concentration between 0.6 to 1.2 ppm (ISI 1983 & WHO 1994.)Present investigation was study Fluorids icon concentration of Bore Well water samples of Green Park colony Pandharkawada in Yavtmal District.

Material & Methods

The Bore Well Water samples were collected from the study during Jun and November 2018. The bore well water samples were analyzed for there Physico – chemical properties in water.

Bore Well water samples ware collected in Bislary plastic bottle 1 liter samples were collected from Bor well after running them for 15 min so as to avoid errors due to contained with in the pipe.

The analysis of bore well water samples was done for parameter like PH, TDS,Turbidity Alkalinity. Total Hundreds calcium chloride, fluoride and Nitrate adopting Tal Nephelo turbidity meter model 132 of systronics, measured turbidity,Total hardness, calcium ,chloride etc were estimated by titrimetric method and spectrophotometer method were employed determine fluoride concentration.

Result and Discussion

Bore Well water samples examined are given in Tabctal-1 turbidity, PH, TDS,Alkalinity total hundreds, Calcium, Chloride , Nitrates have been found to be with in permission limit with reference to the ISI-1991 Standards.

Fluorides

In the present study the fluoride concentration was found to be 1.78 mg/l before rainy season and 1.98mg/l after rainy season. The is acceptable limit. For fluoride is ---1_1.5 mg / L. phosphoric Fertilizers like super phosphate and rock phosphate being extensivety used in India. Various sources of fluoride, enterning the humen being are drinking water, food air industrial exposure ,drugs, cosmetics, toothpaste and mouth rinses (Khoshoo- 1989).Excess an percentage of fluoride causes dental, skelrtal and non skeletal fluorosis has been consider as one of the incurable diseases. Hence Prevention is only solution for the disease (Hem 1985).

Table : Water quality data of the Physico-chemical parameter of study area

Sr.No	Physico-Chemical Parameters	June	Nove	ISI 1991	
				Desirable Limit	Max Limit
1	PH	7.8	7.52	6.5	8.0
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Inverse relationship between fluoride and HCo3 is in deeper (CO3+2) and (OH) are demmated complain to HCO3 the sources of Fluoride in the groundwater of this area to be these OH mineral in present area. High fluoride percentage occur in Bore well water.

Conclusion

The area are under study contain fluoride above permissible limit. Prescribe by ISI 1991 the Study suggested that area of fluoride concentration is high so there is need to artificial recharge for creation of same drinking water source.

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- **APHA** (1995) Standard method for examination of water and waste water : 18th Edition, AWWA,WPCF,Washingtone DC.
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- **Khoshoo T.N. (1989)** Integral approach to fluoride pollution in J. Mohan (ed) environment and pollution management . Ashish publishing house New Dilhi -80-82
- **Rao and Venkateshvarlu** (2000) Physico-chemical characterstics of under ground water in Nagercoil Town (South). IJEP 24 (i) 53-56.
- **WHO 1994** Gaudline of drinking water quality vol II Recommendation. 2nd Edition. World Helth Organizarion Geneva p. 192.

2. Pharmacognostical and Phytochemical Evaluations of Thespesia populnea (L.) Soland EX Corr

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Abstract

Pharmacognostic studies of Thespesia populnea (L.) Soland ex Corr. were carried out in order to establish parameters for its identification and to check adulteration by other species of Thespesia. Morphological characteristic of the plant and its anatomical parts (leaf and stem) were studied by organoleptic evaluation. Powder drug study of the plant parts were also carried out and various structures of the powder drug were also observed. This study also includes quantitative leaf microscopy, extractive values and quantitative data. The results of this study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of this plant.

Key words: Thespesia populnea, Pharmacognosy and Phytochemistry.

Introduction

Plants are the only economic source of number of well established and important drug. Pharmacognostist today also continue to work in a direction of their procedures in a establishing standard whereby quality of commercial plant materials can maintained (Mathappan et al., 2010). Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy (Chopra et al., 1956). As late as the beginning of present century, pharmacognosy had developed mainly on the botanical side being particularly concerned with the description and identification of drug both in their whole state and in powder form. This technique can be established for the correct botanical identification of plant (Kokate et al., 2005).

Thespesia populnea (L) Soland ex. Corr. Commonly called as 'Palas Pimpal' belongs to family Malvaceae. This plant is having much ethnomedicinal importance. They are used in the treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaint, antioxidant, anti-implantation, anti-inflammatory activity, analgesic and

antipyretic properties, antioxidant, anti-diabetic activity, and anthelmintic (Vasudevan et. al., 2006; Vasudevan and Parle, 2006 and Shrivastava, 2009). So, the present work thus attempts to analyze the wide potential traditional plant Thespesia populnea (L.) Soland ex Corr. which includes the standardization of crude materials with the use of different modern techniques based on the available literature and knowledge.

Materials and Methods

Collection and identification of plant material: The selected plant for the study i.e. Thespesia populnea (L.) Soland ex Corr. was collected during the period of flowering and fruiting from Arni region during November 2017. The herbarium specimens of selected plant was prepared, identified with the help of standard floras (Naik, 1998; Almeida, 2001; Singh and Karthikeyan, 2001) and the voucher specimen was deposited in Department of Botany, Bharti Mahavidyalaya Arni, district Yavatmal (MS) India.

Pharmacognostical studies: The organoleptic evaluation, anatomical study, powder microscopy, stomatal study, extractive values and chemical analysis were carried out by using standard methods mentioned in (Pratt and Chase, 1949; Anonymous, 1966; Mukharjee, 2002; Trease & Evans, 2002; Kokate et. al., 2005).

Qualitative and quantitative phytochemical analysis: It involves testing of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug (Wallis, 1990; Harborne, 1998; Kokate, 2005; Sadashivan and Manickam, 2005). The extracts were analyzed for the presence of phytoconstituents like carbohydrates, cardiac glycosides, alkaloids, flavonoids, tannin, phenolics, steroids, coumarins and saponin. The crude quantifications of major phytochemicals were done using standard method (Mukharjee, 2002). Each sample was analyzed in triplicates. Only Alkaloids, flavonoids and tannin from the plant under study were quantified.

Results and Discussion

The pharmacognostical study is the major and reliable criteria for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostical evaluation of Thespesia populnea (L) Soland ex Corr would give valuable information for the future studies.

Oganoleptic evaluations: The organoleptic evaluations of Thespesia populnea (L) .Soland ex. Corr. shown in table 1.

Table 1: Organoleptic evaluation of powder of T. populnea

S.N.	Particulars	Plant parts	
		Leaves	Stem
1	Colour of Powder	Dark green	Cream
2	Odour	Characteristics	Mild
3	Taste	Astringent	Bitter
4	Texture	Smooth	Rough

Anatomical study

This study enables us to give a picture of all tissue distribution in the plant under study.

T.S of leaf: The T. S. of leaf passing through the midrib projects strongly at the lower side and elevated at the upper side and lamina is dorsiventral. The leaf has prominent and thick abaxial midrib and lateral veins. The lamina is thin with wide shallow glandular pits. It consists of a short, semi-circular adaxial part and wide, thick abaxial .The epidermis is thin and continuous comprising small, thick walled squarish cells. The leaf shows peeling of the lamina showing abaxial epidermis- with stomata. The leaf shows the presence of glandular trichomes which are peltate type, epidermis lies a layer of palisade cells, the remaining cells of the mesophyll are consisting of 3 to 4 rows of spongy parenchyma traversed with obliquely cut vascular bundle.

T.S of Stem: The T. S. of mature stem shows that cork composed of 7 - 11 layers of cells, which are stratified due to alternate arrangement of 3 layers of large cells. Resin canals are distributed throughout the cortex. The secondary phloem is wide and occupies the greater portion. A tertiary cambium arises in the secondary phloem and gives rise to tertiary phloem and tertiary xylem strands. The xylem vessels are drum shaped with well marked perforation rims. A few vessels are long and cylindrical. They have all bordered pits on the walls. The tracheids are longer than the vessels. These also have bordered pits on the walls and there are no end wall opening. The xylem fibres are long with pointed tapering ends and short lumen. They are however, shorter and narrower as compared to the pericyclic fibres which have pointed ends.

Powder microscopy: The leaf powder of Thespesia populnea (L.) Soland ex Corr. was containing epidermal cell with stomata, trichome, cortical cells and spongy parenchyma cells.

While in stem powder, Cork cell, collenchymatous cells, sclerenchymatous cells, pitted vessels and tracheids were found.

Stomatal Study: Quantitative microscopical study also yielded valuable information regarding specific leaf constant. Stomata frequency is one of the most widely used characters in taxonomy and pharmacognosy. Mostly anisocytic type of stomata is found. In upper surface of leaf, stomata number is higher than the lower surface (table - 2).

Table 2: Quantitative leaf microscopic analysis of T. populnea

S. N.	Parameters		Range	Mean ± SD
1	Stomatal Number	lower surface	20 - 22	21±0.06
		upper surface	12 - 18	15.7±0.12
2	Stomatal Index	lower surface	28.2 – 32.2	30.2±1.04
		upper surface	22.8 – 24.2	23.38±0.54

Extractive values: The extractive values of the drugs are an important parameter for detecting adulteration in the drugs. However, on the basis of polarity of solvents, successive solvent extractive values of Thespesia populnea (L) Soland in various organic solvents was observed as shown in (table 3). The extractive values determine the amount of active constituents in given amount of crude plant material extracted with respective solvent (Mukharjee, 2002). The extraction of any crude drug with particular solvent yields a solution containing different phytoconstituents in that particular solvent depends upon the nature of drug and solvent used which reflect the extractive values of crude drugs. The maximum extractive value observed in more polar solvent. T. populnea leaf showed higher extractive values than stem. The extractive value of crude powder was maximum in water followed by ethanol and chloroform and minimum in petroleum ether and benzene.

Table 3: Extractive values of Thespesia populnea (L) Soland ex. Corr.

S.N.	Parameter studied	Leaf (% w/w)	Stem (% w/w)
1	P. Ether	1.08%	1.21%

2	Benzene	4.12%	3.91%
3	Chloroform	11.05%	9.14%
4	Acetone	9.2%	8.68%
5	Ethanol	10.82%	10.8%
6	Water	15.2%	13.24%

Chemical behavioral analysis: The crude drug of various parts i.e. stem, leaf and petiole of T. populnea showed different colours when it was treated with various chemicals. The crude drugs consist of various phyto-constituents in different forms shows characteristic reactions with various reagents. This phenomenon is used for qualitative examination of crude drugs. This technique can be used for the standardization and detection of adulterant in crude drug (Wallis, 1990). The crude powder of plant under study reacts with various chemicals and behavioral characteristic of powder was observed as shown in (table-4).

Table 4: Behavioral characteristics of powder of T. populnea with different chemical reagent.

S.N.	Reaction	Stem	Leaf	Petiole
1	Powder as such	Pale brown	Green	Pale brown
2	Powder + Conc. H_2SO_4	Red	Orange Red	Red
3	Powder + Conc. HNO_3	Pale Yellow	Light green	Pale Yellow
4	Powder + Conc. HCl	No change	Pale Green	No change
5	Powder + 10% NaOH	No change	No change	No change
6	Powder + Iodine solution	No change	No change	No change
7	Powder + 5% $FeCl_3$	No change	Dark Green	No change
8	Powder + KI	Brown	Green	Brown

9	Powder+ Ethyl acetate	Brown	Green	Brown

Phytochemical analysis

Qualitative phytochemical screening: Phytochemicals are also known secondary metabolites of plants which play important role in herbal crud drugs. Biologically diverse active phyto-constituents present in the plant sample which were investigated by phytochemical screening of various plant parts like leaf and stem indicated the presence of different constituents as shown in Table 5.

The result of preliminary phytochemical screening of leaf in six different extracts i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water revealed the presence of carbohydrate, proteins, cardiac glycosides, tannins, alkaloids, flavonoids and phenolic compounds. The majority of phytoconstituents were found in ethanol and water extracts. In leaf, Steroids are present only in benzene and water extract. Saponin & Anthroquinones glycosides were totally absent in all the six extracts. Flavonoid was present in all the extract except petroleum ether and benzene. While, in stem showed that there were presence of carbohydrate, proteins, cardiac glycoside, tannins, alkaloids, Saponin, flavonoid and phenolic compounds. The majority of phytoconstituents were found in ethanol extracts. Steroids are present dominantly in the stem. Anthroquinones glycosides were totally absent in all the extracts (table- 5).

Table 5: Qualitative phytochemical screening of Leaf and stem of Thespesia populnea (L) Soland ex. Corr.

S. N.	Constituents	Chemical Tests	Extracts											
			Leaf						Stem					
			P	B	C	A	E	W	P	B	C	A	E	W
1	Alkaloids	Mayer's Test	+	+	+	+	+	+	--	--	+	+	+	+
		Dragendroff's	--	+	+	+	--	+	--	--	+	+	+	+
2	Carbohydrates & Glycosides	Fehling's Test	--	--	+	--	+	+	--	+	+	+	+	+
		Benedict's test	+	--	+	+	+	+	+	--	--	+	+	--
		Molisch's Test	--	+	+	+	+	+	--	--	+	+	+	+
3	Steroids	Salkowski Test	--	+	--	--	--	+	--	+	+	+	--	+
4	Saponin	Foam Test	--	--	--	--	--	--	--	--	+	+	+	--
5	Phenolics & Tannin	$FeCl_3$ Sol. Test	--	--	+	+	+	--	--	--	+	+	+	+
		Lead Acetate	+	--	--	+	+	+	+	--	+	+	+	-

6	Oil & Fats	Spot Test	--	--	+	+	+	--	--	+	--	+	+	--
7	Proteins	Biuret Test	--	--	--	+	+	--	--	--	+	--	+	+
		Million's Test	--	--	--	+	+	+	--	--	--	+	+	+
8	Anthraquinone glycosides	Borntrager's Tes	--	--	--	--	--	--	--	--	--	--	--	--
9	Cardiac glycosides	Keller-Killiani	--	+	--	--	+	+	+	-	+	+	+	+
10	Flavonoids	Lead Acetate	--	--	+	+	+	+	+	--	--	--	+	+

{Where, P= Petroleum ether, B= Benzene, C= Chloroform, A= Acetone, E= Ethanol and W= Water.}

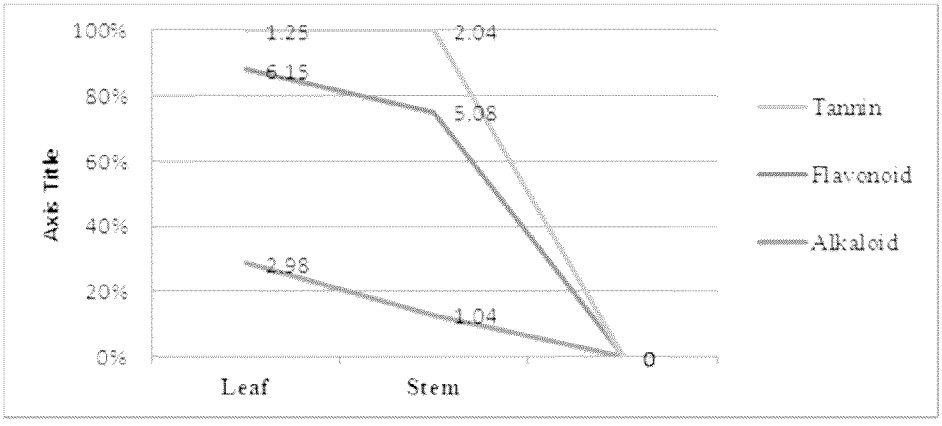
Quantitative phytochemical analysis: The secondary metabolites in plant under study were found in appreciable concentrations (Table-6).

Table 6: Quantitative phytochemical screening of T. populnea

S. N.	Phytochemical	Leaf (g/100g)	Stem (g/100g)
1	Alkaloid	2.98 ± 0.12	1.04 ± 0.30
2	Flavonoid	6.15 ± 0.10	5.08 ± 0.60
3	Tannin	1.25 ± 0.26	2.04 ± 0.04

Where, results are depicted as mean ± SD of three determinants.

The quantification of secondary metabolites like alkaloids, flavonoids and tannin in T. populnea leaf was showed percentage of alkaloids 2.98 ± 0.12g/100g, flavonoids 6.15 ± 0.10g/100g and tannin is 1.25 ± 0.26g/100g. While stem was showed percentage of alkaloids 1.04 ± 0.30g/100g, flavonoids 5.08 ± 0.60g/100g and tannin 2.04 ± 0.04g/100g.



Graph-1: Quantitative phytochemical screening of T. populnea

Highest quantity of alkaloids was recorded in the leaves. However the stem constituted comparatively less amount of alkaloid. Out of all these three quantified phytochemicals, concentration of flavonoids was higher than the alkaloids and tannin. Tannin was present in highest amount in stem than in leaf. Quantitative analysis of plant showed maximum quantity of flavonoids and alkaloids in leaf and minimum in stem.

Conclusion

A Pharmacognostic study on T. populnea has brought to light certain microscopic features as well as preliminary phytochemical data of diagnostic value. Anatomy of the leaves is helpful for identification of fragmentary samples. Collective microscopical data of leaves have proved to be simple technique of identification. Quantitative microscopic data such as stomatal index have been highly relied upon by pioneer pharmacognocists. It is believed that these features are constant for given species and can be employed for inter specific identity of drugs. Physicochemical constants such as successive extractive values of the drug are corroborative evidences in drug standardization. Chemical behavioral analysis of the drug powder as well as drug extract is other test for standardization of the drug. Thus, the anatomical characters coupled with preliminary phytochemical results are specific for the identification of the commonly used and medicinally potent drug T. populnea. Qualitative and quantitative parameters that can serve as an important possible source of information for the identity and to determine the quality and purity of the plant material. These information will also be helpful to differentiate Thespesia populnea from the closely related other species and varieties of Thespesia. Also, this work could be useful for the adulterants resolution of doubtful materials of T. populnea and compilation of a suitable monograph for its proper identification.

References

- Almeida, M.R (2001). Flora of Maharashtra. Vol. III B, Orient Press Mumbai.
- Anonymous (1966). The Indian Pharmacopoeia, 2nd Edn., Government of India Publication, New Delhi.
- Anonymous (1989). Useful plants in India. Publications and Information Directorate, CSIR, New Delhi.
- Chopra, R.N., Nair, S.L. and Chopra, I.C. (1956). Supplements of glossary of Indian medicinal plants. CSIR Publication, New Delhi.
- Harborne, J.B. (1998). Phytochemical Methods. 3rd Edn., Chapman & Hall Publication, London.

- Kamble, SY; Patil, S.R.; Sawant, P.S.; Pawar S.G. and Singh E.A. (2010). Studies on plant used in traditional medicines by Bhilla tribe of Maharashtra. Ind. J. Trad. Know. 9(3):591-598.
- Khare, P.C. (2007). Indian Medicinal Plants. An Illustrated Dictionary Springer - Verlag Berlin/Heidelberg publication, p. p. 60.
- Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. (2005). Pharmacognosy, Nirali Prakashan, Pune.
- Mathappan, R.; Felix, V.; Prasanath, V.V. and Varirappan, K. (2010) Pharmacognostical and preliminary phytochemical studies of Urena lobata linn. Int. J. Phytomed., 2:408-411.
- Mukharjee, P.K. (2002). Quality control of herbal drugs: An approach to evaluation of Botanicals. Business horizon pharmaceuticals Publishers.
- Naik, V.N. (1998). Flora of Marathwada. Vol. I & II, Amrut Prakashan, Aurangabad.
- Pratt, R.J. and Chase, C.R. (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Pharm Assoc., 38: 324-333.
- Sadashivan, S. and Manickam, A. (2005). Biochemical Methods. 2nd Edn., New Age International (P) Ltd., Publisher, New Delhi.
- Shrivastava, S. (2009). Anti-psoriatic and phytochemical evaluation of Thespesia populnea bark extract. Int. J. Pharm. & Pharmc. Sci., 1(1):176-185.
- Singh, N.P.; Lakshminarasimhan, P.; Kartikeyan, S. and Prasanna P.V. (2001) Flora of Maharashtra State. Vol. II, Botanical Survey of India, Calcutta.
- Trease, G.E. and Evans, W.E. (2002). Phamacognosy, 15th Edn., W. B. Saunders Company Limited, London.
- Vasudevan, M. and Parle, M. (2006). Pharmacological actions of Thespesia populnea relevant to Alzheimer's disease. Phytomed., 13:677-687.
- Vasudevan, M.; Gunnam, K.K. and Parle, M. (2006). Antinociceptive and anti-inflammatory effects of Thespesia populnea bark extract. J. Ethnopharmacol., 109:264-270.
- Wallis, T.E. (1990). Text Book of Pharmacognosy. 5th Edn., CBS Publishers & Distributors, New Delhi.

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Conclusion

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- **Indian Standard Institution (ISI)** (1983) Indian standard specification for drinking water IS:10500.
- **Khoshoo T.N. (1989)** Integral approach to fluoride pollution in J. Mohan (ed) environment and pollution management . Ashish publishing house New Dilhi -80-82
- **Rao and Venkateshvarlu** (2000) Physico-chemical characterstics of under ground water in Nagercoil Town (South). IJEP 24 (i) 53-56.
- **WHO 1994** Gaudline of drinking water quality vol II Recommendation. 2nd Edition. World Helth Organizarion Geneva p. 192.

4. Algal Diversity in Kelapur Tahsil - A Case Study

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Introduction

Biodiversity is intimately related to the function and stability of communities and Ecosystem biodiversity play a vital role from many point of view. It is very much important, as it provides the basic material for food . India lying at the Junction of tropical , Eurasian and Indo Malayan biogeographic realms is among the twelve " Mega biodiversity countries in the world. India has a very rich diversity of flora and fauna . Its share of the global biodiversity is about 8.6% wild flora and faunal species Respectively. In India out of this 8.6% of flora and fauna algae occupies about 2.3% because of onely very suitable atmospheric Condition . They occur in a wide range of habitats and have been distributed all over land and water system. Thus They play a fundamental role in the worlds Ecosystem and Reliable and Modern introduction to their Kaleidoscopic diversity. The Present Study was taken with the objective to have an assessment on its diverse composition of Kelapur Tahsil. This paper highlights the different class, order family and genera in an around the Kelapur Tahsil of Yavatmal district. "

Key words :- Algae, Diveristy Keleidoscopic.

Materials and Methods

The Present study Conducted season wise of different water bodies in Kelapur tahisl of Yavatmal district (Maharashtra) India. Kelapur which is situated at Yavatmal district Maharashtra and its geographical coordinates are 190268.53108 North, $76^0$40'43.71273" east. The area of kelapour tehsil is of 675 sq.km. and including 105 villages. The Climate Condition of the area is generally dry. The Maximum and Minimum temperature recorded are in the order of 47^0c in summer or some time near about 48^0c and 9^0c in winter seasons. The algal sample will be collected from surface water sample of different water bodies in identified sample station of the different areas in Kelapur Tahsil. This collected algal sample will be preserved in 3-4% formalin at the fix sampling station of different water bodies, Then the sample was washed with

2-3% of acetic acid in order to clear the algal material from organic matter sand and silt particles. A common method of obtaining pure culture is serial dilution. A detailed study of algal identification it is use to staining technique with 1% iodin solution and obser under Research Microscope and compare with algal identification key of different author's like the blue green-Arnold Heinemann, Introduction to the algae-Harold. Bold , Michae. J. Wynne and The structure and reproduction of algae-F.E.fritch.

Obersveration and Result

Table – 1 List of Algal Species Identified in different Sample Station in Kelaput Tahsil

Class	Order	Family	Genera
Cyanophyceae	Chlorococcales	Chlorphyceae	Microcystis Kutz, Chlorcoococcus
	Nostocales	Ocillatoriaceae	Spirulina, Trichodesmium
		Nostocaceae	Anabaena, Nostoc, Trichormus
		Scytonematceae	Plectonema, Tolypothrix
		Revudariaceae	Calothrix
Chlorophyceae	Volvocales	Chlomydomonadacease	Chalmydomonas, Chloromonas
		Volvocaceae	Vilvax 1
		Hydrodictyaceae	Closteridium Hyderodictyon, Pediastrum
		Oocystaceae Utorichaceae	Chlorella ulotchrix kutz
	Cladophorales	Cladophoraceae	Cladophora kutz
	Oedogonales	Oedogonaiceae	Oedogonium
	Conjugales	Zygnemaceae	Spriogyra, Zygenma
		Desimidiaceae	Closterium, Cosmalium, Desmidium
	Siphonalses	Vauchariaceae	Vaourcheria DC
	Charales	Caraceae	Chara L Nitella
Xathophyceae	Heterosiphonales	Botrydiaceae	Botrydium
Eyglenophyceae	Euglenales	Euglenaceae	Eyglena
		Peranemaceae	Scytomonas
Phaeophyceae	Ectoarpales	Ecotcarpaceae	Ectocarpus
Rhodophyceae	Batrachospermales	Batrachospermoceae	Batrachospermum Lemanea
Bacillariophyceae	Bacillariales	Bacillariaceae	Bacillaria Denticula
Dinohyceae	Peridiniales	Glenodiniaceae	Glenodium

Cryptophyceae	Chattonellales	Vocuolariaceae	Hornellia

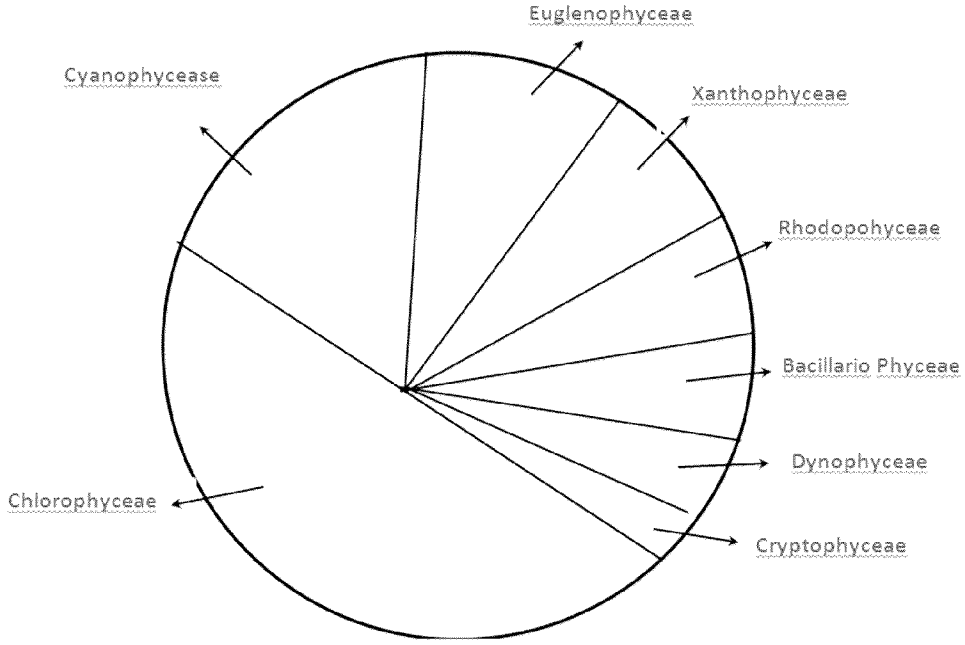

Fig. Percentage of different Phytoplankton in Kelapur Tahsil

Discussion

From the above Observation it was observed that the different water bodies in Kelapur Tahsil have a collection of different algal genera and classes. It was observed that genus belong to the class cyanophyceae with 10 Number of spices i.e., Micorcystis, Chlorocoococcus, Spirulina, Tricholdesmium, Anabaena, Nostc, Trichormus, Plectonema, Tolypothrix, Calothrix, Chalmydomonas, Chloromonas, Volvox, Cladophora , Oeadogonium, Sprigogyra, Zynema ,Closterium, Cosmarium, desmidium Vaucheria, Chlorella , Nitella, that belong to the genus euglena and scytomonas, that belong the class Euglenophyceae with 2 Number of Species and other classes i.e. Phaeophyceae, Rhodophyceae, Bacillariophyceae, Dinophyceae, Cryptohyceae with single number of spices.

Conclusion

From the above data is was concluded that the different water bodies in Kelapur Tahsil a diversified algal flora in which Clorphyceae Members were most dominant in a every sampling

station in a different season followed by cyanophyceae, Euglenophyceae, Xanthophyceae, Rhodophyceae, Bacillariophyceae, Dinophyceae, Cryptophyceae and phaeophycea.

Reference

1. Desikachary, T.V. 1986-1989 Allas of Diatoms Madras Science foundation Mardras.
2. Gupta, P. 2012, Algae of India Vol. I A.Checklist of Cyanoprokaryota (Cyanophyceae) Botanical Survey of India, Kolkata
3. Hazarika , D. and Baruklal, J. (2012) occurance and distribution of blue green algae in Paddy fileds of Golaghat Distrcit Assam, India.
4. Jafari, N.G. Gunale, V.R. (2006) J. Appl. Sci, Environ. Mgt. 10 (2): 153-158
5. Munnawar M, (1970) Hyderobiologia, 35 (1) : 127-162
6. Suxena, M.R. 1918 A Taxonomic and Systematic Survey of diatoms taxa and other synonyms describe from India and its adlacent countries Dept. of Botany Osmania Univeristy Hyderbad.
7. Suresh K. Dhameja- Environmental Studies (2005)
8. www.bsienvis.nic.in (Algae in India).

5. Anti - Cancer Properties of Oroxylum Indicum (L) Vent. - Review

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Abstract

Cancer is the one of the most common cause of mortality since years oroxylum indicum vent contain secondary metabolites which have anty cancer properties O. Indicum contain Baicalein, Chrysin, Oroxylin A. From traditional to recent studies baicalein show an anti cancer properties inducing opoptosis. In traditional medicine oroxylum indicum decotion cure Nasopharyngeal Cancer. In recent studies baicalein induce apapotosis in leukemia and breast cancer cells.

Key Words: Oroxylum Indicum, Baicalein, Nasopharyngeal Cancer, Leukemia, Breast Cancer.

Introducation

Oroxylum Indicum (L) Vent is a medium size tree belonging to family Bignoniaceae. It is distributed through out india in most deciduous to ever green forest upto an 1200 m altitude. Oroxylum indicum an endangered tree has great importance for its attributed medicinal properties. It has widely use in Ayurveda and other traditional system of Medicine. It is a well know member of *dasamula* group and ingredient of several important Ayurvedic fomulations used to treat various diseases. – *Worrier PK, Nambiar VPK (1995)*

Every Plant part contain Medicianal values, Steam, Root and Fruit has been used to cure ulcers, Vata, Morbid, Kapha, Improve Digation, Pharangial Cancer, Cardiac Dissorder, Brochintis. – *Worrier PK, Nambiar VPK (1995).*

Phytochemical Compounds Present in this species are baicalein, Oroxylin A, Chrysin and its derivative are isolated by *Chen LJ, Games DE (2003).* This bioactive Phytochemical Compounds have shown therapeutic potential in some areas such as anti-cancer, anti-inflammatory, antibacterial and anti-viral.

Jones J, Chen LJ, Games DE, (2001) Isolated baicalein (5,6,7 Trihydroxyflavonoid) are isolated from steam bark, root bark, fruit and seeds. Oroxylin A (5,6,7 Dihydroxy 6 Methoxyflavone) from steam and root. Chrysin (5,7 dihydroxyflavone from stem bark, root, seed.

Discussion

Oroxylum Indicum was used from since Ayurvedic Medine till today in many medicinal product. Bangladeshi folk medicine and Indian traditional Medicine this species is widely use to cure many disease. Which is the mean cause many researches where attracted towards this plants for its medicinal use.

In many recent reports many plant extract have been investigated for their possible therapeutic role against disease affecting human health. Effective plant extract are desired as a natural way to combat disease including cancer. Use medicinal plant as an approach in prevention and treatment of cancer is being followed since thousands of years.

Traditional Therapy for Nasopharyngeal Cancer

Vaidus of senapati district of Manipur state gives the medicine (decotion of steam bark) of oroxylum indicum for nasopharyngeal cancer patient (Noram Naga Villager) and cured by this docotion. – *A A Mao (2001)*. Dicotion prepare use in 1 kg of steam bark boiled in 5 liters of water for 30-40 minutes. The cup of this dicotion should give three times a day.

For Leukemia Cancer Cells

In *Malay K. Roy (2006)* in his study he showed that baicalein effectively inhibited the proliferiton of a leukemia cell line, HL-60 by demonstrating the role of baicalein in decreasing the number of cells, in the induction of apaptosis interfearing with cell cycle arrest at S and G 2 Phase of the cell cycle. The degradation of nuclear DNA into nucleosomal unites is one of the feature of apaptotic cell death. In his study he show that fragmentation of nucleosomal DNA appeared in cells treated with 10,20,40 μm**,** baicalein after 48 hours. TUNEL assay thus cancer as a high conformity the presence of fragmented oligo nucleosomal DNA within apapototic cell.

For Breast Cancer Cells- Oroxylum Indicum Non-polar extract where primarly selected for cytotoxicity to cancer cells. For this reason the evolution employed two cell lines a cancer cells and normal cells (MDA- MB 231 Human breast adnocarcinomal) and (WRL 68 normal cells). O indicum extracts where able to selectivity target cancer cells in its due course of cell inhibition- *D.R. Navin Kumar (2012)*

References

1. Gokhale M, Bansal YK. An avowal of importance of endangered tree Oroxylum indicum (Linn.). Vent Nat Prod Rad 2006; 5(2):112-114.
2. Warrier PK, Nambiar VPK, Ramamkutty C. Indian medicinal plants. Vol. 4, Orient Longman Pvt Ltd, Chennai, 1995, 186-190.

3. Sivarajan VV, Balachandran I. Ayurvedic drugs and their plant sources, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, 1994, 464-465.
4. Lambertini N, Diva R, Khan MTH, Lampronti I, Bianchi N, Borgatti M and Gambari R, Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor α gene. Inter. J Oncology 2004; 24, 419-423.
5. Lotufo CLV, Khan MTH, Ather A, Wilke DV, Jimenez PC, Moraes ME et al. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J Ethno pharmacol 2005; 99:21-30.
6. Khandhar M, Shah M, Santani D, Jain S. Antiulcer activity of the bark of Oroxylum indicum against experimental gastric ulcers. Pharmaceutical Bio 2006; 44(5):363-370.
7. Prasad G, Reshmi MV. A mannual of medicinal trees. Agrobios, Jodhpur, 2003, 1-32.
8. Rasadah MA, Houghton PJ, Amala R, Hoult JRS. Antimicrobial and anti-inflammatory activity of extracts and constituents of Oroxylum indicum Vent. Phytomedica 1998; 5:375-381.
9. Ravikumar K, Ved DK. Hundred red listed medicinal plants of conservation concern in Southern India. 1 st Ed, Foundation for Revitalisation of Local Health Traditions (FRLHT), Anugraha, and Bangalore, India. 2000, 195.
10. Rajasekharan PE, Ganeshan S. Biotechnology and conservation of medicinal plants in India. In (Eds.) Govil JN, Ananda Kumar P, Singh VK, Recent progress in medicinal plants, Vol. 4, Biotechnology and Genetic Engineering, Studium Press, LLC, USA, 2004, 1-19.
11. Subramaniam SS, Nair AGR. Flavonoids of the leaves of Oroxylum indicum and Pajanelia latifolia. Phytochem 1972; 11:439-440.
12. Subramanian SS, Nair AGR. Flavonoids of the stem bark of Oroxylum indicum. Curr Sci 1972; 41(2)62-63.
13. Chen LJ, Games DE, Jones J. Isolation and identification of four flavonoid constituents from seeds of Oroxylum indicum using by high-speed counter-current chromatography. J Chromatogr A 2003;

6. Antibacterial Activity of Some Medicinal Plants against S. Aureus

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Abstract

In present study we have selected *Justicia adhatoda*, *Catharanthus roseus* and *Aloe vera* for evaluation of their antibacterial activities against *Staphylococcus aureus*. Traditionally these plants are used in Siddha, Ayurvedic and Unani systems of medication. Due to their medicinal properties, extracts of these plants are now being included in beverages, skin lotions, cosmetics and pharmaceutical ointments. Maceration and infusion methods were used for the preparation of extract of the plants selected by us. The filtered extract from *Justicia adhatoda*, *Catharanthus roseus* and *Aloe vera* were used for evaluation of antibacterial activity against *Staphylococcus aureus* by agar well diffusion method. Maximum inhibition was shown by *Justicia adhatoda* followed by *Aloe vera* and *Catharanthus roseus* against *Staphylococcus aureus.* Therefore the extracts from these plants can be used to treat Staphylococcal infections.

Keywords: Antibacterial activity, *Justicia adhatoda*, *Catharanthus roseus, Aloe vera*

Introduction

Justicia adhatoda is a medicinal plant commonly known as adulsa**.** The leaves of *Justicia adhatoda* contain phytochemicals such as alkaloids (vasicine and quinazoline), tannins, saponins, phenolics and flavonoids [1]. Traditionally it is used in Siddha Medicine, Ayurvedic and Unani systems of medication. *Aloe vera* is stem less or having short stem plant from Asphodelaceae family. Due to its medicinal properties, it is now being included in beverages, skin lotions,

cosmetics and pharmaceutical ointments [2]. *Catharanthus roseus* is an important medicinal plant of family Apocynaceae. Alkaloids from this plant is known to have anticancer, antibacterial, antifungal, antidiabetic, and antiviral activities [3]. In present investigation we have selected three aforementioned medicinal plants for evaluation of their antibacterial activities against *Staphylococcus aureus*.

Materials and methods

Collection of plant materials

Leaves of *Justicia adhatoda*, *Catharanthus roseus* and *Aloe vera* were collected and air dried under shadow. The dried materials were pulverized and sieved individually to form the fine powder (Figure 1).

a. **b.** **c.**

Figure 1: Selected medicinal plants (a.: *Justicia adhatoda*, b.: *Catharanthus roseus* c. :*Aloe vera*)

Formulation of extract

Maceration and infusion methods were used for the preparation of extract. In this method, 1 gm of powdered plant material was added in 4 ml distilled water individually and allowed to stand at room temperature for 3 days with frequent agitation. The wet materials then macerated and heated in boiling water bath for 20 min. The mixture was then cooled and filtered. The filtrate was used for evaluation of its antibacterial activity [4].

Evaluation of antibacterial activity

The filtered extract from *Justicia adhatoda*, *Catharanthus roseus* and *Aloe vera* were used for evaluation of antibacterial activity against *Staphylococcus aureus* by agar well diffusion method. In this method, fresh culture of *Staphylococcus aureus* was spread on nutrient agar medium and wells were formed. Extract (100 µl) from *Justicia adhatoda*, *Catharanthus roseus*

and *Aloe vera* was added in well individually. The plates were incubated at 35°C for 24 h in an incubator [5].

Results and discussion

Extracts from *Justicia adhatoda*, *Catharanthus roseus* and *Aloe vera* have shown remarkable antibacterial activity against *Staphylococcus aureus*. The size of zone of growth inhibition of *Staphylococcus aureus* by these extracts has been given in Table 1. Maximum inhibition was shown by *Justicia adhatoda* followed by *Aloe vera* and *Catharanthus roseus* (Fig.2).

Most of the bacterial pathogens are developing resistance against many of the currently available anti microbial drugs. Therefore the extracts from these plants can be used against these bacteria.

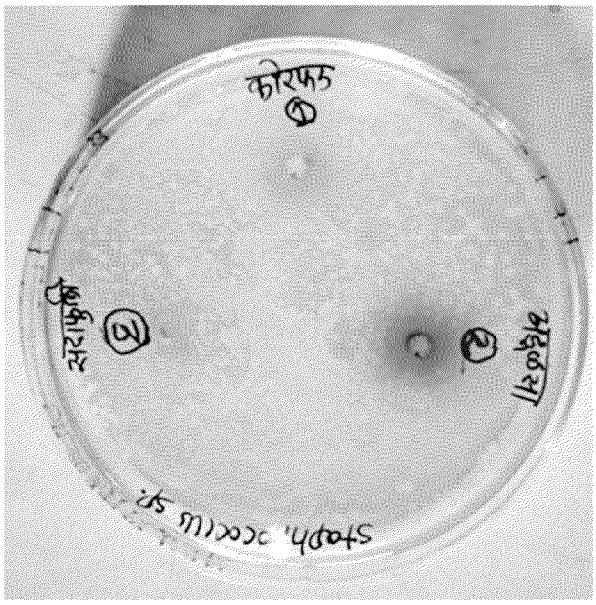

Fig. 2: Antibacterial activity of *Justicia adhatoda*, *Aloe vera* and *Catharanthus roseus* against *Staphylococcus aureus*

Table 1: The size of zone of growth inhibition by

Selected plants	Size of zone of growth inhibition (mm) including diameter of well 5 mm.
Justicia adhatoda	12
Aloe vera	10
Catharanthus roseus	07

Conclusions

This study indicates clear evidence supporting the traditional use of *Justicia adhatoda*, *Aloe vera* and *Catharanthus roseus* in treating diseases related to bacteria particulary Staphylococcal infections. Further research needs to be carried out for the identification of specific phytochemical responsible for the antibacterial activity of these extract.

Acknowledgements

We are thankful to Hon. Dr. Rafiq Shaikh, Chairman and Shaikh Hasarat Pasha Treasurer of M.E.C.H. & W. Society, Parbhani for providing infrastructure and necessary facilities.

References

1. Pa R., Mathew L. Antimicrobial activity of leaf extracts of *Justicia adhatoda* L. in comparison with vasicine, Asian Pacific Journal of Tropical Biomedicine, 2(3) 1556-1560.
2. Ronald M. S., Aloe Vera Its Chemical and Therapeutic Properties, Int. J. Dermatology 30(10) 679-683.
3. Patil P.J.and Ghosh J.S., (2010) Antimicrobial Activity of *Catharanthus roseus* – A Detailed Study, British Journal of Pharmacology and Toxicology 1(1): 40-44.
4. Index of Horticulture: Extraction Technique as last viewed dated 16/02/2019 on site http://agritech.tnau.ac.in/horticulture/extraction_techniques%20_medicinal_plants.pdf.
5. Aneja K.R. (2017) Experiments in Microbiology, Plant Pathology and Biotechnology .New Age International Publisher.

7. In-Vitro Micropropagation of Angelonia Angustifolia (L.): A Rich Source of Secondary Metabolites

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Abstract

In the present study the protocol for callus induction and regeneration in *Angelonia angustifolia* L. was standardized. Young apical leaves and nodes were used as explants for callus induction on Ms Medium containing 2, 4-D and Kinetin, 2, 4-D and BAP, IAA and BAP and IBA and Kinetin in different concentrations. The maximum percentage of callusing was observed on the medium supplemented with 0.5mg/L IBA and 0.5mg/ Kinetin was found to be 100% for leaf & 100% for node explants. The calli in most of the cultures were whitish green and soft in nature. Initiation of shooting of *Angelonia angustifolia* established from leaf explants on MS medium supplemented with combination of hormones IAA 0.4 mg/L & IBA 0.4 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. This plant is also the ware house of secondary metabolites and therefore callus will be the source of extraction of these many secondary metabolites for the therapeutic drugs.

Key words: In-vitro Micropropagation, Regeneration, Organogenesis, *Angelonia angustifolia*.

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 provides 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).

Angelonia angustifolia L. is a probe for anti-inflammatory properties due to its uses in traditional latin American medicine. Its importance as a medicine ranges from anti-inflammatory, analgesic, anti-hyperlipidemic etc.The high concentration of Lupeol found to be present in aerial parts of this plant. Although this plant is a rich source of several secondary metabolites. Therefore it has attracted the attention of Botanists, Chemists, and 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 *this plant*. Keeping entire importance of 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.

Material and Methods

Angelonia angustifolia L . plant used in the present study was collected from the wild population from Nawegaon Bandh, dist. Bhandara (MS). More ever the medicinal importance of the plant has also been documented. Different explants were used for establishing callus including apical leaf and nodes. They were washed thoroughly under running tap water for 10 min. subsequently sterilization was carried out in laminar air flow cabinet under aseptic 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. All cultures were maintained at 27 ^{0}C with 16-18hr. photoperiod.

Results and Discussion

The MS medium supplemented with various concentration of 2, 4-D and Kinetin, 2, 4-D and BAP, IAA and BAP and IBA and Kinetin in different concentrations inducing callusing. The MS medium supplemented with all this combination showed brown and soft and brown white callus induction. The maximum percentage of callusing was observed at the medium supplemented with 0.5 mg/L IBA and 0.5 mg/L kinetin was found to be 100% for apical leaf & 100% for node explants. 100% callus induction for the apical leaf was found to be in MS medium supplemented with 0.5 mg/L 2,4-D + 0.5 mg/L Kinetin, 0.4 mg/L IAA + 0.4 mg/L BAP and followed by MS medium supplemented with 0.6 mg/L 2,4-D + 0.6 mg/L Kinetin i.e. 90% and 0.4 mg/L 2,4-D + 0.4 mg/L Kinetin i.e. 72% respectively. Apical leaf explants were found to be more responsive for the induction of the callus than that of the nodal explants in all hormonal combination which were tested (Table 1 and photo plate 1).

Table No.1: Induction of callus on MS media supplemented with different concentration of hormones.

Sr. No.	Hormone concentrations	Explants Used	% of Callus induction	Duration of induction Of callus in days	Colour and Nature of the callus
1	0.4 mg/L 2,4-D + 0.4 mg/L Kinetin	Apical Leaf	72 %	16	Brown and Soft
		Node	-		
2	0.5 mg/L 2,4-D + 0.5 mg/L Kinetin	Apical Leaf	100 %	15	Brown and Soft
		Node	20 %	15	Brown and Soft
3	0.6 mg/L 2,4-D + 0.6 mg/L Kinetin	Apical Leaf	90 %	12	Brown and Soft
		Node	-		
4	0.6 mg/L 2,4-D + 0.6 mg/L BAP	Apical Leaf	90 %	13	Brown and Soft
		Node	-		
5	0.4 mg/L IAA + 0.4 mg/L BAP	Apical Leaf	100 %	9	White and Soft
		Node	-		
6	0.5 mg/L IBA + 0.5 mg/L Kinetin	Apical Leaf	100 %	15	White and Soft
		Node	100 %	15	White and Soft

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 apical leaf explant callus. Shoot were initiated from indirect organogenesis. The best result of shooting (2.5 cm) was observed with MS medium supplemented with the combination of 0.4mg/L IAA and 0.4mg/L BAP after 13th day with good and long morphology in which 36 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).

Table 2: Effect of different concentration of hormones on shoot regeneration.

Sr. No.	Hormonal concentration	No of shoot per treatment	Shoot length in cm.	Shoot morphology
1	0.4 mg/L IAA + 0.4 mg/L BAP	6	2.5	Green & long
		5	2.0	Green & long
2	0.5 mg/L IBA + 0.5 mg/L Kinetin	2	1.5	Thin Short
		3	2.0	Thin Short

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 *Angelonia angustifolia* that is apical leaf, and nodes, apical leaf was found to be better for callusing on 0.5 mg/L IBA and 0.5 mg/L kinetin supplemented in MS medium. Shoot were induced at concentration 0.4mg/L IAA and 0.4mg/L BAP after 13th day from apical leaf explants callus indirectly..

Acknowledgements

Author is thankful to the Principal N. H. College, Bramhapuri for providing the Tissue culture Laboratory Facility & valuable guidance.

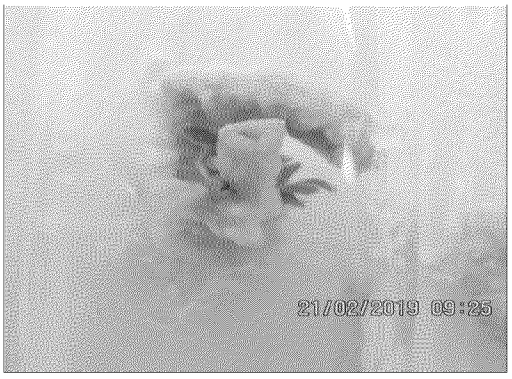
Photo plate 1: Showing habit, habitat and different stages of *In-vitro* regeneration of *Angelonia angustifolia*

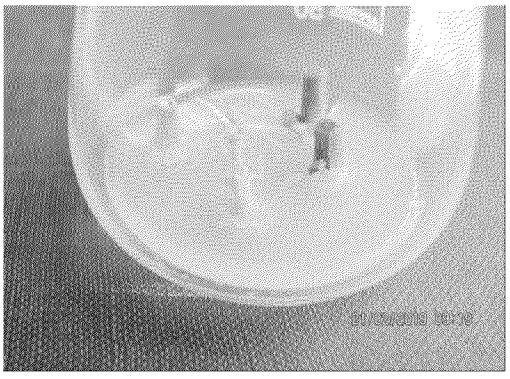
Habit and Habitat

Callus induction from leaf

Callus induction from node

Callus induction from node





Shoot regeneration and multiple shooting

References

- Atal CK and Kapur BM (1982 a) Cultivation and Utilization of Aromatic plants. Regional research Laboratory CSIR, Jammu Tawi.
- Atal CK and Kapur BM (1982 b) Cultivation and Utilization of Medicinal plants. Regional research Laboratory CSIR, Jammu Tawi.
- Bera TK, Roy SC (2000) Plant tissue culture. A tool for rapid multiplication of medicinal plants. tylophora indica – the asthuma herb. *J. of the national botanical Society*. 50(1-2): 27-34.
- Datta PC (1993) Biotechnology and Tissue culture of some medicinal plants. InGovil JN, SInghVK, Hashmi S (eds) Glimpses in plant research, Medicinal plant, New Vistas of research XI (II): 337-342.
- Ghosh B, Sen S (1994) Micropropagation of AAsparagus cooperi Baker as affected by growth regulators, Plant Sci 82:119-124.
- Murashige T and Skoog F (1962). *Physiol. Plant* 15:473-497.
- Toker G, lu Memie M, Toker MC and Yeilada E (2003). Callus formation and Cucurbitacin B accumulation in Ecballium eleaterium callus cultures. Fitoterapia 74(7-8):618-623.

8. Assessment of Antibacterial activity of Euphorbia Tithymaloides Latex

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Abstract

The plant *Euphorbia tithymaloids* belonging to the family euforbiaceae is distributed word wide. This latex producing plant is popular for its medicinal properties. Crude extract of this plant is used to stop abnormal bleeding in piles, ulcerative colitis and during menstruation periods (menorrhagia). In present study latex from surface sterilized *Euphorbia tithymaloides* was collected and its antibacterial activity was evaluated. Maximum inhibition by latex of *Euphorbia tithymaloides* was recorded against *Staphylococcus aureus* followed by *Escherichia coli* and *Pseudomonas aeruginosa*. Scientific study of this plant may promote the formulation of new medications.

Keywords: Antibacterial activity, *Euphorbia tithymaloides* , Latex, Medicinal plant

Introduction

Euphorbia tithymaloids is commonly known as naagdon in India. This plant also occurs in north and central America, Japan, Indonesia, Brazil, France and Mexico. Scientifically this plant has been classified under Euphorbiaceae family and Malpighiales order. It is very common ornamental plant and looks very attractive due to its dark green stem and thick wide leaves. *Euphorbia tithymaloids* is one of the latex producing plant. Generally plants which produce latex are poisonous but this medicinal herb is non toxic. Crude extract of this plant is used to stop abnormal bleeding in piles, ulcerative colitis and during menstruation periods (menorrhagia). Local ayurvedic medicine practitioners use this plant for the treatment of scanty

urination, burning sensation during urination, painful urination, abdominal pain, gas, amebiasis, intestinal infection, constipation, joint inflammation, boils, wound and bleeding due to any reason [1]. To this scenario, we attempted the assessment of antibacterial activity of latex of *Euphorbia tithymaloides* against some common opportunistic pathogens.

Materials and methods

Collection of plant material and surface sterilization

Short stems with intact leaves of *Euphorbia tithymaloides* were collected from the border of local farms (Fig. 1). The collected plant materials were washed thoroughly under running tap water to remove the dust particles and other contaminant deposited on stem and leaves [1]. Further, surface sterilization of this plant material was carried out by immersing in 1% sodium hypochlorite solution for 20 min followed by repeated washing in sterilized distilled water to remove all the traces for sterilant. After surface sterilization, this plant material was subjected for latex collection .

Fig. 1: Photographs of *Euphorbia tithymaloides* (a: Naturally occurring at local farm, Parbhani; b: Cultivated as ornamental plant at Dept. of Biotechnology & Bioinformatics, Yeshwant College, Parbhani).

Collection of Latex from Euphorbia tithymaloides

Latex from surface sterilized *Euphorbia tithymaloides* was collected in a laminar air flow chamber by simply detaching the leaves from stem and immediately each drop of latex was collected in a pre-sterilized screw cap tube.

Assessment of antibacterial activity

The collected latex was used for evaluation of its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Fresh cultures of these bacteria were spread on nutrient agar plates individually. Wells were formed with the help of cork borer at the centre of agar surface of each plate. 0.1 ml of latex was added in each well. The plates were incubated at 35°C for 24 h [1-4].

Results and discussion

Latex of *Euphorbia tithymaloides* has shown remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The size of zone of growth inhibition of selected cultures by latex of *Euphorbia tithymaloides* has been given in Table 1. Maximum inhibition was recorded against *Staphylococcus aureus* followed by *Escherichia coli* and *Pseudomonas aeruginosa*.

Choudhari et al. (2012) have previously studied antibacterial activity of different solvent extract of *Pedilanthus tithymaloides* (now called *Euphorbia tithymaloides*) against the same test cultures and reported the antibacterial activity only by the n-butanol extract.

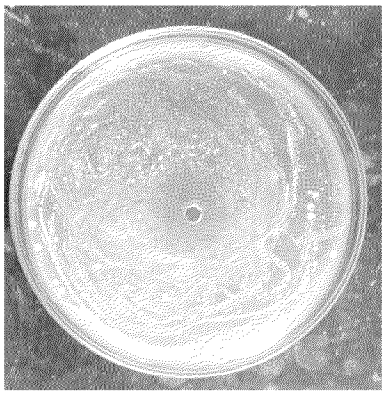

Fig. 2: Antibacterial activity of *Euphorbia tithymaloides* latex against *Staphylococcus aureus*

Table 1: The size of zone of growth inhibition by latex of *Euphorbia tithymaloides*

Test culture	Size of zone of growth inhibition (mm) including diameter of well 7 mm.
Staphylococcus aureus	22
Escherichia coli	20
Pseudomonas aeruginosa	19

Conclusions

Latex of *Euphorbia tithymaloides* has shown remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. This study indicates clear evidence supporting the traditional use of *Euphorbia tithymaloides* in treating diseases related to bacteria. Further research needs to be carried out for the identification of specific phytochemical responsible for the antibacterial activity of latex.

Acknowledgements

We are thankful to Hon. Dr. Rafiq Shaikh, Chairman and Shaikh Hasarat Pasha Treasurer of M.E.C.H. & W. Society, Parbhani for providing infrastructure and necessary facilities.

References

1. Chaudhari Y., Badhe M., Kumar E.P., Mody H., Kokardekar R. An investigation of Antibacterial activity of *Pedilanthus tithymaloides* on different strains of bacteria, *Int.J.Pharm.Phytopharmacol.Res.* 2012, 1(6): 385-386.
2. Murugan, Thiraviam. (2012). Antimicrobial activity of leaves and latex extracts of the herbal plant Calotropis gigantean (Erukku in Tamil). *International Journal of Biology, Pharmacy and Allied Sciences*. 1. 261-270.
3. Shirazi M.H., Ranjbar R., Eshraghi S., Sadeghi G., Jonaidi N., Bazzaz N., Izadi M., Sadeghifard N. An Evaluation of Antibacterial Activity of *Glycyrrhiza galbra* Extract on growth of *Salmonella, Shigella* and *E.coli*. Journal of Biological Sciences. 2007 ;7 (5): 827-829.
4. Aneja K.R. (2017) Experiments in Microbiology, Plant Pathology and Biotechnology .New Age International Publisher.

9. Avifaunal Diversity with Relation to Ecology of Pandharkawada Taluka, India

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Abstract

This study was carried out in Pandharkawda Taluka of Maharashtra state. The present study was made to find out the avian diversity with reference to ecology. We estimate the avian fauna in terms of species richness, diversity and guild structure in different habitats. It was observed that out of 184 species, 116 found were common, 36 were occasionally seen and 32 were uncommon species. The favorable ecological conditions like availability of food, wetlands and roosting places were attracting the various birds. There is need to retain a proportion of natural habitat so as to conserve the bio-diversity.

Keywords: Avian diversity, Pandharkawada Taluka, Maharashtra

Introduction

Biodiversity has central importance in the study of ecology. It is likely to play an important role for ecosystem functioning (Loreau et al., 2001). India has a numerous diversity of plants and animals both domesticated as well as wild in variety of habitats and ecosystems. There is complete interdependence in nature where change in nature causes change in habitat which affects the diversity of the species contained in it. The change in number of assemblage of species affects the nature of habitat. Recent global changes are likely to have strong negative impacts on biodiversity of terrestrial ecosystem (Sala et al., 2000). Today a greatest threat to wildlife is loss of habitat which is widely recognized by ecologists. According to IUCN, globally about one third of all known species are threatened with extinction which includes 29% of all amphibians, 21% of all mammal and 12% of all birds. Out of about 10,000 species of birds worldwide, 1223 (12 percent) are threatened with extinction, and 70 percent of these live in

lowland and mountain tropical forests (IUCN, 2010). Most are primarily endangered by habitat loss and degradation (Sodhi and Smith, 2007).

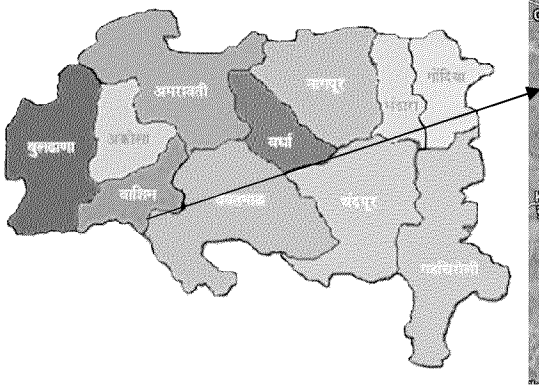
Among all the fauna, birds are use as bio-indicators. The bird species are highly adapted to a specific plant species, community and use to describe the vegetation condition, consequently the ecosystem situation (Jansen & Roberstrom, 2001). The bird populations are usually limited by limiting factors including food supplies, nest and refuge site, competitors, natural enemies and weather (Andrewartha & Birch, 1984, Begon et.al 2006; Brambilla et al; 2010). Guilds are useful in analyzing collective responses of different species to changes in the ecological condition and resources. All the birds live in different habitat on the basis of needs and requirement (U. S. Fish & Wildlife service; 2002). There are growing evidences that climate change will become one of the major diverse of species extinctions in the 21st century. Numbers of published studies have documented a variety of changes attributable to climatic change (IPCC, 2007).

To stop the threats to biodiversity and mass extinction, monitoring programme to measure biodiversity and its threats status are globally recognized as a crucial elements for management. So that in order to identify problems, well structured science-based monitoring and feedback system is needed and essential. Meaningful information that could contribute to conservation planning and management is needed to maintain or improve the structure of various ecosystems. So we selected Pandharkawada Taluka of Yavatmal district (M.S.), India which is first ever scientific attempt to study avian diversity with aspect to ecology.

Material and Methods

Study Site

The study was carried out in Pandharkawada taluka of Yavatmal district of state Maharashtra adjoing the Adilabad distict of state Telangana Pradesh. It is a "Green Oasis" in the southern part of the Maharashtra state. The total area of TWS is 148.63 km^2. It is situated in geographical co-ordinates within the confines of 20º20'00'' (Latitude) to 78º32'00'' East (longitude). It constitutes a compact patch of dense forest cover, having great value from the point of view of wildlife and bio-diversity conservation.



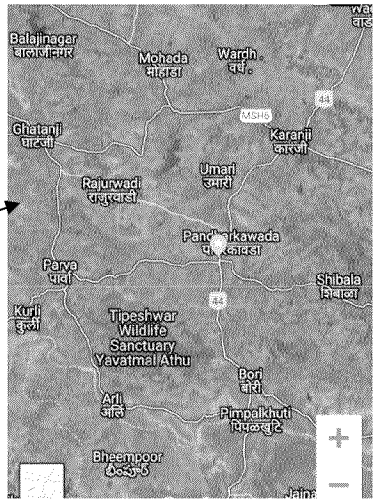


Fig.1.1-Map of Yavatmal District **Fig.1.2-Satellite Map of Pandharkawada Taluka**

Bird Survey

The bird survey was conducted according to a standard point count method (Reynolds et al., 1980; Bibby et al., 2000).We involved audio & visual methods for bird identification within fixed or variable radius plots (Blondel et al., 1970; Hutto et al., 1986).We select different Wetlands such as Lake, rivers & streams & different terrestrial areas. The data collected from the surveys was used to estimate populations of birds, density, diversity and relative abundance or richness of bird species in different habitats. The bird surveys were carried out from January 2017 to January 2019 for 2 years. We used a 25-m/50-m fixed-radius point count method to census the avifauna at each count station (Hutto et al. 1986). Surveys were conducted two days in a week, either from sunrise to 4 hours after sunrise or from 4 hours before sunset until sunset depending on weather conditions. Each count was conducted for 10 minutes, which was further divided into 3 minutes, 2 minutes and 5 minutes respectively.

Materials used for study

For each census, a map of area & satellite map was use to identify sites. So that camera Nikon was used of 45x zoom and 12.6 megapixels. A Tape recorder was also utilized during each survey to record the particular calls, which were later analyzed and used to identify the species. For watching, counting and identifying birds, Binocular (10x50), telescope (25-40x), Notebook, Pen, pencil, Compass, Observation sheet, metal or wooden stakes, permanent Marker, Flagging Tape, Handheld GPS, Guide book of birds of Indian continents, Birds of south India etc were used.

Identification and Classification

For identification and field diagnosis of birds, colored plates of Ali and Ripley (1983), Ali (1996), Grimmett et al., (1998) were used. Classification of birds was made in accordance with Inskipp et al., (1996).

Data analysis

All the diversity of birds was analyzed using biodiversity analysis online software alyoung.

Observation and Result

Biophysical data

Each habitat consists of different combination of physical and biophysical components. The basic factor to be studied of this area on avifauna distribution with aspect to ecology is climate, topography and vegetation. Thus Geology, climate, soil and vegetation types with altitude, latitude and surface topography of particular sites were studied.

Finding of birds

A total of 184 species of birds were observed and recorded during the course of study confined to wetland and terrestrial areas of Pandharkawada taluka. It was observed that out of 184 species, 116 found were common, 36 were occasionally seen and 32 were uncommon species. These belong to 44 Families and 13 orders with family corvidae comprising more bird species following Passeridae and accipitridae.

Diversity of bird community

We found avian diversity index which is most widely used like Shannon-Weiner diversity index, Simpson's index of diversity, Dominance Index, Evenness Index and Hill number N1 and N2. Similarity indices between the same sites of the different habitats were calculated using Jaccard index and Sorenson's index. We calculated the diversity indices for whole Pandharkawada taluka (Table 1.1). The species richness Index i.e. Menhinick index (R1) of this sanctuary was 1.06 and Margalef richness index (R2) was 15.71. The overall Shannon-Weiner index (H^1) was found 4.87 while the Dominance index was 0.017. The two different Evenness measures were calculate namely Shannon evenness (E1) and Sheldon evenness (E2) to measure the evenness of species-abundance which is complimentary to the diversity index concept. The Shannon Evenness (E1) was found 2.21 and Sheldon Evenness (E2) was 0.083. The Similarity Indices were also calculated between the different habitats of the sanctuary namely Jaccard Index

and Sorenson's index which was found 0.03 and 0.06 respectively. The abundances of bird were found higher in July 2017 as compaired to July 2018 . The higher diversity of birds (H^1) was found (4.87) in first year than in second year (4.45). The Simpson index was found 0.029 and 0.022 in two successive years respectively (Table1.2).

Table 1.1 - Overall avian diversity indices of Pandharkawada Taluka

α Diversity	
Species Richness Index (R1)	1.16
Species Richness Index (R2)	14.75
Shannon-Weiner Index (H')	4.89
Simpson's Index (D)	0.017
Hill Diversity N1,N2	5.07, 4.87
Simpson Index of diversity (1-D)	0.98
Evenness Index (Shannon Evenness (E1)	2.21
Sheldon Evenness (E2)	0.083
Reciprocal Simpsons index of diversity (1/D)	57.22
β Diversity	
Similarity Index (Jaccard index)	0.03
Sorenson's similarity index	0.063

Table 1.2 - Diversity indices of two successive years

Year	Shannon diversity (H^1)	Simpson index
Jan 2017- Dec.18	4.45	0.029
Jan. 18- Dec. 19	4.87	0.022

Discussion and Conclusion

Environmental effects on birds were typically assayed by recording changes in population density, abundance or distribution. As there was lowest rainfall in the year 2017, the species richness and abundance was higher in second year because of high rainfall. When rainfall increased, abundance of birds was decreased. This was an inverse relationship which was observed between the rainfall and abundance of birds. A significant negative correlation was obtained between abundance of birds and rainfall. It was seen that, environmental factors affect species richness and diversity. There was more richness and diversity in the undisturbed habitats rather than the disturbed visibility in monsoon. Chilling winds worsen the wet conditions. This

results into low species distribution and low sightings in the study area. Similar observation was detected by bird researcher (Anand et al., 2007). The food availability for these birds was extremely scanty during the monsoon season. This was another reason for their less sighting and low species richness and diversity. Subsequent seasons show better food availability due to increased sunlight and temperature as well as reduction in rainfall. In present study, Bird communities vary among forest types and functional groups. Different vegetation types as well as abundant food resources might be played a greater role in difference in habitat preference by bird species. The rich and high vegetation might be providing heterogeneous and suitable site for nesting, roosting and foraging of bird. Similar finding reported regarding vegetation diversity and richness of wetlands on the bird population richness by Paracuellous (2006). In the study of birds of Uttara Kannada, Daniels et al. (1992) said that birds in teak plantations have species in common with dry deciduous forests. This study also supports the hypothesis. Water birds like, Lesser whistling teal (*Dendrocygna javanica*), Little cormorant (*Phalacrocorax niger*), White-breasted kingfisher (*Halcyon smyrnensis*), Black-winged Stilt (*Himantopus himantopus*), Red-wattled Lapwing (*Vanellus indicus*), Cattle egret (*Bubulcus ibis*) and Indian pond-heron (*Ardeola grayii*) were the common species inhabiting the water bodies, while Purple Heron (*Ardea purpurea*) were rarely sighted. The passage migrant Rosy starling (*Sturnus roseus*) was found everywhere before and after the winter season.

There is need to conserve the avifaunal bio-diversity of this area by protecting its habit conditions especially, the water bodies, riparian areas, edges and streams. Also there is need to generate awareness about the conservation value and ecological role of avifauna and maintainace of natural habitat conditions and ecological balance. Conservation awareness programme among the local peoples is required to sensitize the local peoples about the sustainable use to conserve it for future generations.

References

- Ali S. (1996). The book of Indian Birds (12th and enlarged century edition)/ Bombay. Nat. Hist. Soc. Oxford Univ. Press, New Delhi
- Ali, S. and S. D. Ripley (1983). Handbook of the birds of India and Pakistan. Compact Edition. New Delhi: Oxford University Press. **P:737**
- Alyoung (2013). Online calculator, http://alyoung.com/labs/biodiversity_calculator.html

- Anand, D. Padhye, M. Paingankar, N. Dahanukar and S. Pande (2007). Seasonal and landscape element wise changes in the community structure of avifauna of Tamhini, Northern Western Ghats, India.Zoo's Print Journal, **22 (9):2807-2838.**
- Andrewartha, H. G. and L. C. Birch (1984).The ecological web. Univ. Chicago Press, Chicago, USA.
- Begon, M.,Townsend, C. R. and J.L. Harpe (2006). Ecology: from individuals to ecosystems. Blackwell Publishing, Oxford. UK
- Bibby, C.J., N.D. Burgess., D.A. Hill., and S. H. Mustoe (2000). Bird census techniques. 2nd edition. New York: Academic Press, London. ISBN-10:0120958317
- Blondel, J., C. Ferry and B. Frochot(1970). A method of diversity indices and population abundances (I.P.A.) with relative to avifauna par station count. Alauda **38:55-71.**
- Brambilla, M. Bassi, E. Ceci., C. and D. Rubolini (2010). Environmental factors affecting patterns of distribution and co-occurrence of two competing raptor species.Ibis **152:310-322.**
- Daniels, R.J.R., N. V. Hegde and M. Gadgil (1992). On the relationship between birds and woody plant speicies diversity in the Uttar Kannada district of south India. Proceedings of the National Academy of Science. USA, **89:5331-5315.**
- Grimmet, R. and Inskipp, T. (2007). Birds of Southern India. Om Books International, New Delhi, India.
- Hutto R.L.,S. M. Pletschet and P. Hendricks (1986). A fixed-radius point count method for nonbreeding and breeding season use. Auk **103:593-602.**
- IUCN(2010). IUCN red list of threatened species. Version 2010. Available at http://www.iucnredlist.org
- IPCC Climate Change (2007). The Physical Science Basis.Contribution of working group I to IV. Assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.
- Jansen, A. and A. I. Robertson (2001).Riparian bird communities in relation to land management practices in floodplain woodlands of south-eastern Australia. Biological Conservation **100:173-185**.

- Paracuellos, M. (2006). How can habitat selection affect the use of a wetland complex by water birds. Bio. And Conservation.**15:4569-4582.**
- Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D. and D.A. Wardle (2001). Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science, **294:804-808.**
- Reynolds R.T., J.M. Scott., R.A. Nussbaum (1980). Available circular-plot method for estimating bird numbers Condor **82:309-313.**
- Sodhi, N.S., L.H. Liow and F.A. Bazaz (2004). Avian extinctions from tropical and subtropical forests. Ann. Rev. Ecol. Evol. Syst. **35:323-345**
- U. S. Fish and Wildlife Service (2002).The Four Essential Elements of Habitat.

10. Conservation of Biodiversity with Respect to Development of Country: In India (Aundha [M.S.])

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Abstract

This paper deals with the study of study of biodiversity of Aundha region in Marathwada.Lot of biodiversity is located in the Hingoli district,i.e.first spot means Aundha. As Aundha is a Holy place no of pilgrims visit to this place. So it is our duty to know about the biodiversity of this region. Aundha is a tahasil place of Hingoli district of Marathwada (Maharashtra).Aundha has been attributed with natural beauty .Aundha is covered by forest area with thick vegetation which contain no of variety of plants like herbs, shrubs, climbrs, trees etc. This diversity of plants attract the pilgrims and peoples.In this forest flora consists of large no of Medicinal Plants now a day also *Abrus precatorius,Aclypha indica ,Annona squamosa, Barleria cristata,Butea monosperma,Buchnania lanzan,Cleome viscose,Clitoria ternatea,Cocculus hirtus,Clerodendron serratum,Digera muricata, Dioscorea bulbifera, Dolichandron fulcata, Evolvulus alsinoides,Emblica officinalis, Ficus bengalensis, Gloriosa superba, Helicterus isora etc.* Also there is diversity in fauna which consists of animals like cat, wild pig, fox, deer, Peacock, eagle, crow ,sparrow, different types of snakes, frogs etc. But in some part of this region there is cutting of valuable plants and hunting of some animals and birds, also by some natural scarcity of water ,food which lead to migration or death. Due to this bad activity of human being and natural events, biodiversity of this region becomes loose which is very hazardous for this area. For this we want to take a step to aware the peoples and Government must be involved for the protection of biodiversity. They make a small patch in the forest to grow food. Peoples depend on the forest for. timber, fuelwood, medicine, fodder, leaves etc. Fuel Wood : For the rural population wood is an important source of energy for cooking and heating. Some people cut the trees and sale in the market as a fuel wood. Fodder : Fodder from the forest are important source for cattle and other grazing animals. There are many variety of grasses. Fencing : Fences created with trees, shrubs and thorny plants are preferred to the farms. Shelter ; Forest material like stem , leaves are used to make hat as a house.

Key Words: Medicine, Fodder, Fencing, Biodiversity, Forest ,Food chain. Pilgrims

Introduction

Aundha Nagnath is a holy place due to Lord Jyotirlinga and pilgrims visited thought the year. Majority of peoples depends on forest for their needs. They gradually becomes food grower. They make a small patch in the forest to grow food. Peoples depend on the forest for. timber, fuelwood, medicine, fodder, leaves etc. Fuel Wood : For the rural population wood is an important source of energy for cooking and heating. Some people cut the trees and sale in the market as a fuel wood. Fodder : Fodder from the forest are important source for cattle and other grazing animals. There are many variety of grasses. Fencing : Fences created with trees, shrubs and thorny plants are preferred to the farms. Shelter ; Forest material like stem , leaves are used to make hat as a house. Fruit : Fruit trees are an important source of income of this people Ex.*Annona squamosa* L.,*Annona reticulate* L.Medicine : Peoples have been depending on the forest to cure them of various ailments. Even today man is depend on the forest for herbs and plants to fight against disease.

We know well ,in the universe earth is a planet on which living organisms are from ancient. No evidence that living organisms are not found on any other planet except earth. On the earth at tropical ,temperate and frozen regions living organisms are poor. Different colures, shapes, diversity of natural cycles and their interrelationship ,adaptations earth becomes picturesque universe. Human being and other animals, birds, plants, herbs, shrubs shows variety of existence but live together. From two hundred corore years lot of living organisms are evolved on the earth. Out of them no of organisms are not identified. Some are disorganized and some are added in the diversity. Variety of species are adopting with different environments, like yeast, bacteria. Some living organisms use oxygen and some live without oxygen. Means that any type of organism have an opportunity to live in any type of environment on the earth. For the study human being classified and from that classification we get information related to biodiversity.

Objectives of Biodiversity/Material and Method

To study what is biodiversity and how it ia related with human being.

To realize due to biodiversity earth, living organisms existence on earth and hence human society and environment relationship should be confirm

To discuss if biodiversity is not existed and their hazardous effects on the human life.

Result and Discussion: Biodiversity means all the living organisms on the earth. In the universe different types of biodiversity like Genetic Biodiversity – In this type we know information about living organisms on the basis of heredity. Transfer of genes from one generation to another generation is responsible for biodiversity. Due to heredity we get different types of characters in the same species. Species Biodiversity – living organisms are different from each other and they struggle for existence. Ecosystem Biodiversity –Ecosystem biodiversity means all the living organisms and their interrelationship with environmental factors like light, temp., pressure, soil, water .Domesticated Biodiversity- In our house domesticated plants ,animals also shows biodiversity. man also produced genetically modified plants and animals which shows more diversity. Micro organisms Biodiversity - On the tip of needle we observe no of microorganisms. These are observed under microscope, also enter in animals, plants body by infection and causes different types of diseases. In food chain producers, consumers, decomposers are included which shows diversity in them.

Nature of Indian Biodiversity – In our India different types of soil, environment, temperature, habits, deserts are observed .I f we think related to plants, in our India near about 45000 different types of trees are observed. 7 % types of flowers,15000 species of flowering plants are observed.51 types of grains,104 types of fruits,27 types of condiments,55 types of fruit and leafy vegetables,24 types of fibers,12 types of oil seeds ,tea, coffee, tobacco, sugarcane cash crop also observed. In India 81000 types of animals 57000 insects2546 fishes,204 aquatic animals,428 creeping ,1228 birds 372 vertebrates,20000 invertebrates are observed. 850 bacteria and 12500 fungal types are in existence. No of organism are not steel identified. But for the development of country biodiversities are destructed due to lack of space, pollution of soil, water and atmosphere. Unplanned development ,non awareness regarding biodiversity, ,modern industrialization, weak rules and laws, biodiversity should be in future. Biodiversity should be conserved because no of families are depend for earning source on these biodiversity. For the conservation we want to understand aesthetic value, economic value, ecological factors, religious and cultural factors.

For the conservation of biodiversity international biotic conservation policy, national biotic conservation policy, Environmental protection Act(1986),1897 Fish farming Act,1927 forest Act,,1972 Wild life Conservation Act, from these Acts hunting of animals is

restricted and rare plants are also conserved under these acts. National parks, sanctuaries ,zoo parks should be established

Conclusion

From the study it is observed that due to decrease in biodiversity existence of human being is in the danger zone. We should think about the existence of human being than the development of country. Therefore it is necessary to stop the decreasing percentage of biodiversity. It is observed that in developed countries biodiversity is decreased .Global Warming, Conservation of Biological diversity and Intellectual Property Rights and Patent Acts these three units are influencing on the existence of human being. For the self development of human being , he exploited the nature.

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Bibliography

- Apli Drusti- Aple Dhan Vol-I,III.
- Environmental Science,W.R.Ahirrao.
- TPCC,Climate change report of the intergovernmental panel on climate
- change,WMO/UNEP,Cambridge University press.1990.
- SECP,Mans impact on the global climate,Report of the study of critical Environment
- Problems,the MIT Press Cambridge,Mass 1970.

11. Isolation and Screening of Bacillus spp. for Microbiological Control of Sclerotium Rolfsii Sacc., A Stem Rot Pathogen of Groundnut

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Abstract

Stem rot is one of the most important disease of groundnut caused by *Sclerotium rolfsii* Sacc. which causes major crop losses. The present study was undertaken to search for the effective *Bacillus spp.* for microbiological control of *Sclerotium rolfsii* Sacc. 129 *Bacillus spp.* were isolated from different rhizospheric niches of healthy plants, and screened *in vitro* against *Sclerotium rolfsii,* by dual culture technique. Out of these *Bacillus spp, Bacillus spp. 57* found effective in managing the phytopathogen by dual culture technique.

Key words: Groundnut, Stem rot, *Sclerotium rolfsii, Bacillus spp.*

1.0 Introduction

Stem rot caused by *Sclerotium rolfsii*, a broad host range fungus, is the major soil borne disease of groundnut (*Arachis hypogaea*). In India among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to production and is of considerable economic significance for groundnut grown under irrigated conditions. Stem-rot caused by *S. rolfsii* is sporadic in most of the groundnut growing areas like Tamil Nadu, Andhra Pradesh, Karnataka (Pande, *et al.,* 2000).

The traditional agricultural practice to control the phytopathogen *S. rolfsii* is by using variety of fungicides e.g. Bavistin, Captan etc. but a severe disadvantage of the traditional method is that it is not effective to check the *Sclerotium* during the cropping period (90- 100 days) and is not eco-friendly. Because of the increased usage of chemical fungicides produced concern for the environment and human health, microbial inoculants have been experimented

extensively during the last decade to control wilt and other plant diseases (Siddiqui and Shakeel, 2006; Chakraborty and Chatterjee, 2008; Akhtar *et al.*, 2010).

Bacteria have been explored as microbiological control agents for plant diseases (Gerhardson, 2002) and as plant growth promoters and inducers of disease resistance (Catellan *et al.*, 1999; Bargabus *et al.*, 2002; Bais *et al.*, 2004). Apart from improving plant health, they also meet the increasing demand for low-input agriculture.

The use of antagonistic bacteria is reported as a powerful strategy to suppress soil-borne pathogens due to their ability to antagonize the pathogen by multiple modes and to effectively colonize the rhizosphere. The widely known mechanisms of biocontrol action are competition for an ecological niche or substrate, as well as the production of inhibitory compounds and hydrolytic enzymes that are often active against a broad spectrum of fungal pathogens. Many microorganisms are known to produce multiple antibiotics which can suppress one or more pathogens (Haas and Defago, 2005; Stein, 2005; Ge *et al.*, 2007). For instance, *Bacillus subtilis* produces several ribosomal and non-ribosomal peptides that act as antibiotics such as iturins, surfactins and zwittermycin (Asaka and Shoda, 1996; Stein, 2005) and it secretes also hydrolytic enzymes, i.e. protease, glucanase (Cazorla *et al.*, 2007), chitinase (Manjula *et al.*, 2004), lipase (Detry *et al.*, 2006) and amylase (Konsoula and Liakopoulou-Kyriakides, 2006).

Pseudomonas spp. and *Bacillus* spp. have been applied as biocontrol agents to suppress plant-pathogenic organisms (Koumoutsi *et al.*, 2007; Joseph *et al.*, 2008; Akhtar *et al.*, 2010). In particular *Bacillus* spp. is gaining recognition as safe biocontrol agents in a variety of crops, specifically as seed protectants and antifungal agents (Asaka and Shoda, 1996; Stein, 2005). Moreover, they are spore-formers, which impart a natural formulation advantage over other microorganisms (Emmert and Handelsman, 1999; Haas and Defago, 2005; Romero *et al.*, 2007).

The objective of the current study was to isolate particularly *Bacillus spp.*, from rhizospheric niches of healthy plants such as Neem and evaluate its potential *in vitro* in controlling the soil-borne pathogen, *Sclerotium rolfsii*, causing stem rot of groundnut by dual culture method.

2.0 Materials and Methods

2.1 Stem rot pathogen of Groundnut

Sclerotium rolfsii, Stem rot pathogen of groundnut had been isolated in our laboratory in previous studies (Rakh, 2010). Fungal culture of *Sclerotium rolfsii* was maintained on potato dextrose agar (PDA) by sub-culturing at regular intervals.

2.2 Isolation of *Bacillus spp.* from Rhizospheric niches

Soil from rhizospheric niches of different healthy plants such as neem, soybean, tur etc. were collected in poly-ethylene bags and brought to the research laboratory. 1 gm of soil sample was inoculated into 100 ml nutrient broth and kept for incubation at room temperature for 24 h. For isolation of *Bacillus* isolate, a modified method of Kim *et al.,* (1997) was employed. A 1ml of enriched Nutrient Broth was added to 10 ml sterile distilled water and kept at 80°C for 20 min. later on a loopful of culture was streaked on nutrient agar plates. Plates were incubated at room temperature for 48 hr. Typical white colonies were picked up individually and purified on nutrient agar slants. All the isolates were tentatively named during this research to avoid confusion.

2.3 Screening for Potential Microbiological Control agents

All the *Bacillus spp.* were screened for potential antagonistic activity against *S. rolfsii,* on King's B agar (Ran, *et al.,* 2003) using dual culture technique. (Rangeshwaran and Prasad, 2000) An agar disc (5 mm) was cut from an actively growing (96 h) *S. rolfsii,* and placed on the surface of fresh King's B Agar medium at the one side of the Petri plates. A loopful of actively growing *Bacillus spp.* (each) was placed opposite to the fungal disc. Plates inoculated with phytopathogen and without bacteria were used as control. Each experiment was carried out in triplicates. Plates were incubated at room temperature for 7 days. Degree of antagonism was determined by measuring the radial growth of pathogen with bacterial culture and control and Percent inhibition was calculated by the following equation (Riungu *et al.,* 2008).

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter of pathogen alone (Control)} - \text{Colony diameter of pathogen + Antagonist}}{\text{Colony diameter of pathogen alone}} \times 100$$

3.0 Result and Discussion

3.1 Isolation of Rhizobacteria

In present work, 129 *Bacillus spp.* were isolated from rhizospheric niches of different healthy plants such as Soybean, Neem, Tur etc. All the rhizospheric *Bacillus spp.* were tentatively named as *Bacillus spp* 1 to *Bacillus spp* 129.

3.2 *In Vitro* Screening for Microbiological Control agents against *Sclerotium rolfsii*:

To search for potential microbiological control agent, the entire 129 *Bacillus spp.* screened for their antagonistic activity against *S. rolfsii,* by dual culture method. The present study has shown that *Sclerotium rolfsii,* the stem rot pathogen of groundnut can be controlled by *Bacillus spp 57* recovered from the rhizospheric niche. *Bacillus spp 57* was found effective in inhibiting the phytopathogen, *Sclerotium rolfsii in vitro* (56.66 %) in contrast to other *Bacillus spp.* isolated from various source (Photo Plate 1 & Table 1).

This result was in correlation with the result obtained by Chen *et al. (*2004). Similar findings were also recorded by the study conducted by Souto *et al.* (2004) where mycelial growth of *Sclerotium spp.* was inhibited by application of *Bacillus spp.* using the dual culture technique. Similarly findings were also shown by *Bacillus subtilis* which reduced the growth of *S. rolfsii* effectively on PDA when compared with the control (Keyser and Ferreira, 1988) & also by Gomashe *et al.,* (2014) where *Bacillus subtilis* found effective in controlling *Sclerotium rolfsii* by producing bioactive compound.

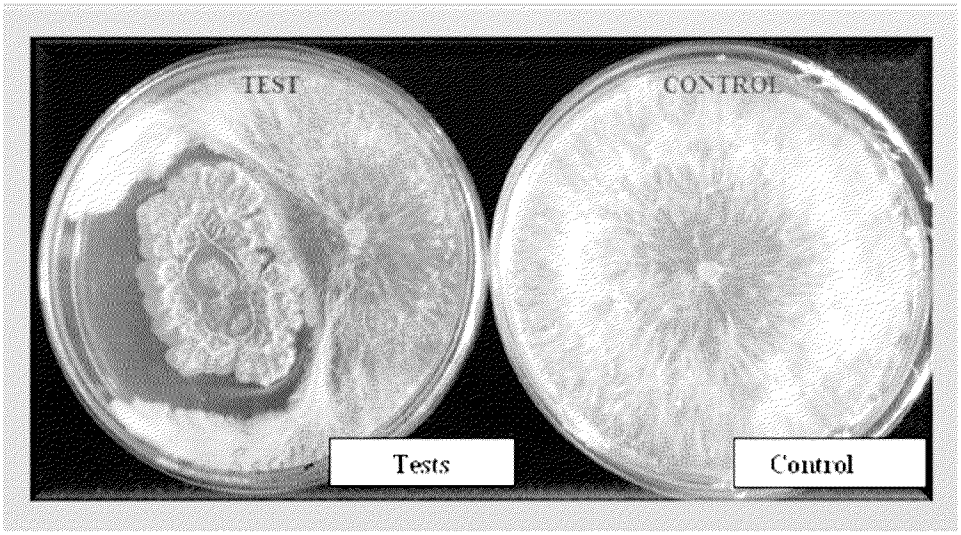

Photo Plate 2: *In Vitro* Antagonism of *Bacillus spp.* 57 against *Sclerotium rolfsii* by Dual Culture Technique

Tentative Name of Bacteria	**Inhibition of** *S. rolfsii* (%)	**Tentative Name of Bacteria**	**Inhibition of** *S. rolfsii* (%)	**Tentative Name of Bacteria**	**Inhibition of** *S. rolfsii* (%)
Bacillus spp. 1	21.2	*Bacillus spp. 44*	29.4	*Bacillus spp. 87*	42..6
Bacillus spp. 2	35.3	*Bacillus spp. 45*	21.8	*Bacillus spp. 88*	24.2
Bacillus spp. 3	43.1	*Bacillus spp. 46*	10.9	*Bacillus spp. 89*	33.1
Bacillus spp. 4	12.1	*Bacillus spp. 47*	16.1	*Bacillus spp. 90*	43.2
Bacillus spp. 5	21.0	*Bacillus spp. 48*	20.5	*Bacillus spp. 91*	41.2
Bacillus spp. 6	30.4	*Bacillus spp. 49*	21.5	*Bacillus spp. 92*	36.5
Bacillus spp. 7	19.0	*Bacillus spp. 50*	32.1	*Bacillus spp. 93*	39.2
Bacillus spp. 8	12.5	*Bacillus spp. 51*	23.1	*Bacillus spp. 94*	14.2
Bacillus spp. 9	21.4	*Bacillus spp. 52*	12.0	*Bacillus spp. 95*	23.6
Bacillus spp. 10	16.9	*Bacillus spp. 53*	19.8	*Bacillus spp. 96*	45.8
Bacillus spp. 11	31.7	*Bacillus spp. 54*	13.1	*Bacillus spp. 97*	34.7
Bacillus spp. 12	33.0	*Bacillus spp. 55*	25.8	*Bacillus spp. 98*	11.5
Bacillus spp. 13	27.0	*Bacillus spp. 56*	8.7	*Bacillus spp. 99*	5.3
Bacillus spp. 14	36.4	***Bacillus spp. 57***	**56.6**	*Bacillus spp. 100*	3.0
Bacillus spp. 15	20.2	*Bacillus spp. 58*	5.8	*Bacillus spp. 101*	0.0
Bacillus spp. 16	16.0	*Bacillus spp. 59*	13.2	*Bacillus spp. 102*	37.5
Bacillus spp. 17	11.0	*Bacillus spp. 60*	28.1	*Bacillus spp. 103*	0.0
Bacillus spp. 18	14.0	*Bacillus spp. 61*	33.7	*Bacillus spp. 104*	16.8
Bacillus spp. 19	23.0	*Bacillus spp. 62*	10.3	*Bacillus spp. 105*	0.0
Bacillus spp. 20	26.9	*Bacillus spp. 63*	35.2	*Bacillus spp. 106*	35.2
Bacillus spp. 21	25.0	*Bacillus spp. 64*	41.7	*Bacillus spp. 107*	18.6
Bacillus spp. 22	18.7	*Bacillus spp. 65*	31.6	*Bacillus spp. 108*	41.5
Bacillus spp. 23	13.4	*Bacillus spp. 66*	46.8	*Bacillus spp. 109*	4.2
Bacillus spp. 24	15.7	*Bacillus spp. 67*	32.8	*Bacillus spp. 110*	0.0
Bacillus spp. 25	25.0	*Bacillus spp. 68*	15.6	*Bacillus spp. 111*	47.9
Bacillus spp. 26	13.8	*Bacillus spp. 69*	14.6	*Bacillus spp. 112*	37.8
Bacillus spp. 27	26.5	*Bacillus spp. 70*	27.3	*Bacillus spp. 113*	0.0
Bacillus spp. 28	34.2	*Bacillus spp. 71*	45.8	*Bacillus spp. 114*	17.4
Bacillus spp. 29	11.8	*Bacillus spp. 72*	41.5	*Bacillus spp. 115*	19.3
Bacillus spp. 30	14.3	*Bacillus spp. 73*	37.4	*Bacillus spp. 116*	28.4
Bacillus spp. 31	31.1	*Bacillus spp. 74*	48.2	*Bacillus spp. 117*	27.6
Bacillus spp. 32	25.1	*Bacillus spp. 75*	26.4	*Bacillus spp. 118*	0.0
Bacillus spp. 33	21.9	*Bacillus spp. 76*	35.8	*Bacillus spp. 119*	19.7
Bacillus spp. 34	23.2	*Bacillus spp. 77*	11.5	*Bacillus spp. 120*	43.7
Bacillus spp. 35	38.3	*Bacillus spp. 78*	23.4	*Bacillus spp. 121*	35.9
Bacillus spp. 36	27.4	*Bacillus spp. 79*	14.7	*Bacillus spp. 122*	31.0

Bacillus spp. 37	11.3	*Bacillus spp. 80*	21.7	*Bacillus spp. 123*	29.7
Bacillus spp. 38	24.8	*Bacillus spp. 81*	29.0	*Bacillus spp. 124*	0.0
Bacillus spp. 39	9.8	*Bacillus spp. 82*	15.2	*Bacillus spp. 125*	17.4
Bacillus spp. 40	17.3	*Bacillus spp. 83*	19.4	*Bacillus spp. 126*	14.9
Bacillus spp. 41	23.1	*Bacillus spp. 84*	23.9	*Bacillus spp. 127*	0.0
Bacillus spp. 42	25.3	*Bacillus spp. 85*	31.4	*Bacillus spp. 128*	38.9
Bacillus spp. 43	13.1	*Bacillus spp. 86*	46.3	*Bacillus spp. 129*	35.7

Table1: *In Vitro* Screening of *Bacillus spp.* against *Sclerotium rolfsii* by dual culture technique

Acknowledgment

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Reference

1. Asaka O., Shoda M., (1996). Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology* **62**: 4081-4085.
2. Bais H.P., Ray F., Vivanco J.M., (2004). Biocontrol of *Bacillus subtilis* against infection of arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. *Plant Physiology* 134: 307-319.
3. Bargabus R.L., Zidack N.K., Sherwood J.E., Jacobsen B.J., (2002). Characterization of systemic resistance in sugar beet elicited by a non pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. *Physiology and Molecular Plant Pathology* 61: 289-298.
4. Cattelan A.J., Hartel P.G., Fuhrmann J.J., (1999). Screening for plant growth–promoting rhizobacteria to promote early soybean growth. *Soil Science Society of American Journal* 63: 1670-1680.
5. Cazorla F.M., Romero D., Pérez-García A., Lugtenberg B.J., Vicente A., Bloemberg G., (2007). Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *Journal of Applied Microbiology* **103**: 1950-1959.
6. Chakraborty M. R., Chatterjee N. C., (2008). Control of *Fusarium* wilt of *Solanum melongena* by *Trichoderma* spp. *Biologia Plantarum* 52: 582-586.

7. Chen CT, Huang CJ, Wang YH, Chen CY (2004) Two step purification of *Bacillus circulans* chitinase A, expressed in *E. coli* periplasm. *Protein expression and purification,* 37: 27-31.
8. Detry J., Rosenbaum T., Lütz S., Hahn D., Jaeger K.E., Müller M., Eggert T., (2006). Biocatalytic production of enantiopure cyclohexane-trans-1,2-diol using extracellular lipases from *Bacillus subtilis*. *Applied Microbiology and Biotechnology* **72**: 1107-1116.
9. Emmert E.A.B., Handelsman J., (1999). Biocontrol of plant disease: a Gram-positive perspective. *FEMS Microbiology Letters* **171:** 1-9.
10. Ge Y.H., Pei D.L., Zhao Y.H., Li W.W., Wang S.F., Xu Y.Q., (2007). Correlation between antifungal agent phenazine-1- carboxylic acid and pyoluteorin biosynthesis in *Pseudomonas* sp. M18. *Current Microbiology* **54**: 277-281.
11. Gerhardson B., (2002). Biological substitutes for pesticides.*Trends in Biotechnology* 20: 338-343.
12. Gomashe Ashok V, Sheikh Neha AM and Gulhane Pranita A (2014) Production of Bioactive Compound by *Bacillus subtilis* and its antagonistic activity against *Sclerotium rolfsii., Int. J. of Life Sciences,* 2(2) : 127-133
13. Haas D., Defago G., (2005). Biological control of soil-borne pathogens by fluorescent Pseudomonads. *Nature Reviews of Microbiology* **3**: 307-319.
14. Joseph B., Dar M.A., Kumar V., (2008). Bioefficacy of plant extracts to control *Fusarium solani* f.sp. *melongenae* incitant of brinjal wilt. *Global Journal of Biotechnology and Biochemistry* **3**: 56-59.
15. Kim, D. S., R. J. Cook, and D. M. Weller (1997) *Bacillus sp.L324-92* for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology, 87: 551-558.
16. Konsoula Z., Liakopoulou-Kyriakides M., (2006). Thermostable α-amylase production by *Bacillus subtilis* entrapped in calcium alginate gel capsules. *Enzyme and Microbial Technology* **39**: 690-696.
17. Koumoutsi A., Chen X.H., Vater J., Borriss R., (2007). DegU and YczE positively regulate the synthesis of bacillomycin D by *Bacillus amyloliquefaciens* strain FZB42. *Applied and Environmental Microbiology* **73**: 6953-6964.
18. Manjula K., Krishna Kishore G., Podile A.R., (2004). Whole cells of *Bacillus subtilis* AF 1 proved more effective than cell-free and chitinase-based formulations in

biological control of citrus fruit rot and groundnut rust. *Canadian Journal of Microbiology* **50**: 737-744.

19. Pande, S. and J. Narayana Rao. (2000). Changing scenario of groundnut diseases in Andhra Pradesh, Karnataka and Tamil Nadu states of India. International Arachis Newsletter 20: 42-44.
20. Rakh, R. R. (2010). Investigation on Biological Control of Major Groundnut (*Arachis hypogaea* Linn.) Diseases, A Ph.D. thesis submitted to S. R. T. M. University, Nanded.
21. Ran L. X., Xiang, M. L., Van Loon, L. C. and Bakker, P. A. H. M. (2003) Siderophore-mediated suppression of Grey Mould in *Eucalyptus urophylla* by Fluorescent *Pseudomonas spp.* 6th International PGPR Workshop, 5-10 October 2003, Calcutta, India. 558-562.
22. Rangeshwaran, R. and Prasad, R. D. (2000) Biological control of *Sclerotium* rot of sunflower. Indian Phytopathology, 53: 444-449.
23. Riungu, G. M., J. W. Muthorni, R. D. Narla, J. M. Wagacha and J. K. Gathumbi (2008) Management of *Fusarium* Head Blight of Wheat and Deoxynivalenol Accumulation using Antagonistic Microorganisms. Plant Pathology Journal, 7(1): 13-19.
24. Romero D., de Vicente A., Rakotoaly R.H., Dufour S.E., Veening J.W., Arrebola E., Cazorla F.M., Kuiper O.P., (2007). The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* towards *Podosphaera fusca*. *Molecular Plant-Microbe Interactions* **20**: 430-440
25. Siddiqui Z.A., Shakeel U., (2006). Use of fluorescent *Pseudomonads* isolates for the biocontrol of wilt disease complex of pigeonpea in green house assay and under pot condition. *Plant Pathology Journal* 5: 99-105.
26. Souto G.I., Correa O.S., Montecchia M.S., Kerber N.L., Pucheu N.L., Bachur M., Garcia A. F. (2004) Genetic and functional characterization of a *Bacillus* spp. strain excreting surfactin and antifungal metabolites partially identified as iturin-like compounds. *Journal of Applied Microbiology,* 97: 1247-1256. Akhtar M.S., Shakeel U., Siddiqui Z.A., 2010. Biocontrol of *Fusarium* wilt by *Bacillus pumilus, Pseudomonas alcaligenes,* and *Rhizobium* sp. on lentil. *Turkish Journal of Biology* 3: 1-7.
27. Stein T., (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular Microbiology* **56**: 845- 857.

12. Bioaccumulation of Heavy Metal Cadmium Chloride in Bivalve, Corbicula Striatella and Potential Risks to Human Health

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Abstract

This study has been carried out to determine the accumulation of heavy metal cadmium chloride in fish, *Corbicula striatella* collected from Tapi river at Bhusawal region. The bioavailability of the metal is highly variable and depends on P^H, presence of organic ligands, water hardness, and numerous other controlling factors. The accumulation of cadmium in the soft tissue of *Corbicula striatella* was analysed. The control group of animals showed minute quantity of cadmium as compared to the experimental group.

Keywards: Bioaccumulation, heavy metal, fish, health effects.

Introduction

Environmental pollution has become one of the most serious problem of today. Industrial wastes increase the amount of unwanted material in our natural environment. The industrial wastes effluent contain an increase amount of toxic heavy metals like *Cadmium, chromium, nickel, copper* and *lead* which affect the people with most of the common disease like *bronchitis, emphysema* and *cardiovascular* problem. The aquatic bodies near the industrial and urban area are more prone to the accumulation of such metals. These elements get concentrated as they progress through the food chain.

Fish is good bio-indicator because it is easy to be obtained in large quantity, potential to accumulate metals, long lifespan, optimum size for analysis and easy to be sampled (Batvari et-al.2007) Fish accumulates metals in its tissues through absorption and human can be exposed to metals via food web. This will cause acute and chronic effect to human (Yi/maz & Dogan, 2007;Fidan et-al.2007) The present study was undertaken to study the concentration of cadmium chloride in soft tissues of fish at Tapi river near Bhusawal region. Therefore, the pattern of accumulation of cadmium chloride was studied by exposing the fish to acute concentrations of the metal.

Material and Methods

Corbicula striatella used in the present experiment were collected from the Tapi river near Bhusawal. (Dist. Jalgaon, M. S. India) and were brought to the laboratory and acclimatized in aquarium for three days to the laboratory conditions. Healthy, active and medium sized fish were selected for the study. These fish were divided into two groups. The first group was the acute treatment group and was exposed to cadmium chloride (LC50/2 of 96hours). The second group which was without any treatment served as the control. The test medium was changed twice a day with the same concentration of the medium. The animals were taken out from experimental water after 24, 48,72 and 96 hours exposure in case of acute treatment. The animals were cleared in water and dissect carefully. After cleaning the soft tissue in distilled water, the tissue was dried at 80c in an oven to constant dry weight, ground to powder and made air tight in specimen bottles by waxing the cork out side. The analysis was carried out on the instrument atomic absorption spectrophotometer at the wavelength of 288,8nm.

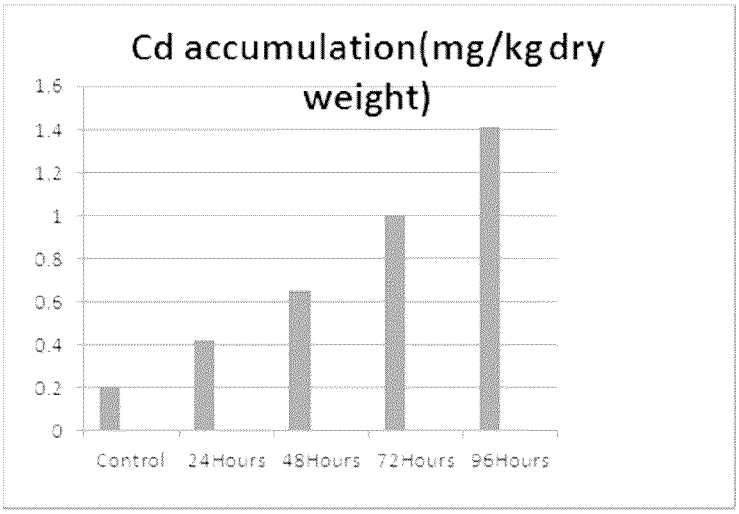
The digestion of samples was carried out as per procedure given in APHA(1985)

Result and Discussion

The accumulation of cadmium in the soft tissue of *Corbicula striatella* was analysed and is presented in table. The cadmium content was exposed in mg/kg dry weight. The control group of animals showed minute quantity of cadmium as compared to the experimental group. The control group showed 0.20mg/kg cadmium in tissues.

In acute treatment after exposure to test concentration (LC50/2 of 96 hours) of cadmium the values of cadmium showed 0.42 mg/kg after 24 hours. At 48hours the tendency of accumulation was 0.65mg/kg. At 72 hours exposure the bioaccumulation was 1.00mg/kg and after 96 hours it was increased to a maximum of 1.41 mg/kg. The rate of accumulation of cadmium was increased with the increase in exposure period and it is also proportional to the concentration of cadmium in water. The values recorded in acute treatment are high up to some exposure period. The values of cadmium from fishes reached 1.41mg/kg dry weight on 96 hours. The cadmium values in tissues are directly proportional to the exposure period.

TABLE: Cadmium content(mg/kg dry weight) in soft tissues of *Corbicula striatella* after acute treatment.



The flow of elements through different trophic level through food chain. Fish are feed on algae, zooplanktons and aquatic plants. Fish are the major bottom feeders in the ecosystem which also have tremendous capacity to accumulate all the microelements present in their food. Fish are considered as the main bioaccumulators of pesticides, heavy metals, toxic chemicals etc. Heavy metals are the class of highly toxic elements, causing great health problem to human life through bioaccumulation from the fish.

Copper bioaccumulation and depuration by red, swamp crayfish, procambarus clarkia was observed by Nagvi et-al. (1998). They concluded that crayfish has a great potential for rapid accumulation and depuration of Cu in freshwater metal concentration in tissues of the freshwater fish, *Capoeta barroisi* from the sehan river was reported by Kargin (1998). Fung et-al. (2004) reported that due to industrial activity the heavy metal concentration such as, As, Cd, Cr, Ni, Pb, Se, Zn, Fe, and Hg, were increased in the body of *Perna viridis* and *Mytilus edulis* in the east coast of china.

Conclusion

The results obtained in this investigation showed great correlation with these obtained by other workers in this field. In response to increased concentrations of cadmium in *Corbicula striatella* accumulates high levels of cadmium.

References

1. APHA (American Public Health Association) American Water Works Association and Water Pollution Control Federation (1985). Standard methods for the examination of water and Waste water 16th Ed., American Public Health Association Washington.
2. Batvari, B.P.D (2007). Heavy metals in two fish species (*Carangoidel malabaricus* and *Belon stronglurus*) from pulicat Lake, North of Chennai, Southeast Coast of India. Environ. Monit. Assess., 145(1-3), 167-175.
3. Fung C.N., Lam J.C.W., zhen G. L ., Connel D.W., Monirith I.,Tanabe S., Richardson B.J., Lam P.K.(2004). Mussel based monitoring of trace metal and organic contaminants along the east coast of china using *Perna viridis* and *Mytilus edulis* . Environ. Pollu. 127(2), 203-216.
4. Fidan, A. F. Cigerci, I.H, Konuk, M.,kucukkurt , I, Aslan, R and Dundar Y. (2007). Determination of some heavy metal levels and oxidative status in *Carassius carrasius* L., 1758 from Eber Lake.Environ. Monit. Assess., 147 (1-3), 35-41.
5. Kargin R. (1998). Metal concentration in tissues of the freshwater fish, *Capoeta barroisi* from the Seyhan River (Tukey). Bull. Environ. Contam. Toxical., 60:822-828.
6. Nagvi S.M., Devalraju I. and Nagvi N.H.(1998) copper Bioaccumulation and depuration by Red swamp Crayfish, *Procambarus clarkia*. Bull. Environ. Contam. Toxicol.61:65-71.
7. Yilmaz, A.B. and Dogan, M.(2007). Heavy metals in water and in tissues of himri (*Carasobarbas luteus*) from Orontes (Asi) River Turkey. Environ. Monit. Assess., 144 (1-3), 437-444.

13. Biotechnology of Microbial Polysaccharide: A Review

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Abstract

Microbial polysaccharides are known to have tremendous important biotechnological applications in pharmaceutical industries, food industries, medical industries. Some industrially important polysaccharide producers are *Leuconostoc mesenteroides, Halomonas eurihalina, Rhizobium meliloti, Agrobacterium tumefaciens, Xanthomonas* sp., *Pseuomonas* sp. and *Azotobacter* sp. Microbial polysaccharides are used as viscosifying, stabilizing, emulsifying and gelling agents in food and pharmaceutical products. Xanthan, gellan, dextran, curdlan, xylilan, pullulan and alginate are among the common microbial polysaccharides in current use.

Keywords: Microbial polysaccharide, Xanthan, Gellan, Dextran, *Xanthomonas Campestris*

Introduction

Polysaccharides are the carbon sources and represent the major constituents of carbohydrates from plants. However some of the microorganisms can produce the polysaccharides as capsular polysaccharide and exopolysaccharides.

Industrially important polysaccharide producers from microbial origin

Several bacterial species are being exploited for commercial production of microbial polysaccharide. Some industrially important polysaccharide producers are *Sphingomonas elodea, Leuconostoc mesenteroides, Halomonas eurihalina, Rhizobium meliloti, Agrobacterium tumefaciens, Agrobacterium radiobacter, Xanthamonas sp., Xanthomonas Campestris,*

Sphingomonas paucimobilis, Zymomonas mobilis, Gluconacetobacter xylinum, Pseudomonas aeruginosa, Pseudomonas putida, Aureobasidium pullulans, Acetobacter xylinum, Azotobacter chroococcum, Alcaligenes faecalis, Azotobacter vinelandii and Leuconostoc Dextranicum [1-8].

Microbial polysaccharides

Some commercially important microbial polysaccharides and their functions are given in Table 1.

Table 1: List of Some commercially important microbial polysaccharides and their uses [9, 10-15]

Polyssaccharide	Microbial source	Use
Xanthan	*Xanthomonas Campestris*	For Salad dressings, in dairy products, syrups, sauces, baked an frozen foods
Gellan	*Sphingomonas paucimobilis*	In jellies, jams and confectionery
Pullulan	*Aureobasidium pullulans*	In snack foods and confectionary decoration
Dextran	*Leuconostoc Mesenteroides*	In Ice cream, frozen and dried foods
Xylinan	*Acetobacter xylinum*	In confectionary product
Alginates	*Azotobacter chroococcum*	Confectionary, Dairy products, Beverages, Jams, Soups, Sauces, Meat, Fish
Curdlan	*Alcaligenes faecalis*	In Freeze-dried foods, Gellies and processed food

Biotechnological applications

Microbial polysaccharides have tremendous important biotechnological applications in pharmaceutical industries, food industries, medical industries and various other related industries [9].

Application of microbial polysaccharides in food industries

Most of the polysaccharides can disperse spontaneously in water and forms hydrocolloids. This property of polysaccharides ensures their uses as stabilizer, emulsifier, thickener, and gelling agent in food industries where products such as bread, sauces, syrup, ice cream, instant food, beverages, and ketchup are manufactured.

Application of microbial polysaccharides in pharmaceutical industries

In the pharmaceutical industry, polysaccharides in the form of hydrocolloids are used in capsule formation, hydrogel, nanoparticles, microsphere, and matrix tablets [10].

Microbial polysaccharide as an alternative to gelatin

Some groups of consumers do not prefer gelatin that is derived from animal sources in the food and pharmaceutical products [11]. In the search of an alternative to gelatin, many researchers have discovered the potential of microbial polysaccharides as viscosifying, stabilizing, emulsifying, or gelling agents that can be used in food and pharmaceutical products [12]. Many microbial polysaccharides exhibit antitumor, antioxidant, antibacterial, antiulcer and cholesterol lowering activities [10, 13].

Robust nature of microbial polysaccharide

Polysaccharides of microbial origin are nontoxic, biodegradable, eco-friendly, and active at extreme temperature, pH, and salinity. Due to such superior and extraordinary properties, polysaccharides from microbial origin are good alternatives to synthetic and natural water soluble gums [17].

Patents on microbial polysaccharide

Many researchers have started for patent filing on production, purification and application methods of microbial polysaccharides from aforementioned microorganisms. Recently the inventors from China have patented the purification method of curdlan polysaccharide from the liquid culture of *Agrobacterium* sp. [18].

Conclusions

Most of the polysaccharide producing bacteria are Gram negative. Microbial polysaccharides are used as stabilizer, emulsifier, thickener, and gelling agent in food industries. They also used in drug formulation systems in pharmaceutical industries. Polysaccharides from microbial origin are boon for mankind since they are having many applications in food and pharmaceutical industries in today's modern era of food and pharmaceutical biotechnology.

Acknowledgements

We are thankful to Hon. Dr. Rafiq Shaikh, Chairman and Shaikh Hasarat Pasha Treasurer of M.E.C.H. & W. Society, Parbhani for overall perpetual inspiration, help and support.

References

1) Chouly C, Colquhoun I.J., Jodele A. (1995) NMR studies of succinoglycan repeating-unit octasaccharides from *rhizobium meliloti* and *agrobacterium radiobacter*. *International Journal of Biological Macromolecules*.17 (6), 357-363.

2) Kaneda I., Kobayashi A., Miyazawa K., Yanaki T. (2002) Double helix of *Agrobacterium tumefaciens* succinoglycan in dilute solution. *Polymers,* 43, 1301-1305.

3) Bekers, M., Upite D., Kaminska E., Laukevics J., Grube M., Vigants A., Linde R. (2005) Stability of levan produced by *Zymomonas mobilis*. *Process Biochemistry*, 40, 1535-1539.

4) Majumder A., Goyal A. (2009) Rheological and gelling properties of novel glucan from *leuconostoc dextranicum* NRRL B-1146. *Food Research International, 42*, 525-528.

5) Huang H.C., Chen L.C., Lin S.B., Hsu C.P., Chen H.H. (2010) In situ modification of bacterial cellulose network structure by adding interfering substances during fermentation. *Bioresource Technology*, *101*, 6084-6091.

6) Schurks N., Wingender J., Flemming H., Mayer C. (2002) Monomer composition and sequence of alginates from *pseudomonas aeruginosa*. International Journal of Biological Macromolecules , 30, 105-111.

7) Celik G.Y., Aslim B., Beyatli Y. (2008) Characterization and production of the exopolysaccharide (EPS) from *Pseudomonas aeruginosa* G1 and *Pseudomonas putida* G12 strains. *Carbohydrate Polymers*, *73*, 178-182.

8) Wu X.C., Chen Y.M., Li Y.D., Li O., Zhu L., Qian C.D., Tao X.L., Teng Y. (2011) Constitutive expression of Vitreoscilla haemoglobin in *Sphingomonas elodea* to improve gellan gum production. *Journal of Applied Microbiology*, *110*, 422-430.

9) Ramalingam C., Jhanu P., Shraddha M. (2014) Applications of Microbial Polysaccharides in Food Industry. *Int. J. Pharm. Sci. Rev. Res*., 27(1), 322-324.

10) Nurul H. A., Shuhaimi M., and Yaakob B. Che Man (2015) Microbial Polysaccharides and Their Modification Approaches: A Review. *International Journal of Food Properties*, 18, 332-347.

11) Maddern, Porcine E.C. (2008) Bovine surgical products, Jewish, Muslim, and Hindu perspectives. Archive Surgery 143 (4), 366-370.

12) Schrieber R, Gareis H. (2007) *Gelatine handbook*, Wiley-VCH GmbH & Co.: Weinhem.

13) Rico C.W., Shin J.H., Um I.C., Kang M.Y. (2011) Cholesterol-lowering action and antioxidative effects of microbial gum in C57BL/6N mice fed a high fat diet. *Biotechnology Bioprocess Engineering*, 16, 167-172.

14) Shetty K., Paliyath G., Pometto A., Levin R.E., Food Biotechnology, Biotechnology of Microbial Polysaccharides in food, Second Edition-Ian w. Sutherland-1.09, 210-211.

15) GarcoAa-Ochoaa F., Santosa V.E., Casasb J.A., GoAmeza E., (2000) Xanthan gum: production, recovery, and properties, *Biotechnology Advances*, 18, 549-579.

16) Bhavani A.L., Nisha J., Dextran, The polysaccharides withversatile uses, International Journal of Pharma and Bio Sciences1(4), 2010.

17) Khattar J.S., Jindal N. (2018) Chapter 4 - Microbial Polysaccharides in Food Industry Biopolymers for Food Design Handbook of Food Bioengineering ,95-123.

18) Patent on 'An isolated polysaccharide curdlan purification method from the microbial broth' (2018) Patent no. CN108752499A.

14. Preliminary Phytochemical Analysis, Antioxidant and Tyrosinase Inhibition Activities of Seeds of Lepidium sativum L.

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Abstract

The present study was to evaluate the phytochemical constituents, antioxidant and tyrosinase inhibition activities of aqueous extract of *Lepidium sativum* L. seeds. Phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, amino acids, reducing sugars, saponins etc. Antioxidant activity was evaluated by using in-vitro antioxidant assay model like DPPH assay and percentage of activity of plant sample was calculated. The present study was revealed that *Lepidium sativum* L. shows significant antioxidant activity. The tyrosinase inhibition activities were also done by using the modified dopachrome method with L-DOPA as a substrate. Assay was conducted in 96-well microtitle plate and a plate reader was used to measure the absorbance at 417 nm. This study of tyrosinase inhibition assay was clear that seeds of *Lepidium sativum* L. were shown inhibition activity against tyrosinase. The phytochemical composition of these plant seeds were tyrosinase inhibition activities due to busting the inclusion of an antioxidant composition.

Keywords: *Phytochemical, Antioxidant Assay, Tyrosinase, L-DOPA* (L-3,4 dihydroxyphenylalanine).

Introduction

Lepidium sativum L. is an yearly herb locally known as halim in India but commonly known as cress. Cress, otherwise known as garden cress, garden pepperwort or garden cress pepper weed, in India in Hindi it is called Halim, Chandrashoor or Asalu. It's a fast growing herb belonging to the family Brassicaceae that is native to Egypt and west Asia but is widely cultivated in temperate climates throughout the world for various culinary and medicinal uses

(Gokavi, S. S.et. al, 2004). *Lepidium sativum* L. Since old times in India, seeds have been used in traditional medicine (McConnell et al., 2007). Numerous studies on plant derivatives suggest that diets rich in phyto constituents and antioxidants have protective roles against various health troubles and diseases (Lampe JW, 1999). Herbal drugs are the oldest form of health care known to mankind (De-Smet and PGAM, 1997). Plants are the rich source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Sofowora, 1993). These plant derived antioxidants might play an important role in combating oxidative stress associated with many degenerative diseases such as cardiological diseases, diabetes, cancer Alzheimer's disease, diabetes and aging (Wong SP et.al., 2006; Naczk M and Shahidi F,2006.) In India, the plant is regarded as a cure for asthma, dysentry, bleeding piles, as a diuretic, and to enhance sexual desire (Dymock, Warden and Hooper, 1890; Chopra, Nayar and Chopra, 1956). Herbal drugs are the therapeutic herbs used to prevent and treat diseases and ailments or to support health and healing (Gossell et al., 2006).

Materials and Methods

Collection of plant material

The *Lepidium sativum* L. seeds under investigation were collected from the local villages of Nanded during 2015. The collected seeds were washed thoroughly first in tap water and then rinsed with distilled water. After this, it was sterilized by using absolute alcohol and dried completely in shade at room temperature. The plant seeds were crushed and blended to fine powder in an electronic grinder and stored in polythene bag till further use.

Preparation of extract

The seeds of the *Lepidium sativum* L. were collected, dried, powdered and did soxhlet extraction successively with distilled water. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until further use.

Phytochemical screening

The presence of various phytochemicals in the plant extract was determined by preliminary phytochemical analysis as per Thimmaiah (2004).

DPPH radical scavenging activity

The antioxidant activity of the extracts was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method (Brand-Williams *et al.*, 2007) with slight modification. Briefly, 2 mL of DPPH solution (0.1mM in methanol) was incubated with 1mL of

extract at concentration of (20 mg/ mL). The reaction mixture was shaken and incubated for 30 min.in dark condition and at room temperature. The control was prepared as above without plant extract. The absorbance of the solution was measured at 517nm against a blank. The free radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated.

Scavenging effect (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

Ascorbic acid (0.1mM) was used as a reference standard.

Results and Discussion

Phytochemical screening

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, reducing sugar, saponins and amino acids in aqueous extracts of *Lepidium sativum* L. seeds. These results exposed that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it was reported that most active components in plants are mostly flavonoids, saponins, glycosides and alkaloids. Further work will be possible to investigate the specific phytoconstituents responsible for these activities (Table- 1).

Table 1: Preliminary phytochemical screening of aqueous seed extract.

Sr. No	Phytochemical test	Aqueous extract	Mucilage extract
1	Alkaloids	++	++
2	Carbohydrates	++	--
3	Flavonoids	++	++
4	Glycosides	++	++
5	Reducing sugars	++	--
6	Saponins	--	--
7	Steroids	--	--
8	Tannins	--	--
9	Amino acids	--	++

++ Presence of constituent; -- Absence of constituent.

DPPH radical scavenging activity (Antioxidant activity)

The DPPH radicals scavenging assay were used for preliminary screening of the aqueous seed extract for the antioxidant activity. The free radical scavenging activity is known as an important mechanism of antioxidants. These results indication towards the seed extract were

found to interact with the stable free radical DPPH, which indicates their potent radical scavenging ability. The aqueous extract exhibited antioxidant property (71.17%), whereas, Mucilage extract showed (60.84%) and the standard used as ascorbic acid showed (86.12%) (Table- 2).

Table 2: Percentage of antioxidant activity of seed extracts.

SR.NO	PLANT NAME	% ANTIOXIDANT ACTIVITY
1	*Lepidium Sativum* L. (Aqueous extract)	71.17±0.31
2	*Lepidium sativum* L. (Mucilage extract)	60.84±0.28
3	Ascorbic Acid (Standard)	86.12±0.22

Values are expressed in means ± S.D. of two separate experiments.

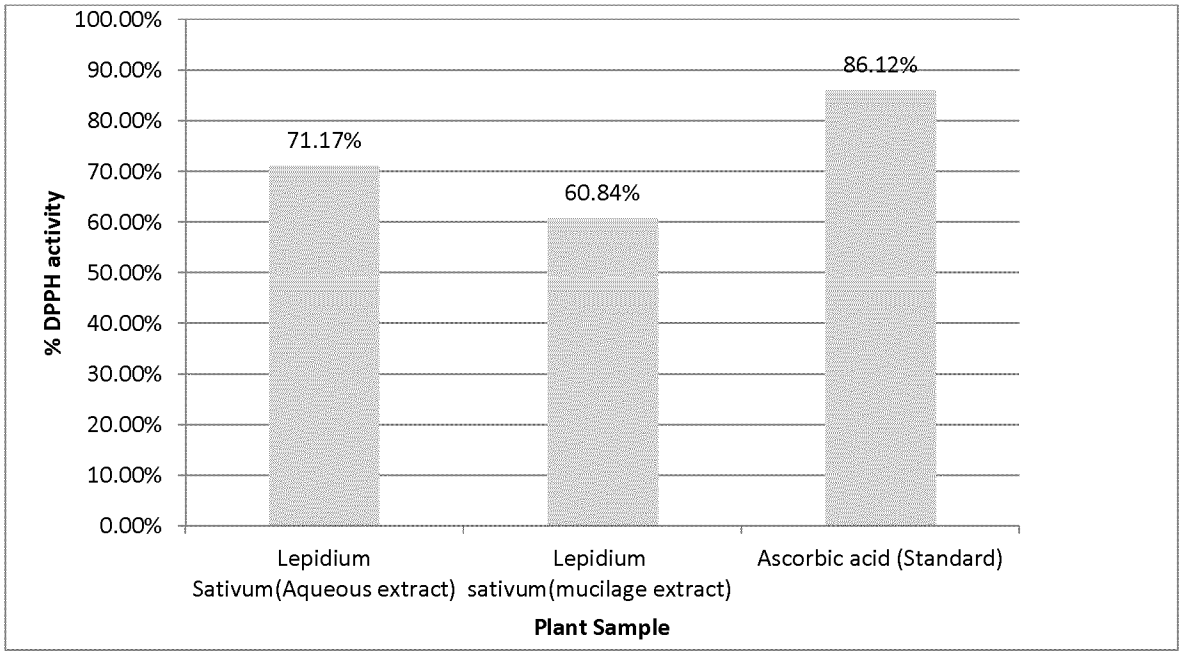

Fig: Graph showing percentage of antioxidant activity of seed extract.

Tyrosinase inhibition:

Tyrosinase involved in the formation of melanins because it facilitates melanin synthesis by catalyzing reaction from tyrosinase to DOPA and from DOPA to DOPA -quinine. Tyrosinase inhibitors have becoming more important as cosmetic and medicinal product, primarily to control melanin pigmentation. Melanin synthesis inhibitors are typically used for treating localized hyper pigmentation in humans such as lentigo, nevus, ephelis, post inflammatory state and melanoma of pregnancy. Mushroom tyrosinase enzyme was used for the

assay because it is gamely available. Since the mode of reticence depends on the structure of both the substrate and inhibitor, L-DOPA was used as the substrate in this experiment, unless otherwise specified. So, the inhibitors discussed in this method are inhibitors of diphenolase activity of mushroom tyrosinase, and their inhibition activity on the enzyme was determined by spectrophotometry, based on dopachrome formation at 417 nm. In the present study the aqueous seed extract exhibited almost good percentage of inhibition for Mushroom tyrosinase at (31units/ml) (Table- 3).

Table 3: Tyrosinase inhibition activity of seed extract.

SR No.	PLANT SPECIES	% TYROSINASE INHIBITION AT CONC.(mg/mL)					
		4	2	1	0.5	0.25	0.125
1	***Lepidium sativum* L.**	81.02±0.12	67.35±0.22	62.12±0.14	56.65±0.30	31.28±0.26	13.85±0.02
2	**Ascorbic acid (Standard)**	85.74±0.21	73.86±0.13	68.23±0.23	60.70±0.32	37.66±0.25	18.23±0.22

Values are reported as means ± S.D. of two separate experiments.

Conclusion

The present study shows aqueous seed of *Lepidium sativum* L. could be used as food supplement in human diet as it possess potent antioxidant activity. It also helps to reduce oxidative stress in human body. The tyrosinase inhibitory activity of *Lepidium sativum* L. seed extract were increased with increasing concentrations. The inhibition of tyrosinase activity might depend on the hydroxyl group on the phenolic compounds of the seed extract, which may form hydrogen bonds with an enzyme site, leading to lower enzymatic activity. Some tyrosinase act through hydroxyl group that bind to the tyrosinase active site, resulting in altered conformation. Ascorbic acid is an effective tyrosinase activity inhibitor. The antioxidant activity mechanism may also be an important reason for tyrosinase inhibition activity. Further pharmacological and clinical studies are required to understand the actual efficiency of these herbal extracts and in future it may be used for ingredients in the formulations of cosmetics and skin whitening agents.

References

- De-Smet and PGAM. The role of plant derived drugs and herbal medicines in healthcare drugs, Indian Drug. 1997; 54: 801-840.

- Gossell M, Simon OR and West ME. The past and the present use of plants for medicines. West Indian Medical Journal, 2006; 55:217.
- Lampe JW: Health effects of vegetables and fruits: assessing mechanism of action in human experimental studies. Am J Clin Nutr 1999, 70:475–490.
- Wong SP, Leong LP, Koh JHW: Antioxidant activities of aqueous extracts of selected plants. Food Chem 2006, 99:775–783.
- Naczk M, Shahidi F: Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharm Biomed Anal 2006, 41:1523–1542.
- Sofowora A. Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd., Ibadan, 1993.
- Gokavi, S. S.; Malleshi, N. G. and Guo, M. Plant. Food. Hum. Nutr. 59 (2004)105–111.
- McConnell et al. (2007). Physical characteristics of vegetable foodstuffs that could influence bowel function. J Sci Food Agric, Vol 25, Issue 12, Pages 1457–1464.
- Chopra, R. N., Nayar, S. L. and Chopra, I. C: Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, 152, 1956.
- Dymock, W., Warden, C. J. H. and Hooper, D.: Pharmacographia Indica, A History of the Principal Drugs of Vegetable Origin. Kegan Paul, Trench, Trubner and Co., Ltd., London 1 2 0 - 1 2 1 . 1890.
- *Thimmaiah SR. Standard methods of biochemical analysis, 2nd ed. New Delhi: Kalyani Press, 2004; 169.*
- Brand-Williams, W., Cuvelier, M.E., Berset, C., *Lebensm Wiss Technol* BSBI Botanical Society of Britain and Ireland. Retrieved, 2007, -10-17.

15. Chromosomal Analysis in Chlorophytum Tuberosum (Roxb.) Baker from Melghat Forest of Amravati District, Maharashtra

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Abstract

Chromosomal Analysis was carried out in *Chlorophytum tuberosum* collected from Melghat forest of Amravati district, Maharashtra. Results revealed considerable variation in karyotypic characteristics and meiotic behavior from the previous investigations. The karyotype is symmetrical but showed some asymmetric characters. There were many chromosome configurations observed during the analysis but from them few are shown here which showed the high percentage of occurrence. The telomeres and the interstial region of the chromosomes in *C. tuberosum* were deeply stained by O-banding and showed a clear zonation of enhanced and reduced type of fluorescence at interstitial region when stained with Quinacrine mustard.

Keywards: *Chlorophytum tuberosum*, Chromosomal analysis, Banding patterns

Introduction

Chlorophytum tuberosum is the member of family Liliaceae, one of the largest plant families with about 240 genera and 4,000 species distributed throughout the world. The genus *Chlorophytum* comprises about 234 species distributed in tropical and subtropical regions; out of these more than a dozen species occur in the Indian subcontinent **(Hooker, 1892; Santapau and Fernandez, 1955).** *Chlorophytum tuberosum* (Roxb.) Baker is one of several species of *Chlorophytum* used in Ayurvedic as well as the traditional medicine. It is a perenial herb distributed throughout India and found in abundance in natural forest areas and commonly known as 'Safed musli'.

The cytological studies has been carried out in *Chlorophytum* which confined that *Chlorophytum* has ploidy from diploid to octaploid with two basic numbers, x=7 and x=8 **(Baldwin and Speese, 1951).** The diploid chromosome number of some species of *Chlorophytum* like *C. tuberosum* is 2n=16 and while other species shows ploidy up to 2n=56 in *Chlorophytum nepalensis* **(Basu and Jha, 2008).**

The species *C. tuberosum* has attracted a number of biologists from time to time to study a range of cytological aspects. According to the literature, most of the studies have been carried out on chromosomal studies in *Chlorophytum tuberosum* during 1960-1980s **(Kumar and Rao,1958 ; Datta and Mitra, 1968; Pahuja and Kumar, 1969; Sheriff and Chennaveeraiah, 1972; Naik, 1977; Chowta and Dyansagar, 1977; Patil *et* al., 1987; Patil and Gandhi, 1988; Jhon *et al.*, 1989; Lekhak *et al.*, 2012)** and the findings of various workers show the variations in karyotype and meiotic chromosome behaviour at every step of analysis but there are very few reports on the banding studies in *Chlorophytum* except in *C. borivilianum* **(Lavania, 2005)** and *C. hyneanum* **(Krisnappa and Nagesh, 1996).** But in the present investigation quinacrine mustard fluorescent banding and orcein banding were carried out to study the differential banding pattern in chromosomal segments of *Chlorophytum tuberosum* which revealed important insights on the numerical and structural chromosome changes involved in the evolution of the genus.

Thus, the present investigation is an attempt to record the chromosomal details in more precise manner with prime objective of chromosome based karyotypic, banding and meiotic variations in *Chlorophytum tuberosum*, the common wild species occurring at Melghat forest of Amravati district and their significance is discussed in the light of modern concepts.

Materials And Methods

Plant Material

Chlorophytum tuberosum was collected from Melghat forest of Amravati district, Maharashtra and grown under suitable condition in the departmental garden. Voucher specimens are deposited in the Herbarium of Botanical Survey of India, Western Circle, Pune (*C. tuberosum* voucher no. SPGCHLT-2).

Cytological Preparations

Root-tips were pretreated with colchicine (0.05%) for about two hours and squashed seperately in 2% aceto-orcein. For morpholgical classification of somatic chromosomes the

nomenclature proposed by **Levan *et* al., (1964)** was followed. Symmetry and asymmetry have been determined according to the system described by **Stebbins (1950).** For meiotic studies, freshly collected flower buds were fixed in a Cornoy's fluid-I and the smears were made in 2% aceto-orcein as well as 2% Acetocarmine. Abnormalities that might impair the meiotic product were taken into account. Orcien Banding (O-banding) and Quinacrine Banding (Q-banding) was carried out by the method prescribed by Vosa, 1976. Photomicrographs were taken mostly from freshly prepared slides using Trinocular Fluorescence Microscope (AXIOSTAR PLUS, M/S Carl Zeiss, Germany).

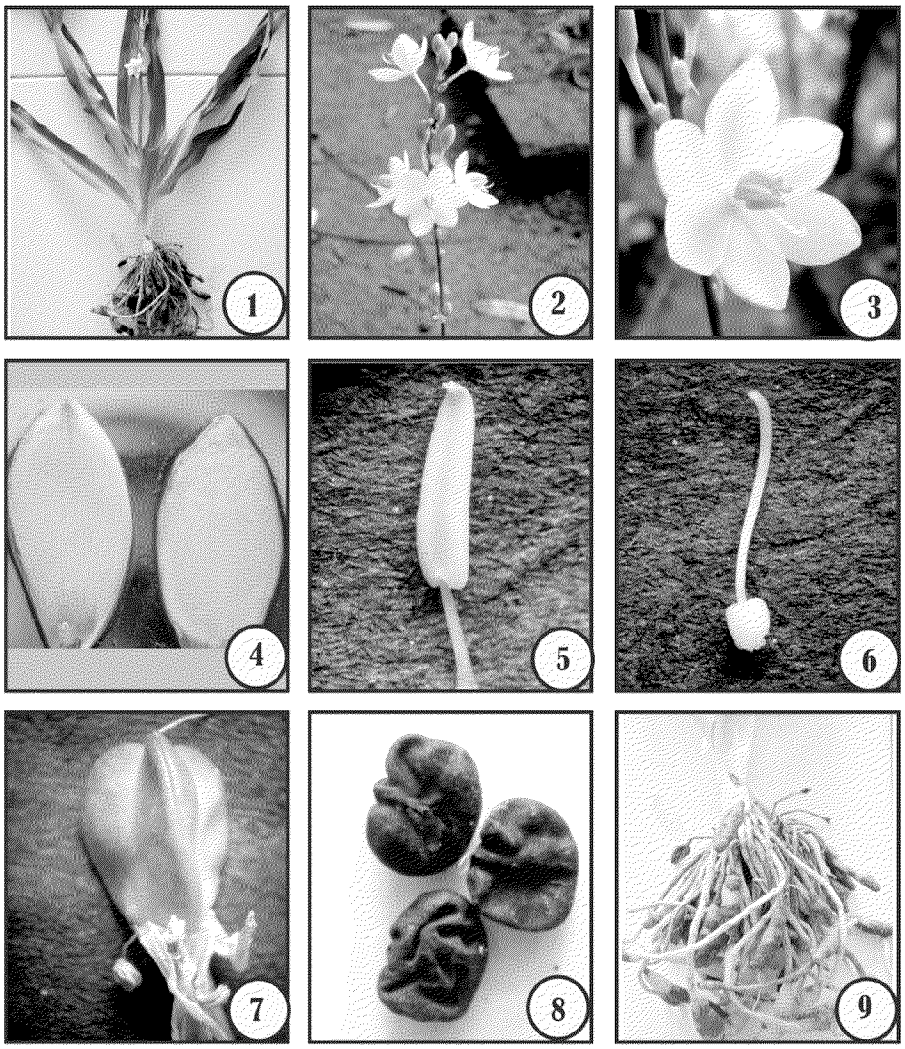

Fig:I-Plant Parts of *Chlorophytum tuberosum*-1) Habit **2)** Inflorescence **3)** Single flower **4)** Outer and Inner tepals **5)** Stamen **6)** Pistil **7)** Capsule **8)** Seeds **9)** Tuberous roots

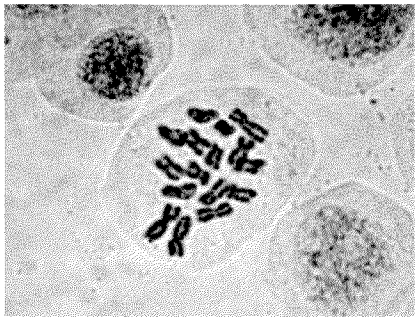

Fig-II-Somatic chromosome count in *Chlorophytum tuberosum* (2n=16)

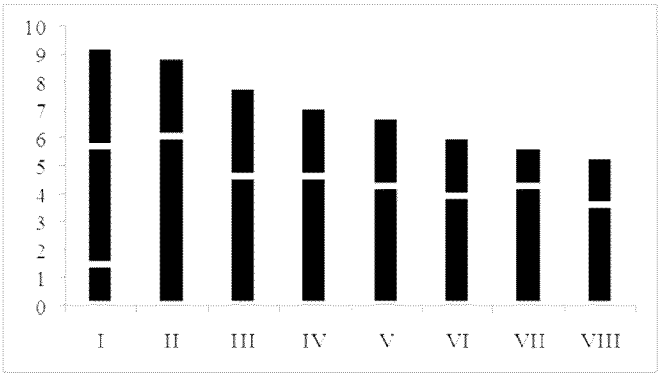

Fig-III-Idiogram of *Chlorophytum tuberosum*

Table 2: Measurements of somatic chromosomes of diploid *C. tuberosum* (2n=16) at metaphase.

Chromosome pair	**(l) µm**	**(s) µm**	**(c) µm**	**(d)**	**(r)**	**(i)**	**RCL %**	**Asymmetry class**	**Centromere Position**
I	4.28+1.42	3.57	9.27	2.13	1.59	38.51	100	C	m
II	6.07	2.85	8.92	3.22	2.12	31.95	96.22	C	sm
III	4.64	3.21	7.85	3.43	1.44	40.89	84.68	C	m
IV	4.64	2.5	7.14	2.14	1.85	35.01	77.02	C	sm
V	4.28	2.5	6.78	1.78	1.71	36.87	73.13	D	sm
VI	3.92	2.14	6.06	1.78	1.83	35.31	65.37	D	sm
VII	4.28	1.42	5.7	2.86	3.01	24.91	61.48	D	st
VIII	3.57	1.78	5.35	1.79	2.00	33.27	57.71	D	sm

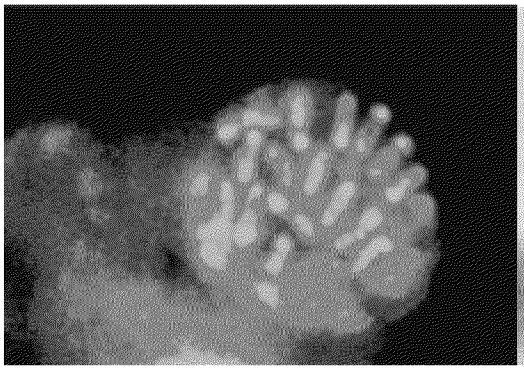

Fig-IV: Q-Banding

Fig-V: Orcien Banding (O-banding)

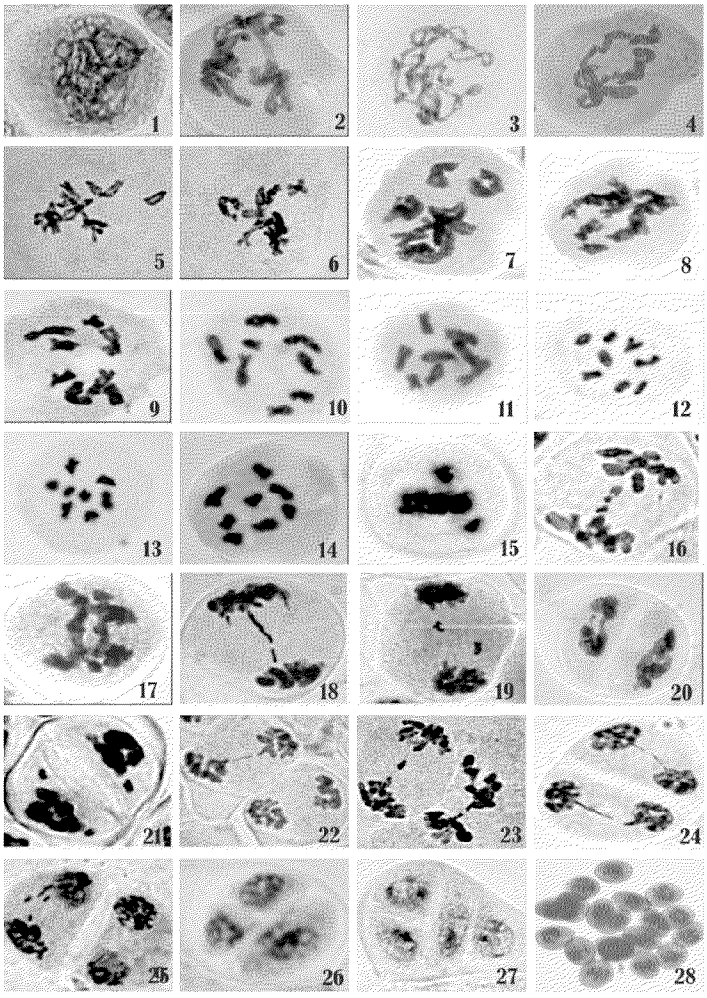

Fig-VI-Meiotic Chromosomal Behaviour in *Chlorophytum tuberosum* (Roxb.) Baker 1-3) Synezetic knot with thicker chromatin strands **4)** Early diplotene **5-8)** Sticky

diplotene with different number of chiasma **9)** Diplotene showing 8II with number of chiasma **10-12)** Diakinesis showing 8II **13)** Metaphase-I showing 1III + 6II +1I **14)** Metaphase-I showing 8II **15)** Sticky Metaphase-I with precocious chromosomes **16)** Anaphase-I with multi laggards forming bridge **17)** Anaphase-I showing dicentric bridge **18)** Late Anaphase-I with single chromatin bridge **19)** Telophase-I with laggards **20)** Metaphase-II with vertical movement of chromatin material **21)** Metaphase-II with lagging chromosomes **22)** Anaphase-II with single chromati bridge **23)** Anaphase-II with bridge in one cell and laggard in another cell **24)** Telophase-II with chromatin bridges **25)** Telophase-II with laggards **26)** Triad (tetrad with unreduced gametes) **27)** Tetrad showing change in orientation **28)** Microspores

Table-1: PMCs showing meiotic irregulararities in *C. tuberosum*

Sr.No.	Phases	No. of PMCs analyzed	Percentage of abnormal PMCs	Abnormalities
	Prophase-I	68/211		Synezetic knot
1.	Metaphase-I	120/227	52.8%	M-I with multivalent, stickyness micronuclei
2.	Anaphase-I	143/197	72.5%	Bridge formation Laggards Chromosome
3.	Telophase-I	102/220	46.3%	Bridge formation micronuclei
4.	Metaphase-II	98/177	55.3%	Change in orientations micronuclei
5.	Anaphase-II	78/120	65%	Bridge formation Laggards Chromosome
6.	Telophase-II	53/189	28%	Bridge formation micronuclei
7.	Diplotene	161/234	68.8%	Sticky diplotene number of chiasma per chromosome
8.	Diakinesis	101/158	63.9%	Multivalent chromosome configurations
9.	Tetrads	77/253	30.4%	Unreduced gametes

Observations and Results

Karyomorphology of *Chlorophytum tuberosum*

The somatic chromosome count of *Chlorophytum tuberosum* was found to be (2n=16) (Fig. II). A somatic chromosome complement consists of 8 pairs of chromosome represented as

an idiogram (Fig. III). The length of chromosome varied from 5.35 to 9.27 µm. The long arm length ranged from 3.57 to 6.07 µm while short arm varied from 1.42 to 3.57 µm. Chromosome pair II showed highest long arm and chromosome I with satellite on its long arm with highest short arm (Table-2). The absolute length of I pair was noted 9.27 µm with centromeric position placed at median position. II pair measured 8.92 µm with centromeric position at sub median position. III pair showed 7.85 µm absolute lengths with centromeric position at median position. The absolute length of IV pair was noted 7.14 µm with centromeric position at sub median position. The absolute length of V pair was 6.78 µm showed sub median position of centromere. VI pair showed 6.06 µm absolute lengths with sub median position of centromere. VII pair measured 5.70 µm in absolute length with centromere at sub terminal position and VIII pair showed 5.35 µm absolute lengths with centromere at sub median position. In this species chromosomes were categorized in C and D type on the basis of their absolute length. Out of all pairs II,IV,V,VI and VIII pair was found to be sub median, VII pair was found to be subterminal and I and III pair was median in position. Arm ratio (r) varied from 1.44 to 3.01. Similarly, centromeric index (i) showed lowest range 24.91 and highest 40.89. The relative chromosome length (RCL %) vary from 57.71 to 100. Hence the smallest chromosome was found to be about 1/2 of the longest one. The total chromosome length (TCL) was recorded 57.07 µm. The total form percent (TF%) was found to be 34.99 and karyotype asymmetry index (As K%) was 68.51. From the above observations the karyotype formula suggested for *C. tuberosum* was $1C(m)^{(sat)}$+1C(m)+2C(sm)+1D(st)+3D(sm). The karyotype is symmetrical but showed some asymmetric characters.

Banding pattern in *Chlorophytum tuberosum*

The application of fluorescent dyes such as quinacrine and its mustard to cytological studies (**Caspersson *et al.*, 1969**) has advanced the understanding of the linear differentiation of the chromosomes of various organisms. The somatic chromosomes of *Chlorophytum* stained with quinacrine showed heterochromatic bands as intense fluorescent bands. The present study focused on the variation of bands in *Chlorophytum tuberosum* which would help for identification of species. But in some chromosomes bands were not observed clearly. Mostly the constitutive heterochromatin i.e. telomere stained darkly and some regions near to centromere were not sharp in Orcein banding in *Chlorophytum tuberosum* (Fig: V). In present study the *Chlorophytum* showed typical QM banding pattern. Q bands were observed as enhanced bands at

distal end in *C. tuberosum* (Fig: IV) and both types of bands i.e. enhanced and reduced bands at intermediate regions were also noticed**.** In Orcein banding preparations, a few cells occasionally show a differentiation of the two types of staining, with the enhanced segments very darkly stained and the reduced segments somewhat lighter stained. The telomeres and the interstial region of the chromosomes (Fig:V) in *C. tuberosum* were deeply stained by O-banding and showed a clear zonation of enhanced and reduced type of fluorescence at interstitial region (Fig:IV) when stained with Quinacrine mustard.

Meiotic analysis of *Chlorophytum tuberosum*

The meiotic behavior of chromosomes in *C. tuberosum* was studied by observing more that 1000 microsporocytes showed normal as well as abnormal meiotic behavior. The different meiotic irregularities from diplotene to tetrads are represented in Table-1. In some PMCs the pairing of chromosomes was perfectly normal and 8 bivalents were seen at diakinesis and metaphase-I in case of *C. tuberosum* (Fig-VI: 10, 11, 12 and 14 respectively). The occurrence of two univalents may be due to the failure in their pairing during prophase-I. Early disjunction of a bivalent at diakinesis would also lead to a similar situation. Multivalent associations are formed and the number of quadrivalents, trivalents, bivalents and univalents formed varied widely from cell to cell. There were many chromosome configurations observed during the analysis but from them few are shown here which showed the high percentage of occurrence.

As the chromosome number of *C. tuberosum* is 2n=16 i.e. n=8 showed different chromosome association at diplotene, diakinesis and metaphase-I. The different number of chiasmata was observed on the bivalent in diplotene and diakinesis. High amount of chromosome stickiness was another abnormality was observed in maximum stages of meiosis (Fig.VI: 5, 6, 7, 8, 15, 16, 17, 18, 20, 21, 22, 23 and 24). Precocious chromosome accessions and non congressed bivalents were observed at M-I (Fig. 15). Lagging of chromatin material in the form of 1-2 laggards (Fig. 16, 19, 23 and 25) and chromatin bridges (Fig. 17, 18, 22, 23 and 24) in A-I/T-I and A-II/T-II and late disjunction of some bivalents were also observed in some PMCs (Fig. 20 and 21). These percentages were high compared to other abnormalities. After the completion of T-II, the daughter nuclei may divide or the division in one may fail resulting in the formation of triads. Fig No. 26 shows three microspores of varying sizes. Few tetrads showed change into orientation (Fig. 27) and the microspores (Fig. 28).

Along with the normal meiocytes the frequency of aberrant meiocytes was high in A-I, M-I, A-II, M-II and T-II than the earlier stages. Unequal distribution of chromatin material is a significant parameter seen. This resulted in abnormal end products in microspores form. This tends to uneven size of chromatin.

Discussion

The *Chlorophytum tuberosum* investigated presently showed gametic number of n=8, which is confirmed from the 8II and different configurations of chromosomes. The transfer of chromatin material during microsporogenesis caused various meiotic abnormalities such as interbivalent connections, chromosome stickiness, laggards, bridges, late disjunction, pyknotic chromatin and unorganized chromatin threads **(Singhal and Kumar, 2008)**

The meiotic analysis of *Chlorophytum comosum* showed meiotic abnormalities like chromosome stickiness, irregular spindle and meiotic aberrations such as laggards, chromosomal bridges, micronuclei, abnormal cytokinesis and chromatin pulling. The meiocytes showed single microspores (monad), dyad to polyads and variations in orientation of triad. The formation of linear tetrads and unequal distribution of chromatin material in polyads was also observed in *C. comosum* **(Gudadhe *et al*, 2012).** Such type of irregularities was also visible in *C. tuberosum* except the tetrad variation. The formation of unreduced gametes is of evolutionary significance in that it can lead to the production of plants with higher ploidy through polyploidization **(Villeux, 1985)** such condition was found in *C. tuberosum*.

Occurrence of lagging univalents may be the direct consequence of presence of univalents which failed to disjunct to the opposite poles in an orderly fashion. Unpaired chromosomes (Univalents) in diploid taxa are reported to be the main cause of abnormal distribution of chromosomes at anaphase-I and that of chromatids in anaphase-II **(Sybenga, 1972; Singh, 1993).** The extra chromatin masses present in the PMCs do not pair with the main chromatin and remain in the cell as a separate mass. The fate of such additional masses of chromatin is not known, but they probably form micronuclei **(Bhat *et al*, 2006).** The species showed precocious chromosome segregation in metaphase-I and II (Fig. 15 and 21), lagging chromosomes in anaphase-I and II (Fig. 16 and 23) and micronuclei in telophase I and II (Fig. 19 and 25).

Chromosome stickiness is caused due to genetic and environmental factors and several agents have been reported to cause chromosome stickiness **(Pagliarini, 2000)**. **Gaulden (1987)**

postulated that stickiness may result from the defective functioning of one or two types of specific non histone proteins involved in chromosome organization which are needed for chromosome separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them (hereditary stickiness) or by the direct action of mutagens (induced stickiness). The phenomenon of chromosome stickiness was recorded from early prophase-I and persisted till the second meiotic division. However it was more frequent in MI, AI and AII (Fig. 15, 17, 22 and 23) Due to this, the chromosome lost their individuality completely. In severe cases, chromosome stickiness resulted in thick chromatin bridges was the next anomaly encountered in most of meiocytes which include linking chromosomes together in metaphase and causing them to form bridge (s) in the anaphase (Fig. 16, 17 and 18), a process which could continue up to the telophase (Fig. 24) and also showed the change in orientation of metaphase II (Fig. 20). The thickness of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes. This leads to loss of genetic material and give rise to polyploids.

C. tuberosum (Fig. III) showed symmetrical karyotype, satellite was present on longer arm of Ist pair of median chromosome which is highly asymmetric character. **Stebbins (1950)** stated that the satellite is borne on the short arm of a chromosome with the subterminal centromere or in one of the arms of V-shaped chromosome and usually a pair of satellites is observed in diploid plants like *C. tuberosum* (**Pahuja and Kumar, 1969**). The *Chlorophytum tuberosum* differs from other species of the genus in having the satellite born on the longer arm of submedian chromosomes (**Chowta and Dnyansagar, 1977**) such conditions has been observed by **AL-Fakhry *et al.* (1964)** in *Penicetum typhodium* and by Sharma and Mukhopadhya, (1965) in *Arisaema tortuosum* and *A. sanguineum.* It is also confirmed by the TF % and As K % values. In *C. tuberosum* there is uniformity in the reports of chromsome number (2n=16) while the number and position of secondary constrictions have been variously described. **Pahuja and Kumar (1969)** and **Sheriff and Chennaveeraiah (1972)** reported two chromosomes with secondary constriction. Further, only **Sheriff and Chennaveeraiah (1972)** have recorded it to be interstitial in the first pair of chromosomes while all others have noted it in the subterminal position in the short arm of second pair. **Patil and Patil (1987)** recorded satellite on the short arm of II pair in *C. tuberosum* and it appeared to be intermediate in the karyotype asymmetry. The karyotype asymmetry index is a good expression of the general morphology of

plant chromosomes. It would therefore be advantageous to have a uniform system whereby the karyotypes of related genotypes and species could be compared (**Paszko, 2006**). There are the variations in chromosome morphology and the number of submetacentric and subtelocentric chromosomes observed in present study and previous reports because the species were collected from different geographical regions. Karyotypic variability in different population of same species inhabiting more or less the same geographical region is suggestive of its possible wide spread occurrence in population endemic to widely different geographical regions may have served as potential factor in evolution of species specially at the diploid level (**Mathew and Thomas, 1974**).

In most cases investigation of plant chromosomes is carried out by the C-banding method. This method gives contrast banding patterns, but the number of bands is not always sufficient to distinguish the differences between banding patterns of non-homologous chromosomes and in many cases does not permit the reliable identification of chromosomes, particularly the small ones.

As reported by earlier Giemsa C-terminal banding was found to be variable in different arms of chromosomes in *C. heyneanum* and the complete absence of centromeric heterochromatic bands which appear to be most common in mammals (**Krishnappa and Nagesh, 1996**).

Cytological features like low TF value, low total chromatin length (TCL) and more submetacentric chromosomes are taken to indicate the relative advancement of a species within a genus (**Stebbins, 1950**). With respect to number of submetacentric chromosomes, TCL and TF value, *C. tuberosum* seems to be more evolved. Evolution in all characters may not progress at the same rates in a single species and therefore a species may have some advance and some primitive features.

References

1) Al-Fakhry, A. K. Sarvella P. And Gregan C. O. (1964) Somatic Chromosome of *Babala. J. Hered* 55: 57-60.
2) Baldwin J.T and Speese B. M. (1951) Cytogeography of *Chlorophytum* in Liberia. *Amer.J.Bot*, 38: 153-156.
3) Basu S. and Jha T. B. (2008) *Chlorophytum nepalense* Baker. an unexplored plant of potential economic value. *Curr. Sci.* 95 (4): 439.

4) Bhat T.A., Parven S and Khan A.H. (2006) MMS-induced cytomixis in pollen mother cells of board bean (*Vicia faba* L.); *Turk. J. Bot.* 30: 273-279

5) Caspersson T.; Zech L.; Modest E. J.; Foley G. E.; Wagh U. and Simonsson E.

6) (1969) Chemical differentiation with fluorescent alkylating agents in *Vicia faba*

7) metaphase chromosomes. *Expl. Cell. Res*. 58: 128-140.

8) Chowta C.D And Dnyansagar V.R (1977) Karyotype Analysis In *Chlorophytum tuberosum* Baker. *Journal of The University of Bombay* 35-43.

9) Datta N. and Mitra K. (1968) Karyotype analysis of *Chlorophytum tuberosum* Baker. and *Chlorophytum laxum* R.Br. *Bulletin of Botanical Survey of India* 10: 228.

10) Gaulden, M. E. (1987). Hypothesis: some mutagenes directly alterspecific chromosomal proteins (DNA) topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis* 2:337-365.

11) Gudadhe, S. P., Nathar, V. N. And Dhoran, V. S. (2012). Meiotic Abnormalities In *Chlorophytum comosum* (Thunb) Jacq. *International Journal of Research in Plant Science* 2 (2): 29-34.

12) Hooker, J. D. (1892). *The Flora of British India*. 23-A, Connaught Place, Dehra dun, India 6: 227-362.

13) John C. K.; Somani V. J. and Thengane R. J. (1989) Some interesting features of male meiosis in *Chlorophytum tuberosum*. Buker. *Curr. Sci.*58 (5): 254-255.

14) Krishnappa D. G. and Nagesh N. (1996) Priliminary studies on Giemsa C-banding pattern in chromosomes of *Chlorophytum attenuatum*. *Journal of Cytology and Genetics* 31(2): 221-223.

15) Kumar L. S. S. and Rao H. K. S. (1958) Chromosome number in *Chlorophytum tuberosum* Baker. *Curr. Sci*. 27**:** 406-407.

16) Lavania U. C.; Basu S.; Srivastava S.; Mukai Y. and Lavania S. (2005) *In situ* chromosomal localization of rDNA sites in "safed musli" *Chlorophytum* Ker-Gawl. and their physical measurement by fiber FISH. *Journal of Heredity* 96 (2): 1-6.

17) Lekhak M. M.; Adsul A. A. and Yadav S. R. (2012) Cytotaxonimical investigations in to the genus *Chlorophytum* from India. *Kew Bulletin*. 67: 1-8.

18) Levan A.; Fredga K. and Sandberg A. A. (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

19) Mathew P. M. and Thomas K. J. (1974) Cytological studies on *Chlorophytum. New Botanist* 1(1+2): 34-45.

20) Naik, V.N (1977) Cytotaxonomic Studies of Six Indian Species of *Chlorophytum .Bot. J. Linn.Soc.*74:297-308.

21) Pagliarini, M. S. (2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genetics and Molecular Biology* 23: 997-1002.

22) Pahuja A.N And V. Kumar (1969) Cytogeography Of Three Species Of *Chlorophytum. Curr.Sci*. 5(19):469-471.

23) Paszko, B. (2006). A Critical Review and a New Proposal of Karyotype Asymmetry Indices. *Plant Systematics and Evolution* 258: 39-48.

24) Patil S.G and V.P Patil (1987) Stomatal Studies in the Genus *Chlorophytum* and their Taxonomic Significance. *Phytomorphology*. 37(2, 3) :155-158.

25) Patil V.P and S. Gandhi (1988) Inter-specific Variations chiasma Frequencies and terminalization in *Chlorophtum* ker-gawl. *Cytologia* (Tokyo) 53:571-575.

26) Santapau H. and R.R. Fernandes.(1955) A new species of *Chlorophytum borivilianum* from Salsette Island. *J. Bombay nat. Hist. Soc.* 52: 897.

27) Sharma A. K. And Mukhopadyay (1965) Chromosome Studies in *Typhonium* and *Arisaema* with a view to find out the mode of origin and affinity of the two. *Cytologia*. 30: 58-66.

28) Sheriff A. And M.S Chennaveeraiab (1972) Karyomorphology of Four Diploid Species of *Chlorophytum. The Nucleus* XV (I):39-45.

29) Singh R. J. (1993) Plant Cytogenetics. CRC Press, Inc. Boca Raton, Florida, USA.

30) Singhal, V.K. and P. Kumar (2008) Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeate* Royle) *J. Biosci*. 33(3): 371–380.

31) Stebbins G.L. (1950) Variation & Evolution In Plants, Columbia University Press.

32) Sybenga J. (1972) General cytogentics. North Holland Publishing Co. Amsterdam

33) Villeux, R. (1985). Diploids And Polyploid Gametes In Crop Plants: Mechanism of Formation and Utilization In Plant Breeding. In: *Janick J (Ed) Plant. Breed. Rev.* 3 AVI, New York: 442.

16. Ethion Induces Behavioral Changes of Fresh Water Fish Clarias batrachus, Godavari River, Nanded, Maharashtra

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Abstract

Ethion is the pesticide used to control the pests of fruits and vegetables which ultimately contaminate the water along with aquatic ecosystem. The fresh water fish, *Clarias batrachus* was selected for present experimentation. The fishes were collected and brought to the laboratory for acclimatization. The freshwater fishes were subjected to different concentrations of Ethion. The LC_{50} value for 96 hours exposure to Ethion was found to be 0.060 µ ml/L. The behavior of fishes was observed for 24, 48, 72 and 96 hours. Alteration in the behavior of the fish *Clarias batrachus*, was studied. In order to protect aquatic ecosystem along with man, awareness must be initiated to the farmer to control agricultural pest by biological methods and using organic manure in proper dose.

Key Words: Ethion Pesticide, Freshwater Fish, *Clarias batrachus,* Behavior, Godavari River, Nanded (Maharashtra).

Introduction

The water contamination and the heavy traces of pollutants is serious problem of recent era. Heavy modernization, industrialization and evolution in farming cause the several changes in the natural water bodies. All insecticides, chemical manure and pesticides consumed to control the pest eventually pollute the water resources and alternately cause the serious hazards to aquatic life (Konar, 1975).

The pesticide pollution is harmful to human, animals and wild life fauna by directly through pesticides or their degraded products. Any changes in chemical composition of the natural aquatic biota generally affect the behavioral and physiological systems of the inhabitants (Edward, 1973).

To cope of this situation the exact loss should be studied and hence in present investigation fresh water fish *Clarias batrachus* has been used as an indicator organism. *Clarias batrachus* is one of the Indian major carp edible fresh water fish of a great economic importance. The effect of Ethion Pesticide on behavioral alterations has been studied in detail.

Material and Methods

The fresh water fish *Clarias batrachus* were collected through fisherman from Godavari River, Nanded. They were brought to the laboratory and cleaned by using 0.1% Potassium Permanganate solution to avoid dermal infection. Only healthy fishes ranging between 100-125 gms were selected for experiments. They were acclimatized in glass aquarium for 07 days and water in the aquaria was replaced every day.

The assessment of toxicity of Ethion with reference to aquatic biota especially fresh water fish is crucial in toxicity evaluation. The LC_{50} value for 96 hours exposure to Ethion was found to be 0.060 μ ml/L.

Results

Various symptoms of poisoning have been observed from studies of determination of LC_{50} as well as many alteration in the behavior of the fish *Clarias batrachus* were remarkably noted. Dube et.al, (2012) showed acute toxicity of mercuric chloride with behavioral changes on fresh water crustacean *Barytelphusa guerini.*

Behavioral Observations of Control and Experimental Fish

Animal behavior is the highly specialized characteristics of every animal. Normal behavioral of animal is sign of healthy animal. Aquatic biota also exhibits various normal behaviors of animal including fishes. In present investigation, the control set of experiments were showed various normal behavioral activities of fresh water fish *Clarias batrachus* such as they were very active with their well co-ordinated movements. They were very alert at slightest disturbance. They were swimming and breathing normally.

But at sub lethal concentration of Ethion, they become irritable and hyper excited at initially along with jumping movement. They showed changes in their skin color at sides and mouth, barbeles becomes reddish and on the caudal fins black spots seen. The fishes were became restlessness and finally fish turned upside down. At last period of lethal dose the mucus secretion and loss of equilibrium were also observed. They slowly become sluggish and finally they settle down at the bottom prior to death. Schein and Cairns (1966), Panigrahi and Konar

(1990) studied and shown that the abnormal behavioral by test fish may be attributed to impairment of nervous and sensory system at fish to pesticide.

Discussion and Conclusion

The problem of water pollution is burning issue of recent era and human being has to face the crucial situation. The aquatic life is strongly influenced by agro-pollutants including insecticides, pesticides like Ethion and frequently cause serious hazards to aquatic life. Among the aquatic fauna, fishes are affected significantly than other aquatic animals. Many people have worked on the effects of different pesticides and heavy metals on the physiology and biochemical aspects of different animals (Tungare et.al. 2000; Mali, 2010; Jagtap, 2009; Shailaja, 2008 and Rajaiah, 2007) toxicity evaluation.

Ethion is used as effective pesticide on citrus and apples. It is used to kill aphids, mites, scales, leafhopper, maggots & foliar feeding larva. Ethion is a colorless and insoluble in water. It has very disagreeable odor. It is highly toxic by inhalation, dermal exposure and ingestion. It is very irritating to the eye and caused slight inflammation and redness in the eye & skin. It is used on wide variety of food, fiber & ornamental crops including green house crops, lawns and turf. The chemical formula of Ethion is $C_9H_{22}O_4P_2S_4$. Ethion pesticide is helpful to the farmer to control the pests, but farmers are not using in proper format. They are not using the pesticide with recommended dose. This excess amount of pesticide ultimately causes damage to soil and water.

The toxicity evaluation showed that excess use of pesticide residues alters the molecular, cellular, biochemical and behavioral changes in fresh water fish *Clarias batrachus*. It may be dangerous the human being if fruits or vegetables or water contaminated by Ethion used in daily life.

Hence, it is suggested that the Ethion is not safe to non-target organisms like fishes. In order to protect the whole aquatic ecosystem along with man, awareness must be initiated to the farmer to control the agricultural pest by biological methods and using organic manure properly, somehow if there is no alternative to use the Ethion, farmer should use it in only recommended dose.

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References

1. **Dube K. V. and R. P. Mali (2012):** Acute toxicity of mercuric chloride on freshwater crab, *Barytelphusa guerini*. J. Environ and Sociobiology: 9(2): 121-124.
2. **Edward C. A. (1973):** In: *Environmental pollution by pesticides.* (Plenum Press New York)
3. **Jagtap, A. R. Afsar, S.K. Kothole, S.D. and Mali, R.P. (2009):** Effect of pollutant from car washing centre on oxygen consumption in freshwater fish, *Channa punctatus, J. Aqua. Biol.*24 (2): 189-192.
4. **Konar, S. K. (1975).** Pesticide and aquatic ecosystem. Ind. J. Fish., 22: 80-85.
5. **Mali, R. P. Afsar, S. K. and Jagtap. A. R. (2010):** Impact of cadmium induced alterations in the glycolytic potential of freshwater female crab *Barytelphusa guerini. Geobios*. 37:100-102.
6. **Panigrahi, A. K. and Konar, S. K. (1990):** Sbulethal effects of mixture of crude petroleum oil and anionic detergent on fish. Env. J. Col., 8: 877-822.
7. **Rajaiah, V. and Venkaiah Y. (2007):** Effect of parathion on esterase patterns of *Channa punctatus. J. Aqua. Biol*. 22(1): 181-185.
8. **Schein, A. and Cairns, J. Jr. (1966):**. Persistence of gill damage in *Leopomis gibbosus* following brief exposure of alkyl benzene sulfonate. Not. Nat. Acad. Nat. Sci., Philadelphia, 392: 1-8.
9. **Shailaja, V., Madhuri, E., Ramesh Babu, K, Rama Krishna, S. and Bhasker, M. (2008)**: Study of exposure to altered pH media. *Ecology and Fisheries.* 1(1): 85-92.
10. **Tungare, S.M. and A.D. Sawant (2000):** Physiological effects of heavy metals on prawns. International conference on Probing in biological system in Mumbai Feb. 2000 pp. 139.

17. Toxicity Evaluation of Ethion Pesticide on Fresh Water Fish Clarias batrachus from Godavari River, Nanded, Maharashtra

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Kadam M. S.

Abstract

Aquatic Toxicity is burning issue of today's life. The toxicity of pesticide, Ethion to the fresh water fish *Clarias batrachus* was studied for 24, 48, 72 and 96 hours. This toxicity with reference to aquatic biota especially fresh water fish is crucial in toxicity evaluation. The LC_{50} value for 96 hours exposure to Ethion was found to be 0.060 µ ml/L. Ethion is the pesticide use to control the pests of fruits and vegetables which ultimately contaminate the water along with aquatic ecosystem. In present study, various symptoms of poisoning has observed and used for determination of LC_{50} of the fish *Clarias batrachus,* was studied. In order to protect aquatic ecosystem along with man, awareness must be initiated to the farmer to control agricultural pest by biological methods and using organic manure in proper dose.

Key words: Ethion Pesticide; Freshwater Fish, *Clarias batrachus;* LC50.

Introduction

The effect of pesticides, organic and inorganic substances on aquatic animals like crabs, fishes, amphibians and other animals are the important aspects of chemical contamination of environment. Many chemical pesticides such as organochlorine, carbamate, organophsophate, fungicides, herbicides etc. are useful in agriculture and equally important against pest that causes diseases of animals and human beings. But a enormous use of such pesticides or chemicals give the research workers their attention towards aquatic pollution (Mali et.al., 2002; Sastry et.al., 1979; Tungare et.al, 2000).

The water contamination and the heavy traces of pollutants is serious problem of recent era. Heavy modernization, industrialization and evolution in farming cause the several changes

in the natural water bodies. All insecticides, chemical manure and pesticides consumed to control the pest eventually pollute the water resources and alternately cause the serious hazards to aquatic life (Konar, 1975).

To cope of this situation the exact loss should be studied and hence in present investigation fresh water fish *Clarias batrachus* has been used as an indicator organism. The toxicity evaluation after exposure to Ethion has been studied in detail.

Clarias batrachus is one of the Indian major carp edible fresh water fish of a great economic importance. In present work, the impact of Ethion is studied on behavioral changes of the given species.

Material and Methods

The fresh water fish *Clarias batrachus* were collected through fisherman from Godavari River, Nanded. They were brought to the laboratory and cleaned by using 0.1% Potassium Permanganate solution to avoid dermal infection. Only healthy fishes ranging between 100-125 gms were selected for experiments. They were acclimatized in glass aquarium for 07 days and water in the aquaria was replaced every day.

Results

The percentage mortality of fish in various concentration of Ethion was determined at 96 hours exposure was estimated by Fenny et, al. The fishes divided into 10 groups and each group having 10 fishes were exposed to different concentration of Ethion ranging from 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09. One control group was also maintained along with experimental set simultaneously.

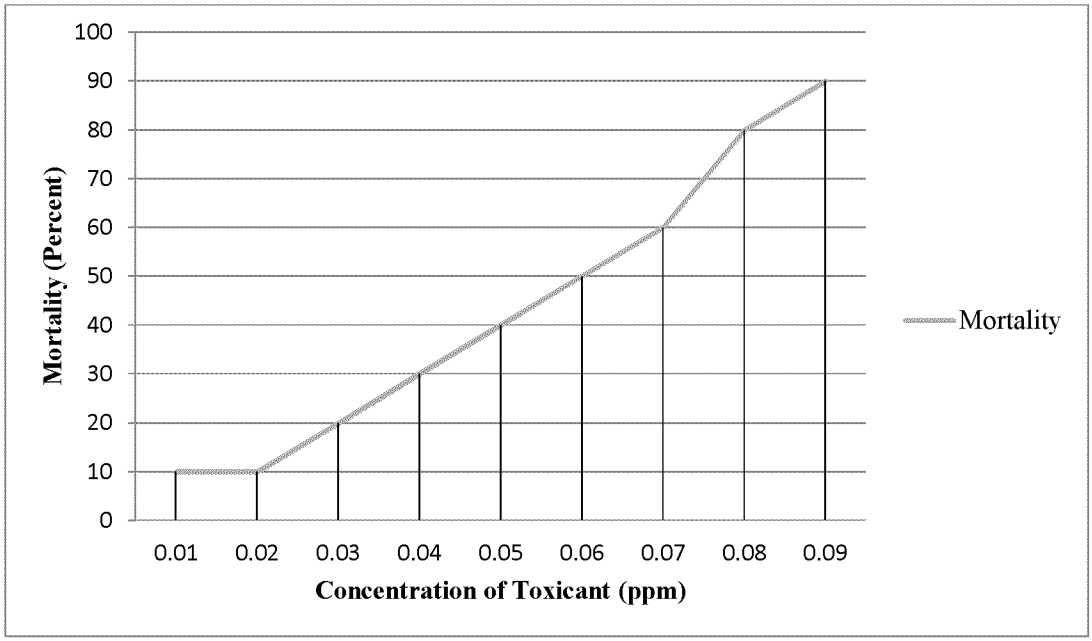
Toxicity Evaluation

Sr. No.	Conc. at Toxicant (PPM)	No. of animals	Exposure Period (Hours)				Dead Animals	Mortality (Percentage)
			24	48	72	96		
1	0.01	10	-	01	-	-	01	10
2	0.02	10	-	-	01	-	01	10
3	0.03	10	-	01	01	-	02	20
4	0.04	10	-	01	01	01	03	30
5	0.05	10	-	01	01	02	04	40
6	0.060	10	01	01	02	01	05	50
7	0.07	10	01	02	02	01	06	60

8	0.08	10	01	02	01	04	08	80
9	0.09	10	01	02	03	03	09	90

Graphical Representation

Graphical representation showing Pesticide Ethion concentration (in ppm) Vs Mortality (in percent) in fresh water fish, *Clarias batrachus*.



The assessment of toxicity of Ethion with reference to aquatic biota especially fresh water fish is crucial in toxicity evaluation. The LC_{50} value for 96 hours exposure to Ethion was found to be 0.060 µ ml/L.

Various symptoms of poisoning have been observed from studies of determination of LC_{50} fresh water fish *Clarias batrachus* were remarkably noted. Dube et. al. (2012) showed acute toxicity of mercuric chloride on fresh water crustacean *Barytelphusa guerini*

Discussion

The contamination of pesticides in ecosystem constitutes an immense environmental stress. This results in pesticides on river, lakes and streams etc. Many pesticides reaches the aquatic environment following spraying operations. Therefore, usage of pesticides has impact on environment, leading to the development of various types of adjustments, adaptations, such as

morphological, physiological, biochemical and behavioral etc., in aquatic life at various levels of organization in the organisms to suit their environment. The rapid increase in use of insecticides in agriculture poses serious hazards to aquatic animals (Edward, 1973, Barde, 2014).

The problem of water pollution is burning issue of recent era and human being have to face the crucial situation. The aquatic life is strongly influenced by agro-pollutants including insecticides, pesticides like Ethion and frequently cause serious hazards to aquatic life. Among the aquatic fauna, fishes are affected significantly than other aquatic animals. Many people have worked on the effects of different pesticides and heavy metals on the physiology and biochemical aspects of different animals (Mali, 2010; Jagtap, 2009; Sailaja, 2008 and Rajaiah, 2007) toxicity evaluation. In the study of aquatic toxicology, fish is used as indicator fish and play an important role in toxicity testing and evaluation.

Ethion is used as effective pesticide on citrus and apples. It is used to kill aphids, mites, scales, leafhopper, maggots & foliar feeding larva. Ethion is a colorless and insoluble in water. It has very disagreeable odor. It is highly toxic by inhalation, dermal exposure and ingestion. It is very irritating to the eye and caused slight inflammation and redness in the eye & skin. It is used on wide variety of food, fiber & ornamental crops including green house crops, lawns and turf.

Even though it is helpful to the farmer to control the pests, but farmers are not using in proper format. They are not using the pesticide with recommended dose. This excess amount of pesticide ultimately causes damage to soil and water. The toxicity evaluation showed that excess use of pesticide residues alters' the molecular, cellular, biochemical and behavioral changes in fresh water fish *Clarias batrachus*. It may be dangerous the human being if fruits or vegetables or water contaminated by Ethion used in daily life.

Hence, it is suggested that the Ethion is not safe to non-target organisms like fishes. In order to protect the whole aquatic ecosystem along with man, awareness must be initiated to the farmer to control the agricultural pest by biological methods and using organic manure properly, somehow if there is no alternative to use the Ethion, farmer should use it in only recommended dose.

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References

1) **Ambore N. E. (1976):** Studies on some aspects of physiology of a fresh water crab with special reference to sex and size. Thesis submitted to Marathwada University, Aurangabad.
2) **Barde. R. D. (2014):** Cardiac Physiology of Freshwater Water Male Crab, Barytelphusa guerini under Sumidon and Acephate Stress, International Journal of Biolife; Vol. 2 (4), pp. 1199-1202
3) **Edward C. A. (1973):** In: *Environmental pollution by pesticides.* (Plenum Press New York)
4) **Jagtap, A. R. Afsar, S. K. Kothole, S. D. and Mali, R. P. (2009):** Effect of pollutant from car washing centre on oxygen consumption in freshwater fish, *Channa punctatus, J. Aqua. Biol.* 24 (2): 189-192.
5) **K. V. Dube and R. P. Mali (2012)**: Acute toxicity of mercuric chloride on freshwater crab, *Barytelphusa guerini*. J. Environ and Sociobiolgy: 9(2): 121-124.
6) **Konar, S. K. (1975).** Pesticide and aquatic ecosystem. Ind. J. Fish., 22: 80-85.
7) **Mali R. P. and Ambore N. E. (2002):** Studies on some aspects of physiology of fresh water female crab, B. Guerini with speical reference to inorganic. pollutants. Ph.D. thesis submitted to S.R.T.M.U. Nanded
8) **Mali, R. P. Afsar, S. K. and Jagtap. A. R. (2010)**. Impact of cadmium induced alterations in the glycolytic potential of freshwater female crab *Barytelphusa guerini. Geobios*. 37:100-102.
9) **Panigrahi, A. K. and Konar, S. K. (1990)**. Sbulethal effects of mixture of crude petroleum oil and anionic detergent on fish. Env. J. Col., 8: 877-822.

10) **Rajaiah, V. and Venkaiah Y. (2007)**. Effect of parathion on esterase patterns of *Channa punctatus*. *J. Aqua. Biol*. 22(1): 181-185.

11) **Sastry, K. V., Gupta, P. K. and Malik, P. V. (1979):** A comparative study of effect of acute and chronic treatement of Hgcl2 on a teleost fish, *Channa punctata. Bull. Environ. Contam; & Toxicol.*, 22; 28-34.

12) **Schein, A. and Cairns, J. Jr. (1966)**. Persistence of gill damage in *Leopomis gibbosus* following brief exposure of alkyl benzene sulfonate. Not. Nat. Acad. Nat. Sci., Philadelphia, 392: 1-8.

13) **Shailaja, V., Madhuri, E., Ramesh Babu, K, Rama Krishna, S. and Bhasker, M. (2008)**: Study of exposure to altered pH media. *Ecology and Fisheries*. 1(1): 85-92.

14) **Tungare, S.M. and A.D. Sawant (2000):** Physiological effects of heavy metals on prawns. International conference on Probing in biological system in Mumbai Feb. 2000 pp. 139.

18. Ethano - Botanical Review on Clerodendrum Serratum Linn. Moon

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Kamble L. H.
Dalvi S. M.

Abstract

Nature comprising kind of flora . These are responsible for diversity and their presence may affect to the mankind. Plants are play an important role in human health. One of them is *Clerodendrum serratum* (Linn.) Moon. It belongs to the family of verbanaceae. It is a shrub which is not much branched with stems. The root of the plants is with different activities like digestive, carminative, anti- inflammatory and many more. It is also helpful in anorxia , cough, asthma, hiccough, inflammations, skin diseases etc. In present review , Identification, Varnacular Name ,Habitat, Morphology, Chemical constituents & different pharmacological activity of *Clerodendrum serratum* were done. Thus ,this paper highlights the various pharmacological activity of Clerodendrum serratum and its further scope for clinical trials.

Keywords : *Clerodendrum serratum,* Barbura, hispidulin, Bronchodilator Property.

1. Introduction

Clerodendrum serratum (Linn).Moon belongs to the family Verbenaceae. It is commonly called as 'Bharangee' in Marathwada region. The parts used are the root and leaf . Its roots are bitter, acrid , thermogenic stimulant, anti- inflammatory, expectorant digestive ,carminative ,stomachic, anthelmintic , depurative, sudorific antiplasmodic, activities & are also helpful in asthma ,bronchitis, hiccough, tumors, dropsy, colic, dyspepsia, consumptions ,appetite,lessens expectoration, chronic inflammation of the nose, skin disease , leprosy ,fever and leucoderma. Leaves are useful as an external application for opthalmia and cephalgia.

2. Taxonomical Identification

Domain : *Eukaryota*

Kingdom :*Plantea*

Sub-Kingdom: Viridaeplantae

Phylum :Tracheophyta

Sub- Phylum : *Euphylophytina*

Infraphylum : *Radiatopses*

Division : *Angiospermae*

Class :Magnoliopsida

Sub-Class : *Lamiidae*

Order : *Lamiales*

Family :Lamiaceae/ Verbenaceae

Sub- Family : *Ajugoideae*

Genus : *Clerodendrum*

Species : *serratum*

3. Vernacular Name

Kannada - Gantubarangee

Hindi - Bharagi

Tamil - Cheruthekku

Sanskrit – Brahmanayashtika, Barbura

Gujarati –Bharangee

Bengali- Bamunhatee , Bamanhatee , Bhuijam

English – Blue glory, Beetle killer

Malayalam – cheruteku

Marathi – Bharangee , Bharang

Oriya –Chinds

Punjabi – Bhadangee

Telugu – Ganttubrabangee

4. Habitat

Clerodenrum serratum is a genus of flowering plants in the *Verbenacee* family estimates of number of species in *Clerodendrum serratum* vary widely, about 450 species. The genus is native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia, but some in the tropical Americas and northern Australia, and a few extending north in to the temperate zone in eastern Asia.

5.Morphology

Habit : woody shrub with branches, 2-8 feet high, annual, aromatic Root : Hard, woody, cylindrical ,solid, smoothy texture, external surface light brown having elongated lenticels.

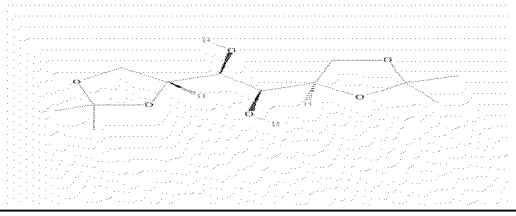
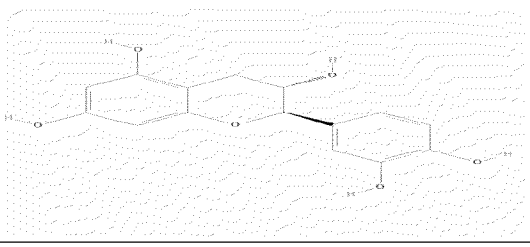
Stem : usually quadrangular.

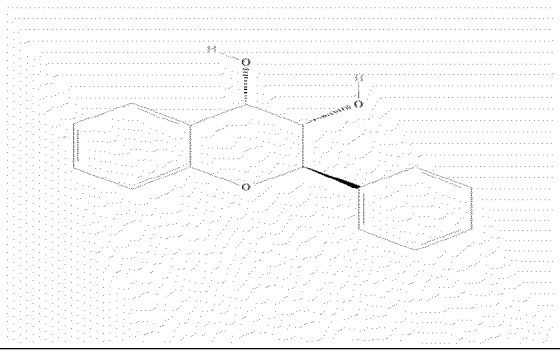
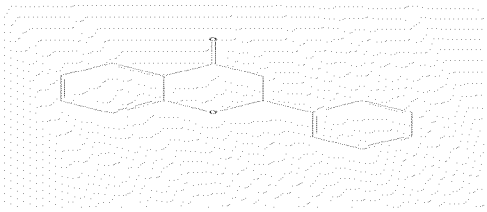
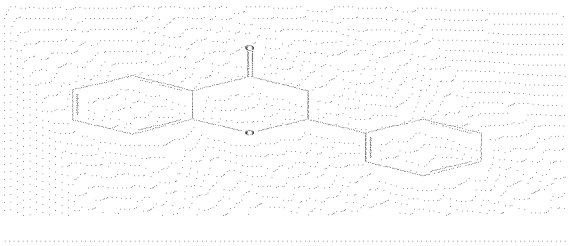
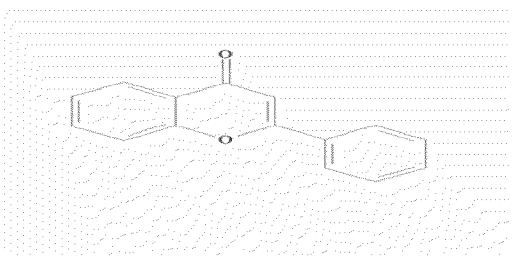
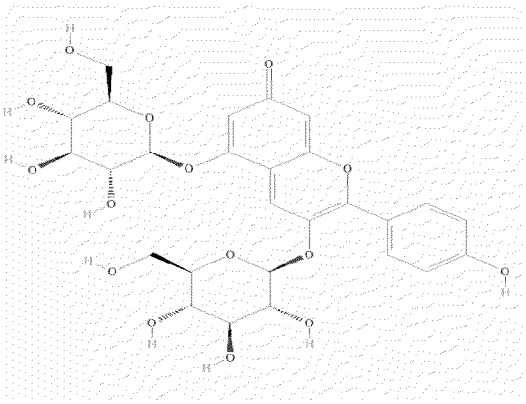
Leaf : three leaves at a node , sometimes opposite oblong or elliptic, serrate, alternate without stipules. Flower : Blue , many in long cylindrical , bisexual, hermaphrodite , zygomorphic , bracteolate,5 petals , stamen epipetalous , anther 1, dehiscing ,disc persistent. ovary superior , 2celled and each cell 2 ovuled and style sub- terminal.

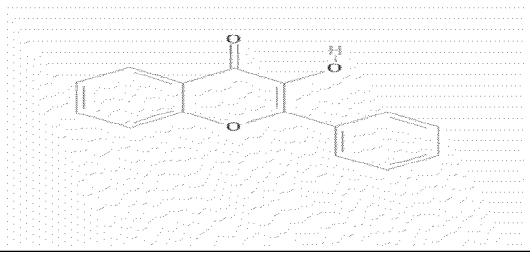
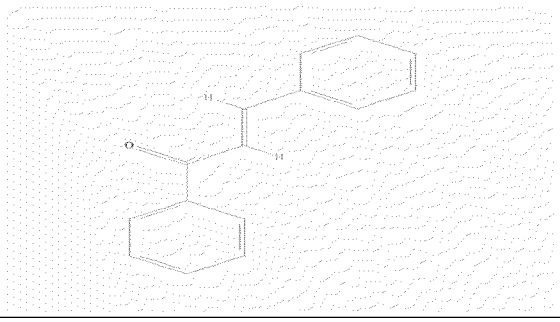
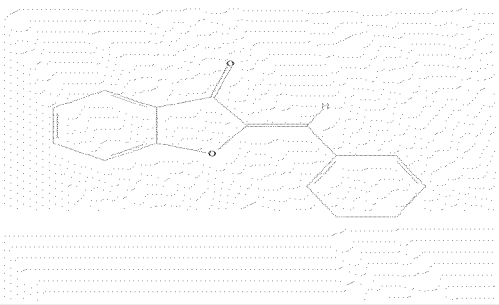
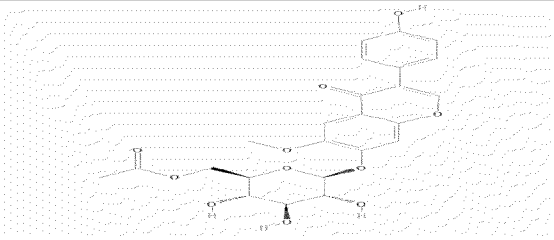
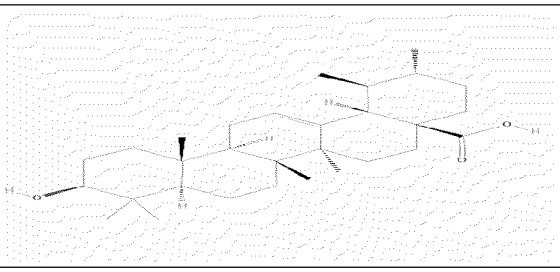
Fruit : Four lobed purple drupe.

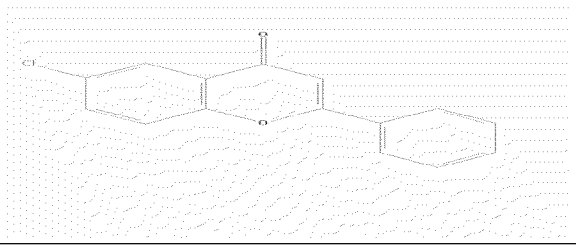
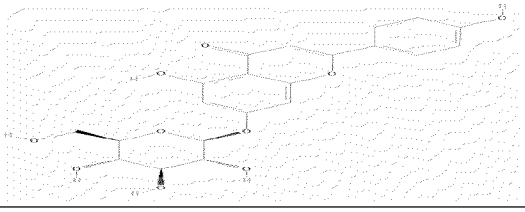
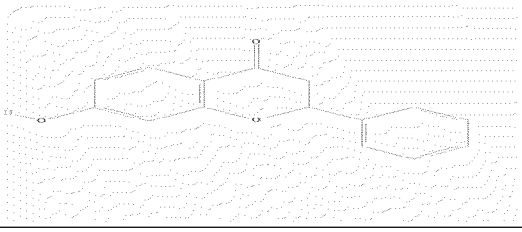
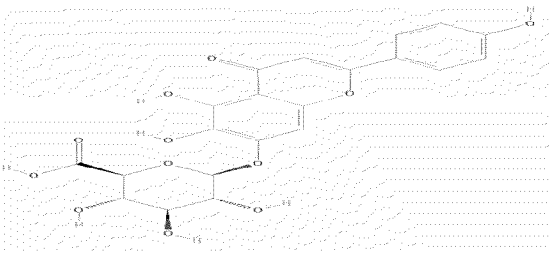
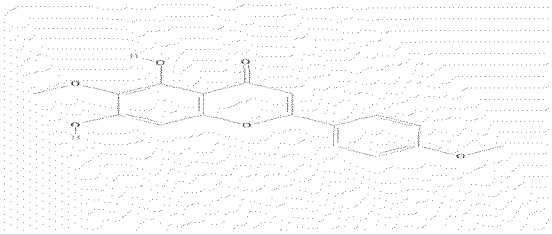
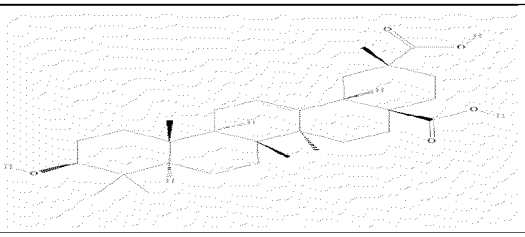
Seed : endospermic seed

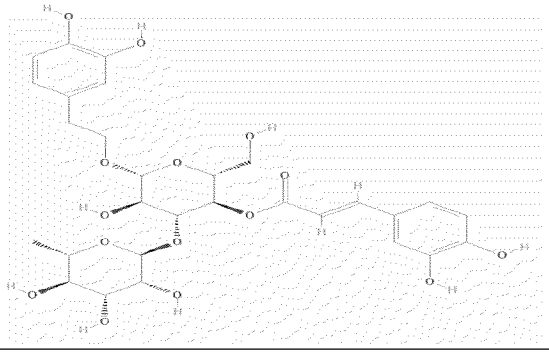
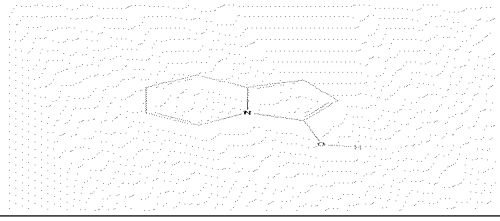
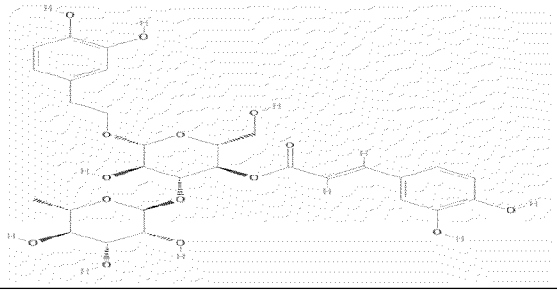
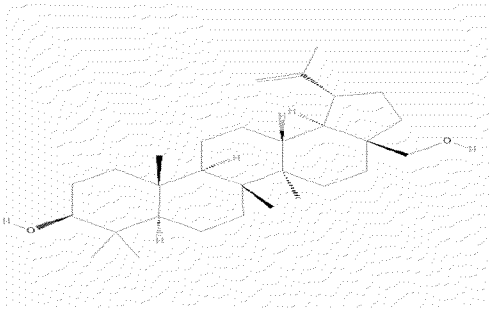
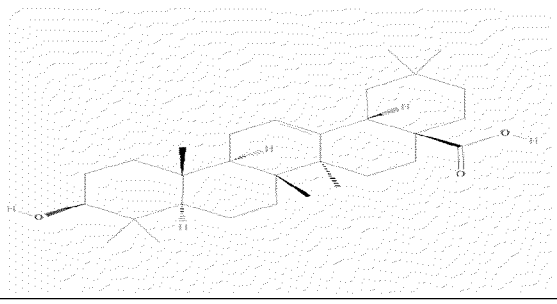
6. Bioactive Compounds

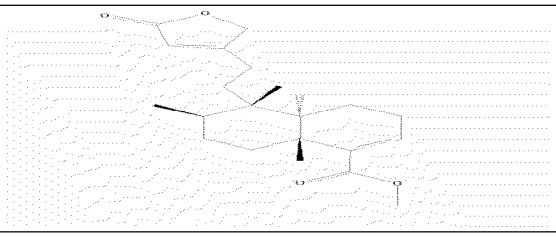
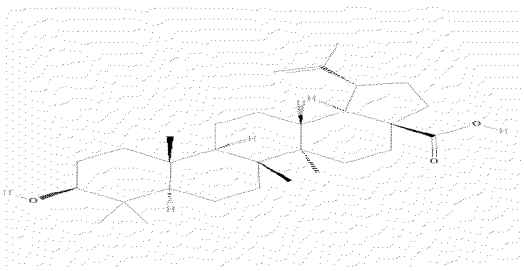
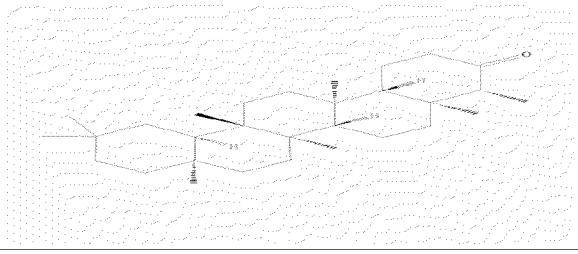
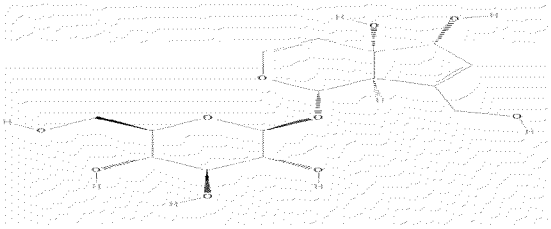
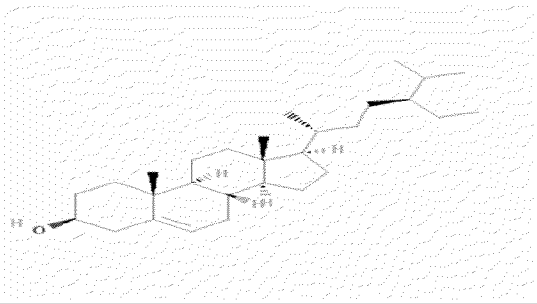
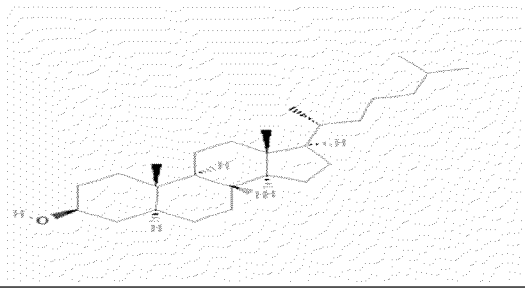
Types	Compound Name	Structure
Carbohydrates	D- Mannitol	
Flavonoids	catechins	

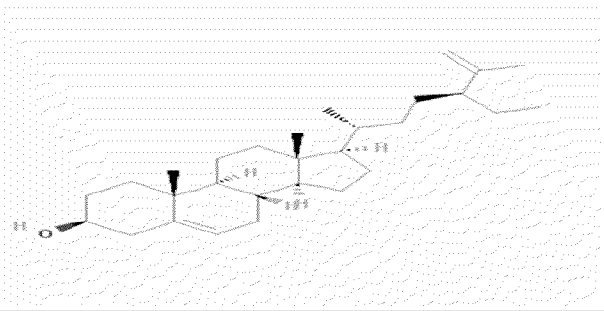
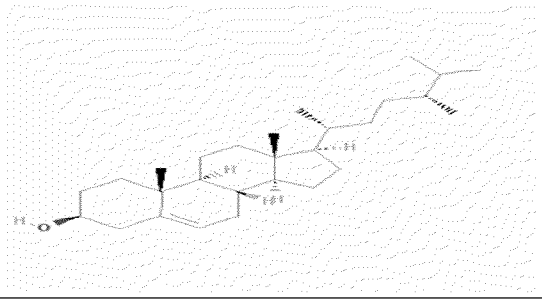
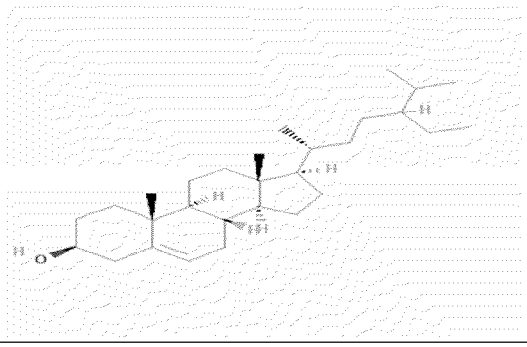
	leucoanthocyanidins	
	flavanones	
	flavanonols	
	flavones	
	anthocynidin	

	flavanols	
	chalchones	
	aurones	
	isoflavones	
	Ursolic	

	cleroflavone	
	apigenin	
	7 hydroxy flavanone	
	Scutellarein acid	
	Pectolinarigenin	
Phenolics	Serratagenic acid	

	acteoside	
	Indolizonoic acid	
	verbascoside	
Terpenes	Betulin	
	Oleanolic acid	

	Clerodermic acid	
	Betulinic acid	
	Friedelin	
	Monomelittoside	
Steroid	Gamma- sitosterol	
	cholestanol	

	clerosterol	
	campesterol	
	24 ethyl cholesterol	

Conclusion

The present review revealed that the clerodendron serratum is a very useful plants . It has unique morphology, it also contain numerous bioactive compounds such as serrategenic acid , gamma sitosterol, etc. Clerodendron serratum acts as thermogenic stimulant , anti – inflammatory, Depurative , sudorific, antiplasmodic, etc. It is also helpful in asthama bronchitis, hiccough, tumors, dropsy, colic, Dyspepsia, appetite, lesion expectorants, chronic inflammation of the nose, skin disease, leprosy, fever and leucoderma, Leaves are useful as an external application for opthalmia and cephalgia. Further intention of this study is to correlate relationship of secondary metabolites to possible biological activities and evaluate C. serratum as a potential source of natural bioactive compounds. **Acknowledgement** :

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Referances

1. Levekar GS *et al.* 2002.Database on medicinal plants used in Ayurveda. Vol 1, Central Council for Research in Ayurveda and Siddha, New Delhi, 73.
2. Kamat SD. Dhanvantari Nighantu. 2002, Chaukhambha Sanskrit Pratishthan, Delhi, 24.
3. Singh M *et al.* Clerodendrum serratum : A clinical approach. JAPS 2012 ; 2(2):11-15.
4. Gupta AK , Tandon N , Sharma M. 2008 .Review on Indian Medicinal Plants. Vol 7, Indian Council of Medicinal Research ,New Delhi ,110-111.
5. Gupta AK , Tandon N, Sharma M. 2008. Review on Indian Council of Medical Research , New Delhi, 127.
6. Gupta AK ,Tandon N, Sharma M. 2008 . Review on Indian Medicinal Plants . Vol 7, Indian Council of Medical Research , New Delhi , 128.
7. Sharma M *et al.* 2009. *In vitro* clonal propagation of Clerodendrum serratum (Linn.) Moon (barangi) : a rare and threatened medicinal plants . Acta Physiologiae Plantarum , 31 (2): 379-83.
8. Bhujbal SS *et al.* 2009 Antioxidant effects of roots of Clerodendrum serratum Linn. Pharmacognosy Research .1(5) :294-298.
9. Saha *et al.* 2012. Evaluation of analgesic activity of ethanolic extract of Clerodendrum serratum Linn leaves in rats. International Research Journal of Pharmaceutical and applied Sciences 2012.2(6) : 33-37.
10. Bhangare NK, Pansare TA , Ghongane BB, Nesari TM. 2012. Screening for anti-inflammatory and anti-allergic activity of Bharangi (Clerodendrum serratum Linn.) in animal. International Journal of Pharma and Bio Sciences 2012 ; 3(4):245-254.
11. Agarwal SK *et al.* 2013. Pharmacological evaluation of hepatoprotective activity of Clerodendrum serratum. International Journal of Pharmacology and Toxicology 2013; 3(2):67-70.
12. Zalke AS et al. In vivo anticancer activity of Clerodendrum serratum (L) Moon. Research Journal of Pharmaceutical, Biological and chemical Sciences 2010;1(3).

13. Shareef MI, Leelavathi S, Gopinath S, Shareef M. Evaluation of in vivo Activity of Clerodendrum serratum L. Against Rheumatism. International Journal of Innovative Research in Science, Engineering and Technology 2013; 2(12):7750-58.
14. Kajaria DK et al .2013 Evaluating polyherbal compounds – bharangyadi for Antiplatelet aggregating effect and Steroidogenesis activity . Journal of Pharma Research 2013; 2(3):1-3. Cited on 25.9.2014.
15. Sharma et al. In vitro clonal propagation of clerodendrum serratum (Linn). Moon (barangi):a rare and threatened medicinal plant. Acta Physiologiea Plantarum 2009; 31(2):379-83.
16. Gupta A, Prajapati PK. Effect of different Avaleha in the management of Tamaka Shwasa (Bronchial Asthma). Journal of AYU 2011;32(3):427-31.
17. Gupta AK ,Tandon N, Sharma M. Review on Indian Medicinal Plants. Vol 7, Indian Councilof Medical Research , New Delhi,2008, 127.
18. Kirtikar KR, Basu BD. "Indian Medicinal Plants. Vol.III."Periodical Experts D-42, Vivek Vihar Delhi.1975.
19. Bhavamishra, Bhavaprakasha Nighantu, G.S.Pandey editor,Haritakyadi varga, Shloka 182.Reprint Varanasi. Chaukanbaha Bharati Acadamy; 2006.p 101.
20. P.C. Sharma. M.B. Yelne, T. J. Dennis Database on medicinal plants used in Aurveda Vol I , New Delhi: Ministery of health and family welfare ,Govt. Of India ; Reprint 2002.p.74.
21. The Ayurvedic pharmacopoeia of India,Part I, Vol III, First edition. New Delhi :Ministery of health and family welfare ,Govt of India ;1999.p25.
22. Rastogi R.P, Mehrotra B. N,Compedium of Indian Medicinal Plants, Vol I, Second reprint.New Delhi: Central Drug Research Institute and national Institute of Science Communication ;1999. P- 113.
23. K. Narayana. Poisonous and Medicinal Plants; first edition Benglore. Jayashri Publication; 2003 P-243.
24. Singh Mukesh Kr, Khare Gaurav, Iyer Shiv Kr , Sharwan Gotmi and Tirupati DK. Clerodendron serratum: a clinical Approach; Journal of applied Pharmaceutical Science ;2012;2(2);11-15.

25. Nal Bhujbal, Santosh S et al. Protective effect of Icosahydropicenic acid isolated from the roots of Clerodendron serratum(L). Moon on experimental allergic asthma , Journal of Complementary and integrative medicine ,2010;(7).
26. Jayaraj F Chinchali, Rajeshwari D Sanakal and Basaappa B Kali Wal. Evaluation of anticarcinogenic activity of Clerodendrum serratum leaf extract on liver and kidney of 7, 12- dimethylbenz(a) anthracene (DMBA) induced skin carcinogenesis in mice. Pelagia Research Library, Euro, 2011; 1(4):130-141.
27. Bhangare NK , Pansare TA, Ghongane BB and Nesari TM. Screening for antifertility and anti allergic activity of Bhargi (Clerodendrum serratum L. Moon.) in animals, International Journal of Pharma and Bio Sciences 2012 Oct; 3(4): 245-254.
28. Ali Jamale Mohamed et; Antioxidant ,antiangiogenic and vasorelaxant activities of methanolic extract of Clerodendrum serratum (Spreng) leaves ; Journal Of Medicinal Plants Research 2012 ; 6(3) :348-360.
29. Benzie IF.(2000) European Journal of Nutrition , 39, 53-61.
30. Manigauha A, Huma Ali and Uma – Maheswari M.(2009). Antioxidant activity of ethanolic extracts of Clerodendrum serratum leaves. Journal of Pharmacy Research , 2(3),pp.491-494.

19. Impact of Sodium Fluoride on Protein and Lipid Concentration of Fresh Water Fishe Labeo Rohita

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Abstract

The toxicity of Sodium fluoride (NaF) to fresh water fish *L. rohita* was evaluated after exposure to the study of acute (96hrs.) toxicity. The changes of biochemical parameters in muscle, liver, gill and kidney tissues were recorded. Muscle shows the greatest loss of protein due to exposure of NaF followed by liver, gill and kidney. Liver shows significant reduction of lipid and glycogen in comparison with other selected tissues of the experimental fish species.

Keywords: Sodium fluoride; *Labeo rohita*; biochemical; body tissue.

Introduction

Inorganic Fluorides were introduced into the environment as a result of natural emission and anthropogenic sources. Depending on metrological condition and season, gaseous and particulate inorganic fluorides are transported in air and ultimately are deposited on land or open water bodies. Important anthropogenic sources of fluoride to the aquatic environment inclued municipal waste and effluents from fertilized producing plants and aluminum refineries (Woodiwiss and Fertwell, 1974). In water mobility and transport of inorganic fluoride are dependent on pH, water hardness, and the prescience of ion exchange mineral. In water inorganic fluoride remain dissolved in solution under acidic condition, low hardness, and the presence on ion exchange material (Cuker and Shilts, 1979; Sahu and Karim, 1989). Free fluoride level in freshwater is generally low (Skjelkvale 1994; Radic and Barlic, 1995).

Inorganic fluoride are toxic to aquatic organism and may caused adverse biological effect such as change in carbohydrate, lipid, and protein metabolism, reproduction, impairment, reduce embryonic and development of life stage, and alternation size and growth (Samal, 1994).

Aquatic animals such as fish and invertebrates can take up fluoride directly from the water or via food (Hemens and Warwick, 1972; Nell and Livanos, 1988). Fluoride tends to be accumulated in the exoskeleton of invertebrates and in the bone tissue of fishes. Fluoride toxicity

depends upon increasing fluoride concentration in the aquatic medium, exposure time and water temperature (Neuhold and Sigler, 1960; Angelovic et al., 1961; Hemens and Warwick, 1972).

Fluorine interferes with various metabolic activities and alters the levels of protein, lipids, glycogen, and cholesterol of fish (Kumar et al., 2007). The present studies was under taken to evaluate the toxic effect on sodium fluoride on biochemical changes in different tissue such as gill, liver, kidney and muscle of fresh water carp *Labeo rohita*.

Material and Method

The fresh water fishes *Labeo rohita* measuring about 6 to 7 cm in length were collected from state government fish seed rearing center. The collected fish were acclimatized under laboratory condition at 28-30^{0}C for 10 days and then divided into different groups having 10 fishes in each. All the groups except control were transferred to separate plastic container containing different concentration (10 L) sodium fluoride (NaF) to determine toxicity LC_0 and LC_{50} value and fish behavior. Acute toxicity experiment was conducted for 96hrs. Toxic medium was changed at an interval of 24h. During experimentation temperature, pH, oxygen content and hardness of the water were determined using standard methods by APHA. After acute exposure 96hrs fishes were sacrificed to obtained gills, liver, kidney and muscle. The pooled sample of the organ was used for estimation of glycogen, total protein and total lipid.

Biochemical parameters like total protein, lipid and glycogen were estimated by Lowry et al. (1951), Folch et al. (1957), and De, Zwann and Zandee (1972) respectively.

Result

The alteration in total glycogen, protein and lipid were calculated from *Labeo rohita* after acute (96hrs.) exposure to sodium fluoride. The significant changes were observed in the experimental fish. The glycogen content in different tissues of *Labeo rohita* was *in* the order of liver> muscle> gill> kidney. After the acute exposure to sodium fluoride, the glycogen content from all the tissues decreased significantly (Table 1.). The maximum loss of glycogen was recorded in liver, while minimum in kidney.

After the acute exposure to sodium fluoride, the protein content from all the tissues decreased significantly (Table 2). Muscle showed the greatest loss of protein as compared to all other tissues. In acute exposure studies, the muscle protein loss was more significant ($P<0.05$) followed by, liver, gill and kidney.

The lipid content was found to decrease after exposure to acute concentration of sodium fluoride in different tissues of *Labeo rohita.* It was found to decrease in order of liver > muscle > gill > kidney (Table 3).

Table: 1 Changes in glycogen content in different tissues of *Labeo rohita* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute Exposure	
		LC_0 (910ppm)	LC_{50} (935ppm)
Gill	11.98 + 0.865	7.53** + 0.745	6.12*** + 0.579
Liver	15.40 + 1.072	8.82*** + 0.964	6.57*** + 0.932
Kidney	10.49 + 1.102	8.28* + 0.974	7.29** + 0.777
Muscle	11.42 + 0.834	7.32** + 0.675	6.45*** + 0.606

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± SD, Values are significant at P < 0.05 *, P < 0.01**, P < 0.001***

Table:2

Changes in protein content in different tissue of *Labeo rohita* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute dose	
		LC_0	LC_{50}
Gill	17.73 ± 0.33	12.43 ± 0.57 *	9.73 ± 0.64 **
Liver	21.90 ± 0.20	16.85 ± 1.00 *	13.83 ± 0.85 **
Kidney	15.37 ± 0.16	9.77 ± 0.50 *	7.19 ± 0.47 **
Muscle	23.45 ± 0.24	15.22 ± 0.46 *	10.02 ± 0.62 **

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± S. D. values are significant at P<0.05 *, P< 0.01**, P< 0.001***

Table: 3 Changes in lipid content in different tissue of *Labeo rohita* after acute exposure to sodium fluoride (96 hrs)

Tissue	Control	Acute dose	
		LC_0	LC_{50}
Gill	11.95 ± 0.48	9.36 ± 0.54 NS	5.71 ± 0.56 **
Liver	11.64 ± 0.43	8.66 ± 0.41 NS	5.65 ± 0.52 **
Kidney	8.54 ± 0.35	6.31 ± 0.36 NS	3.42 ± 0.25 **
Muscle	9.10 ± 0.55	6.42 ± 0.38 NS	3.67 ± 0.31 **

Each value is the mean of five observations. (Values expressed in mg/100mg wet tissue) ± S. D. values are significant at P<0.05 *, P< 0.01**, P< 0.001***

Discussion

The decreases caused by sodium fluoride (NaF) in protein content of muscle, liver, gill, and kidney was observed, this result is similar to the observation of (Gupta R. 2003). This biochemical changes may due to blocking of the metabolism of amino acid and its preventing cells from synthesizing protein. In fact study has shown that sodium fluoride (NaF) inhibit protein synthesis and interferes with amino acid metabolism (Pandit CG, Narayana RD, 1940). Another possible reason may be depletion of protein for its utilization in conversion to glucose (Sirvastava N, Kaushik N, Gupta P. 2002).

The percentage of glycogen increases significantly was found in the tissue of liver and muscle and decreases in the tissue of gill and kidney. The increased glycogen level in liver and muscle in lethal concentration due to disturbance of carbohydrate metabolism as it has been observed to effect enzyme involved in glycogen turnover at higher sodium fluoride concentration (Strochkova LS, Zhvoronkov AA in 1983). Several other studies have revealed that sodium fluoride inhibit glycolytic enzyme (Camargo JA 2003). The percentage of glycogen decreases may be due to enhanced conversion of glycogen to glucose to meet and increased energy requirement under stress condition of sodium fluoride in liver and muscle.

The total lipid decrease in liver, gill, muscle, and kidney due to inhabitation of lipid synthesis by sodium fluoride as well as increased utilization of storied lipid as a source of energy to conduct regular metabolic function. Sodium fluoride is well known as an inhibitor of various enzyme like lipase, phosphatase, and esterases. The interference of sodium fluoride was also observed in fatty acid oxidation and inhibit the enzyme acyl-co-A synthesis (Batenburg JJ, Vanden Bergh SG. 1972). Thus decreased lipid content in various tissue may be due to the inhibition of these enzyme. Total lipid decreased in muscle, liver, and testis of the fluoride exposed catfish was observed by Sashi et al. in rabbits in 1989.

From the result obtained here , it is cleared that sodium fluoride (NaF) interferes with various metabolic activities and biochemical changes are observed in the level of protein, glycogen, and lipid content in experimental fish *L rohita.*

The initial phase of acute inorganic fluoride intoxication in fresh water species such as rainbow trout and carp is characterized by apathetic behavior accompanied by Neuhold and Sigler 1960 and Newhold 1972). In many cases, the surviving young fish had curved spines (Singler and Neuhold 1972).

References

- APHA.1980.Standered Methods for the Examination of water and Wastewater, Amer-Public Health Association Washington.17th Edition.(1989).
- Batenburg JJ, Vanden Bergh SG. The mechanism of inhibition by fluoride of mitochondrial fatty acid oxidation . Biochim Biophys Acta 1972;280:495-505.
- Camargo JA. Fluoride toxicity to aquatic organism: A review. Chemospher 2003;50(3):251-64.
- Coker,W.B. and W.W. Shilts. 1979. Lacustrine geochemistry around the North shore of Lake Superior: implication for evolution of the effect of acid precipitation. Current Research Part C, Geogical Survey of Canada. Paper 79-1C.
- Damkaer, D.M. and D.B. Dey. 1989. Evidence for fluoride effect on salmon passage at John Day Dam, Columbia River, 1982-1986. N. Am.J. Fish. Management. 9(2): 154-162.
- De, Zwann and D.I. Zandee 1972.Body distribution and seasionl changes in glycogen content of the common sea muscle *Mytillus edulis*. Comparative Biochem. Physiol. Biochemistry 43: 53-55.
- Floch J, Lees M, Sloane- Stalely GH. A sample method for isolation and purification of total lipid fron animal tissue. J Bio Chem 1957;226:497-507.
- Gupta R. Pathophysiological consequences to fresh water fish Channa punctatus induced by fluoride (Phd dissertation). Lucknow: University of Lucknow; 2003.
- Julio A. Camargo. 2003. Fluoride toxicity to aquatic organisms: Volume 50, Issue 3. 251-264.
- Kaplan et. al. 1964. Toxicity of fluoride for frog. Lab. Anim. Care 14: 185-198
- Lowry, O.H., Rosebrough, N.J; Farr, A.B. and Randall, R.J. 1951. Protein measurement with folinphenol reagent. J. Bio.Chem. 193: 265-275.
- Mishra, P.C. and A.K. Mohapatra. Haematological characteristics and bone fluoride content in Bufo melanostictus from an aluminum industrial site. Enviorn. Pollut. 99 (3): 421-423.
- Neuhold, J.M. and W.F. Sigler. 1960. Effect of sodium fluoride on carp and rainbow trout. Trans. Am. Fish. Doc. 89: 358-370.

- Pandit CG, Narayana RD. Endemic fluorosis in south India: Experimental production of chronic fluorine intoxication in monkey (Macaca radiate). Ind. J Med Res 1940;28:559-74.
- Pimentel, R., and R.V. Bulkley. 1983. Influence of water hardness of fluoride toxicity to rainbow trout. Environ. Toxicol. Chem. 2: 381-386.
- Radic, N. and Bralic, M. 1995. Aluminum fluoride complexation and its ecological importance in the aquatic environment. Sci. Total Environ.172(2-3): 237-243.
- Samal, U.N. 1994. Effect of fluoride on growth of certain freshwater fishes .Environ. Ecol. 12(1): 218-220.
- Sashi, Singh JP, Thaper SP. Effect of fluoride in excess on lipid constituents of respiratioy organs in albino rabbits. Fluoride 1989;22(1):33-9.
- Sigler, W.F., and J.M. Neuhold. 1972. Fluoride intoxication in fish: a review. J. Wild> Dis. 8: 252-254.
- Sirvastava N, Kaushik N, Gupta P Zink induce changes in the liver and muscle of fish Channa punctatus (Bloch). J Ecophysiol Occup HIth 2002;197-204.
- Skjelkvale, B.L. 1994b. Waterchemistry in areas with high deposition of fluoride. Sci. Tot. Environ. 152: 105-112.
- Sshu, N.K. ans M.A. Karim. 1989. Fluoride incidence in natural waters in Amreli district Gugarat. J. Geol. Soc. India 33(5): 450-456.
- Strochkova LS, Zhavoronkov AA. Fluoride as an activator of enzymatic systems. Fluoride 1983;16:181-6.
- Woodiwiss, F.S. and G. Fertwell. 1974. The toxicity of sewage effluents, industrial discharge and some chemical substance to brown trout (Salmo trutta). In the Trent River authority area. Water pollut. Cont. (G.B.) 73:396.

20. Water Quality Assessment in Freshwater Lake Ambona near Umarkhed (Maharashtra)

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Abstract

All the freshwater resources are polluted due to discharges from agricultural, domestic and industrial wastes because; the waste water is not treated before discharge. Advanced water treatment methods are necessary to prevent such waste water. The physical chemical and biological characteristics of a water body define the water quality. The correlation between different parameters gives an effective index of water quality. Co-efficient of correlation is an index of the degree of association between two continuous variables. Present study indicates the co-efficient correlation between physico-chemical parameters and phytoplankton's.

Key Words- Phytoplankton, Zooplankton, Fish, Physico-chemical Parameters, Ambona Lake.

Introduction

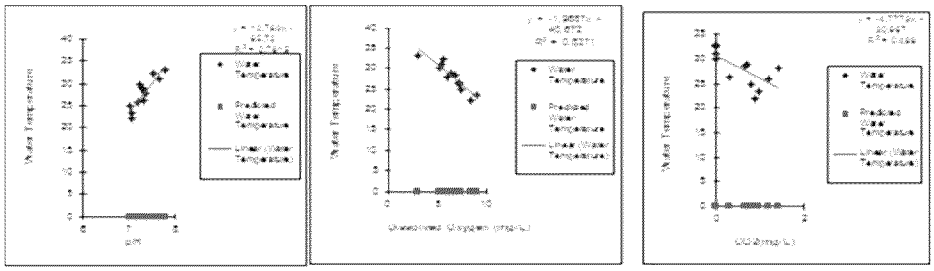
Water resources are polluted because of man's interference in addition of matter to the water or changing the physical, chemical and biological characteristics of water. The quality of water is an index to provide a complete picture of various water bodies. Because of drinking polluted water, health related issues arises like water borne diseases such as cholera, typhoid and kill millions of peoples every year. Like that overuse of water and polluted water destroys the natural environment and increasing risks for many species of life. The water quality and quantity is deteriorating nowadays because of rapid industrialization, urbanization and increasing population growth. Most countries are aware of the necessity of freshwater requirement for life on the earth. Agriculture is the big reason for water pollution because every country using agricultural fertilizers and pesticides that gets contaminated with the ground water as well as surface waters. High levels of nitrates and phosphates in water stimulate the growth of algae leading to deoxygenation which can change the water body towards eutrophication. This can affect the metabolism of the organisms present in water.

In India lake pollution is severe problem due to addition of high amount of pollutants. Thus there is need of continuous monitoring of these water bodies. Recent works on ecological study carried out by Hydrobiological work on Indian lakes by Ghosh and George(1989), Khatavkar et al (1989) on limnological aspects of lentic fresh water bodies of Kolhapur; Tripathi and Pandey (1989) on Chandari pond and Rao and Durve (1984) on Lake Rangasagar, Udaipur Rajasthan and Mathivanan V.; Vijayan P. ; Sabhanayakm, S. and Jeyachitra, O. (2007)on Cauvery river with reference to pollution.

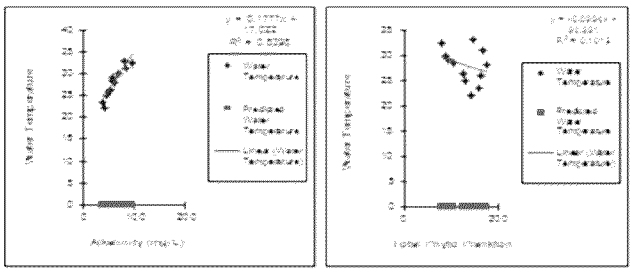
Materials and Methods The water quality parameters of the Ambona Lake were monitored in the period of June 2016 to May 2017. Sampling at four points in the lake was carried out on the day of sampling. The physico-Chemical parameters of waters studied were water temperature, transparency, color, turbidity, pH, Dissolved oxygen, alkalinity, free CO2, nitrogen and chloride. The analysis of water samples was done according to APHA (1998). The chemical parameters except pH (Units) were expressed in mg/lit. Samples of phytoplanktons were collected on monthly basis from sampling stations for study. Filtered samples were fixed and preserved by adding Lugol's Iodine for phytoplankton.

Observation Table

Throughout the period of investigations the maximum and minimum values of various physico-chemical parameters were found to be……



Graphs showing the cor relation between different parameters2016-17



Results and discussions

The twelve months observations on Ambona Lake indicated that the lake is most disturbed due to activities of domestic animals and pollution from agricultural run-off. From June 2016 to May 2017 investigations were carried out to know the physico-chemical and biological condition of the lake. The lake water shows alkaline nature with narrow range of pH. In summer months the low values of dissolved oxygen was recorded. The amount of free co2 was very low may be because of photosynthetic activity or free CO2 combines with water molecule. Phytoplanktons were maximum in late winter, which indicates the temperature transparency, turbidity, alkalinity were favourable in their existence. Water containing pH value of more than 9 or less than 4.5 not suitable for use (Salla and Ghosh, 2014). pH is most important in determining the corrosive nature of water. Minimum pH value, maximum is the corrosive nature of water. pH was positively correlated with electrical conductance and total alkalinity (Gupta, 2009). Planktons play an important role in the food chain of aquatic ecosystems. The planktons form the basic level of food chain and hence the energy transfer is of great importance to the aquatic ecosystems. Oxygen containing in the water is important for many organisms and dissolved Oxygen affect many nutrients in water and therefore the periodicity of aquatic ecosystem (Azmi et al., 2015). Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients, stratification etc. In summer months, the amount of dissolved oxygen lowers due to increase in temperature and also because of increased microbial activity (Patil et al., 2012).Nitrogen cycle represents the transfer of nitrogen to and from atmosphere, biosphere and hydrosphere. Man's interference with nature influence on the water quality by use of fertilizers such as ammonia and nitrogen compounds. Correlation studies between phytoplankton's exhibited the positive correlation with transparency and negative correlation with chlorides. Chlorophyceae was the most dominant group of the total phytoplankton.

Referances

- **A.**P.H.A. (1998) : Standard methods of examination of water and waste water 20th Edition, APHA, AWWA and N.W. Washington D.C
- Azmi N., Anwar M.R. and Kumari M., (2015), Water quality analysis of fish pond of
- Araria district, Bihar, Indian journal of applied research, 5, 587-589.
- Gupta, D. P., Sunita and J. P. Saharan, (2009), Physiochemical Analysis of Ground

- Water of Selected Area of Kaithal City (Haryana) India, Researcher, 1(2), pp 1-5.
- Ghosh, A. and George (1989): Studies on the abiotic factors & zooplankton in a polluted urban reservoir, Hussain Sagar, Hyderabad. Indian Jr. of Env. Health 31 (1), Pg. 49 – 59.
- Khatavkar R.S., N.V. Shah, K.R. Rao and R.A. Navale (2004): Variations in physico – chemical parameters in freshwater Tanks in and around Solapur city, Maharashtra J. Aqua. Biol., Vol 19 (1), Pg. 111 – 114.
- Mathivanan V.; Vijayan P. ; Sabhanayakm, S. and Jeyachitra, O. (2007) : An assessment of plankton population of Cauvery river with reference to pollution J. Env. Biol. 28: Pg. 523 – 527
- Patil. P.N, Sawant. D.V, and Deshmukh. R.N., (2012), Physico-chemical parameters for
- testing of water – A review, International journal of environmental sciences, 3(3), pp
- 1194-1207.
- Rao N.G. and Durve V.S. (1984): Cultural eutrophication of the lake Rangasagar, Udaipur, Rajasthan, India, J. Environ. Biol, 140: Pg. 127 – 134.
- Salla S., and Ghosh S., (2014), Assessment of water quality parameters of lower lake,
- Bhopal, Archives of Applied Science Research, 6(2), pp 8-11.
- Tripathi A.K., Pandey S.N. and Tiwari R.K. (1987): Eutrophication study of Kalyanpur Pond, Kanpur India Proc. Nat. Acad. Sci., India, 57 b (111) : Pg. 279 – 283.

21. Estimation of Quercetin from Different Varieties of Capsicum Annuum L. by using HPTLC Fingerprinting Method

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S. K. Umate
A. H. Jadhav

Abstract

HPTLC method was developed for the estimation of Quercetin from methanol extract of five different varieties of Capsicum annuum L. *an important agricultural crop plant cultivated for its fruit as vital part of condiment in indigenous food preparations. Preloaded silica gel* GF_{254} *is used as stationary phase and mobile phase used is Ethyl acetate: Formic acid: Glacial Acetic acid: Water [10:0.5:0.5:1.3, V/V/V/V]. Detection and quantification were performed densitometrically at wavelength λ 254. The Rf value of standard Quercetin was found to be 0.98. The total peak area percentage of the Quercetin corresponding to standard Quercetin for extract of five different varieties A,B,C,D,E of chilli were compared and the peak area percentage for Quercetin content was estimated to be 17.33%, 17.29%,26.46%, 19.30%, 29.60% respectively.*

Keywords: *Agriculture Crop plant, Condiment, HPTLC, Quercetin. Etc.,*

Introduction

Natural products from plant, animal and minerals have been the basis of the treatment of human disease. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine [1, 2]. *Capsicum annuum Linn.* belonging to the family of Solanaceae is cultivated in almost all the tropical countries. In ayurvedic medicine *C. annuum* is classified as follows [3]:

Classification:

Kingdom-*Plantae*

Division- *Magnoliophyta*

Class- *Magnoliopsida*

Order- *Solanales*

Family- *Solanaceae*

Genus- *Capsicum*

Species- *annuum* L.

Chilli (*Capsicum annuum* L.)

The fruit of chilli contains several related chemicals containing a series of homologous branched- and straight-chain alkyl vanillylamides, collectively called capsaicinoids as their chief chemical entity. The major capsaicinoids present are capsaicin (48.6%) is quantitatively followed by 6, 7-dihydrocapsaicin, minor capsaicinoids that are present is nordihydrocapsaicin (7.4%), homodihydrocapsaicin (2%), and homocapsaicin (2%). The seeds contain the steroidal glycosides capsicoside A through D, all furostanol. C. *annuum* is rich in carotenoid pigments, including capsanthin, capsorubrin, carotene, luteine, zeaxanthin, and cucurbitaxanthin A. Capsicum is carminative, stimulant, antispasmodic, analgesic, alterative, astringent, haemostatic, and antiseptic in nature. Capsicum has a strong effect upon circulation, initially acting upon the heart. It shows a protective effect on the respiratory system. The capsaicin has shown antigenotoxic and anticarcinogenic effects, and is an important dietary Phytochemical with potential chemo preventive activity [4, 5].

Polyphenolic compounds comprise a vast number of biological active compounds that are ubiquitous to medicinally important plants, many of which have been used in traditional eastern medicine. They also constitute an important component of the food preparation. Quercetin exhibit antiulcer, anti-inflammatory, antioxidant, antimicrobial and antiallergic activity [6, 7, 8,

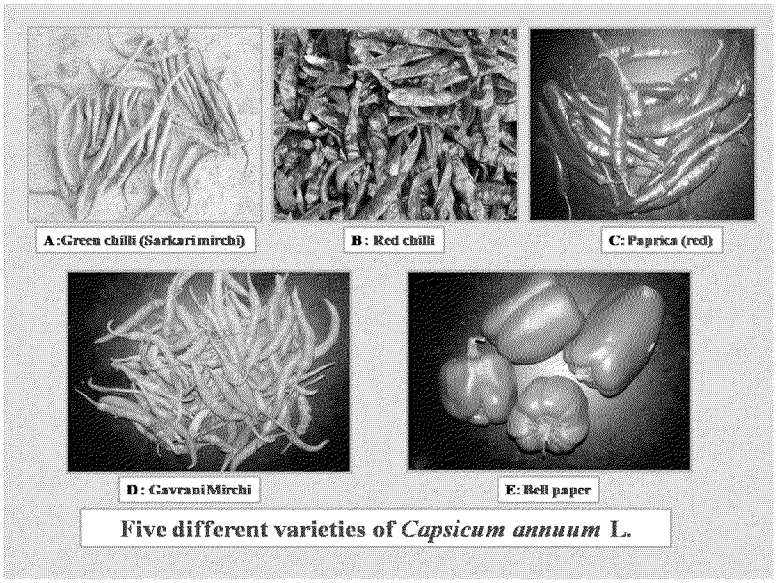
9]. These compounds have been shown regulatory activity of hormones such as transport, metabolism and action of thyroid hormones [10].

High performance thin layer chromatography (HPTLC) is a useful analytical method for screening of chemical constituents present in plant materials [11] .In present study Quercetin which is an important nutraceutical and antioxidant of plants was estimated from fruit of five different varieties of *Capsicum annuum* L. by HPTLC method.

Material and Methods

Collection of Plant Material

For the present study five different varieties of *Capsicum annuum* (A, B, C, D, E), Capsicum Varieties: A= Green chilli (sarkari), B=Red chilli, C= Paprika red chilli, D= Gavrani E=Bell paper were selected. The Chilli fruits were collected from the local market of Nanded separately in polythene bags. The materials were brought to laboratory and are dried separately under the shade until dryness. After complete drying the plant material was powdered using mechanical grinder.



Soxhlet method for extraction

The powdered fruit materials were extracted with methanol using Soxhlet apparatus and the extract obtained was then diluted appropriately with methanol. After extraction the extracts were evaporated to dryness using hot plate. The dried extracts were re-dissolved in 5 ml

methanol and filtered using Whatmann filter. The filtered extracts were later used for HPTLC analysis.

Preparation of standard and sample solutions

Quercetin 10mg were accurately weighed into10mL volumetric flask dissolved in 10 mL of methanol [1mg/mL]. The 50 mg of extract was dissolved in methanol [5mL] then solution was filtered through Whatmann filter paper No.42.

Development of HPTLC Technique

The sample were spotted in the form of bands with microlitre syringe on precoated silica gel plates F_{254} [10 cm x 10 cm with 0.2 mm thickness]using CAMAG Linomat 5 applicator .automatic sample spotter of band width 8mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. The distance was 10 cm .subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC Scanner in absorbance at 254 nm and operated with Win CATS Planar chromatography Manager.

Quercetin estimation

Stationary phase is silica gel F_{254} plates, Mobile phase used is Ethyl acetate: Formic acid : Glacial acetic acid : Water (10:0.5:0.5:1.3 v/v/v/v/v), standard Quercetin 1 mg/ml (5 µl) and sample methanol extract 10mg/ml(10 µl), Migration distance assigned as 100 mm, scanning wavelength at 254 mm, Mode of scanning is absorption (deuterium)[12].

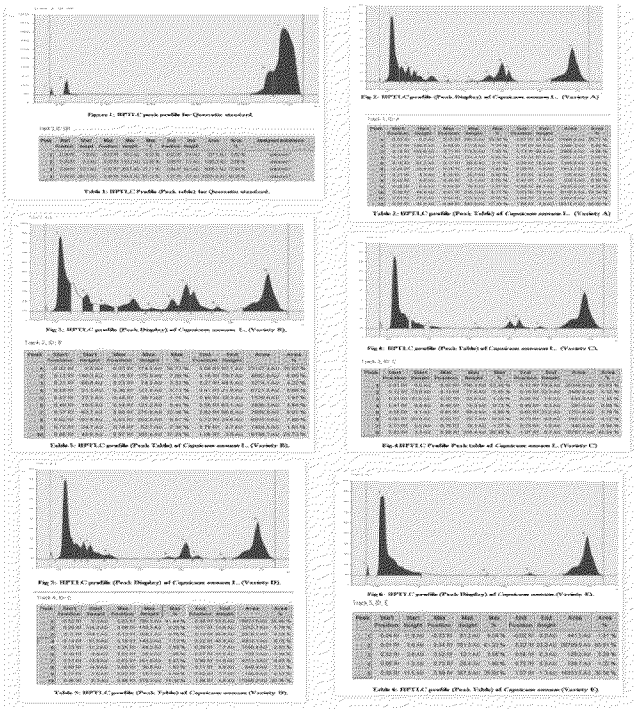
Results and Discussion

In early studies Quercetin were estimated by liquid chromatography from this plant and found to have Quercetin rhamnoside and Quercetin glycosides in abundant proportion. [13]. The Rf values of standard Quercetin was found to be 0.98 and peak area was 8088.1 [fig.1, table.1]. Methanol extract of five different types A,B,C,D,E of chilli plant showed peak profile and corresponding Rf values of different phytoconstituent, Rf 0.98 was coinciding with standard Rf value and which was observed in all varieties.

The results from HPTLC fingerprint scanned at wavelength 254 nm for methanol extract of *Capsicum annuum* Variety A showed twelve polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.07 to 1.06, in which second highest concentration of

the Quercetin was found to be 17.33% and its corresponding Rf value of standard is 0.98(fig.2 and table 2). The *C. annuum* Variety B showed ten polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.08 to 1.06 in which second highest conc. of the phytoconstituents was found to be 17.29% and its corresponding Rf value is 0.97(fig.3, Table 3). The Variety C showed eight polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.10 to 1.07 in which one of highest conc. of the phytoconstituents Quercetin was found to be 26.46% and its corresponding Rf value is 0.98 of standard Quercetin (fig.4, table 4).

Variety D showed ten polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.08 to 1.06, in which highest conc. of the Quercetin was found to be 19.30% and its corresponding Rf value 0.98 of standard (Fig.5, Table.5). The Variety E showed five polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.02 to 1.07 in which highest conc. of the phytoconstituents was found to be 29.60% and its corresponding Rf value of standard Quercetin (Fig.6, Table 6). Out of the five varieties of *C. annuum*, Variety E showed highest concentration (29.60%) of Quercetin among all other varieties.



Conclusion

Using this HPTLC fingerprinting method, quality assessment and evaluation of botanical materials has done for broad number of compounds both efficiently and cost effectively. The antibacterial, antiparasitic, hypoglycaemic and antidiabetic etc., properties of *C. annuum* L. Extract of five different varieties has been proved by the presence of bioactive compound Quercetin, which could be a potential source of natural antioxidant.

References

1. Verma S, Singh SP. Current and future status of herbal medicines. Veterinary World. 2008; 1(11):347-350.
2. Wikipedia, the free encyclopaedia. Capsicum annuum [Internet] 2015 [cited on 2015 June 18]. Available from: https://en.wikipedia.org/wiki/Capsicum_annuum.
3. The Taxonomicon. The Taxonomic on & Systema Naturae 2000[Internet]. 2015 [cited on 2015 May 9]. Available from: http://sn2000.taxonomy.nl/Taxonomicon/Default.aspx.
4. MDidea Extracts Professional. Phytochemicals and Constituents of Capsicum: Cayenne, Red Pepper. [Internet] 2010 [cited on 15 July 2010]. Available from: http://www.mdidea.com/products/new/new00504.html.
5. Syeda Nishat Fathima, 2015; A Systemic Review on Phytochemistry and Pharmacological Activities of Capsicum annuum, *Ijppr.Human, Vol. 4 (3): 51-68.*
6. Agnes W. Boots, Guido R.M.M., Haenen, Aalt Bast. 2008. Health Effects of Quercetin: From Antioxidant to Nutraceutical: *Euro. J. of Pharmaco*.585, 325-337.
7. Aneela Malik, 2014. Pharmacological applications of Quercetin and its derivatives: A Short Review. *Tropical J. of Pharma*. Res.; 13(9):1561-1566.
9. Nidhi Gupta, RS Chauhan, Jatindra Kumar Pradhan.2014. Rutin: A Bioactive Flavonoid; Handbook of Medicinal *Plants and Their Bioactive Compounds*, 51-57.
10. Saurabh Singh, Mayanglambam Bilashini Devi.2015. Vegetables as a potential source of Nutraceuticals and phytochemicals: A Review; *Int. J. of Medicinand Pharmace. Sci.* 5(2), 1-14.

11. Ashok Kumar et al. 2010. Estimation of Gallic acid, Quercetin and Rutin in *Terminalia Chebula* by HPTLC; *Jordan J. of Pharmaceutical Sci*., Vol.3, No.1, pp. 63-67.

12. P.D.Sethi, HPTLC, CBS Publishers and distributors 1st edition, New Delhi, 1996, 39.

13. Marathe Vishal R and Umate Satish K. 2016; Estimation of Gallic acid, Rutin and Quercetin in *Portulaca quadrifida* L. – A potential wild edible plant by HPTLC method, Int. J. of Life Sciences, 2016, Vol. 4 (1): 83-88.

14. Mokhtar M[1], Soukup J, Donato P, Cacciola F, Dugo P, Riazi A, Jandera P, Mondello L 2015, Determination of the polyphenolic content of a Capsicum annuum L. extract by liquid chromatography coupled to photodiode array and mass spectrometry detection and evaluation of its biological activity. *J Sep Sci.* 38(2):171-178.

22. Cultivation of Commercially Important Oyster Mushroom on Inexpensive Substrates

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Abstract

Mushrooms are included often in the healthy diet plan of human beings. Mushrooms have the escalating demand in public especially among vegetarians due to their health benefits. The commercially important oyster mushroom (*Pleurotus florida*) was cultivated by us on inexpensive and easily available cheap substrates viz. soybean husk, rice husk, sawdust, wheat husk etc. The luxuriant growth of fruiting body of selected oyster mushroom was recorded on aforementioned substrates when mixed in equal proportion. Therefore combination of these substrates can be used for large scale production of oyster mushroom.

Keywords: oyster mushroom, *Pleurotus florida*, spawn, sawdust, inexpensive substrates

Introduction

Mushrooms are excellent source of food to address the problem of malnutrition in developing countries. They are good source of protein, vitamins and minerals and known to have lot of applications in food and pharmaceutical industries. Taxonomically mushrooms are classified to Basidiomycetes class and Agaricales order. Mushrooms have variety of taste, flavor and texture. Of the many edible species white button mushroom (*Agaricus bisporus*), shiitake mushroom (*Lentinula edodes*), Oyster mushroom (*Pleurotus* sp.), straw mushroom (*Volvariella volvaceae*) and ear mushroom (*Auricularia* sp.) are being cultivated at commercial scale worldwide, especially in south-east Asia, India, Europe and Africa. In the modern research era,

mushroom cultivation is intended for the degradation of waste plant residues from forests and agriculture to initiate economical and viable biotechnology process [1-4]. In this context we have selected the agricultural waste materials for cultivation of oyster mushroom (*Pleurotus florida*).

Materials and methods

Collection and processing of waste materials

Rice husk, soybean husk and wheat husk were collected from local farmers and sawdust was collected from a sawmill industry present near M.I.D.C. area of Parbhani. All the collected materials were air dried under shadow. The coarse materials were pulverized in a grinder and then sieved [2, 5,6].

[A] [B]

[C] [D]

Figure 1: Materials used for cultivation of oyster mushroom by us.

[A] Rice husk, [B] Soybean husk, [C] Wheat husk, [D] Sawdust

Collection of Spawns

Spawn sample of oyster mushroom *Pleurotus florida* was purchased from Pepper Agro industry, Vimanapura, Bengaluru. This sample was mixed thoroughly in 200 gm of pre-sterilized wet wheat and incubated at 25°C for 72 h [5].

Spawning

Equally weighed (400 gm) flours of rice husk, soybean husk, wheat husk and sawdust and equal proportion mixture (100 gm of each) of all these flours were sterilized and then moistened by adding water. All the moistened materials were filled by seeding spawn layers in plastic bags individually. The distance between two spawn layers was kept 7 cm. All the bags were tied with threads and then bags were punctured from all sides with the help of sterilized needle. The number of puncture count was maintained for all bags [5,6].

Incubation and cultivation

All the bags were transferred in mushroom cultivation room where 25 °C temperature and 80 % air humidity was maintained. Temperature was monitored by a mercury thermometer and humidity was monitored by a hygrometer (Model no. Accutemp IIP-THM-401) [5,6].

Results and discussion

The mycelium of *Pleurotus florida* were spread completely in all bags as the materials turned into white. The growth of fruiting body was initiated after 72 hours of incubation on all substrates (Fig. 2). Then all the bags were uncovered.

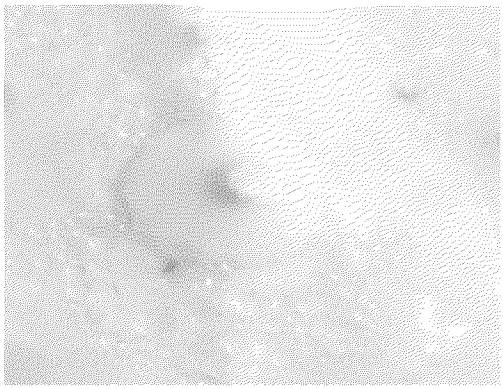
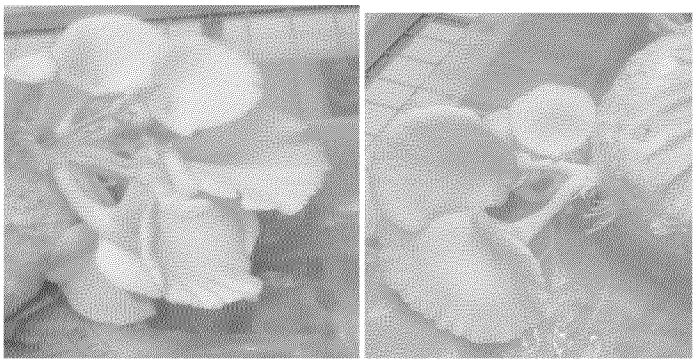
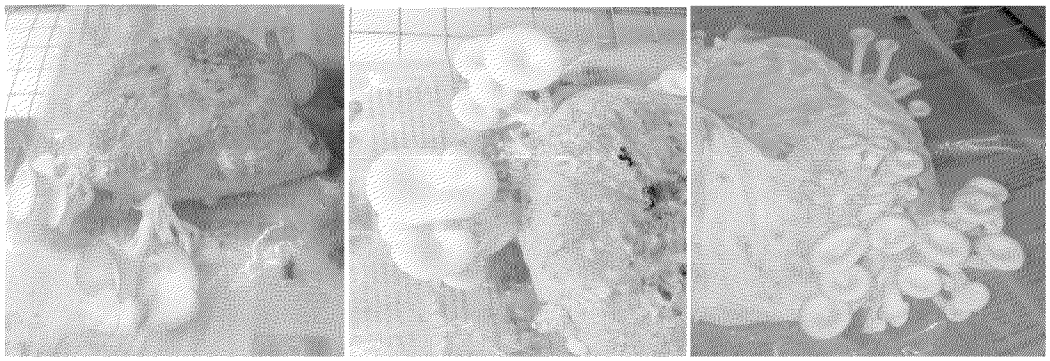


Fig. 2: Initiation of fruiting of *Pleurotus florida* after 72 h.

Complete fruiting was appeared after next 48 h. All fully grown mushrooms were detached simply by twisting. Cultivation of *Pleurotus florida* on mixed substrates, sawdust, wheat husk, soybean husk and rice husk has been shown in Fig. 3a, 3b, 3c, 3d, and 3e respectively.



3a 3b.



3c. 3d. 3e.

Fig. 3 (a-e): Cultivation of *Pleurotus florida* on mixed substrates, sawdust, wheat husk, soybean husk and rice husk.

The average weight of each fruiting body from different substrate has been given in Table 1. Fig. 4 reflects the maximum weight of a fruiting body of *Pleurotus florida* after cultivation on mixture of all selected substrates.

Table 1: Average weight of a fruiting body of *Pleurotus florida* after 5 days on individual and mixed substrates

Substrates	Average weight of a fruiting body (gm) after 5 days
Rice husk	38.19 ± 0.23
Soybean husk	40.13 ± 0.17

Wheat husk	42.76 ± 0.19
Sawdust	45.47 ± 0.26
Mixture of all substrates	52.11 ± 0.31

Fig. 4: Weight of a fruiting body of *Pleurotus florida* cultivated on mixture of all selected substrates;

Conclusions

In conclusion, *Pleurotus florida* was cultivated on sawdust, wheat husk, soybean husk and rice husk. Maximum weight of a fruiting body of this mushroom was recorded after cultivation on mixture of these substrates. Cultivation of this oyster mushroom can be done at commercial level by using these cheaply available agricultural waste substrates. Broad range substrate specificity of this mushroom can promote the bio-utility of industrial and agricultural waste.

Acknowledgements

We are thankful to Hon. Dr. Rafiq Shaikh, Chairman and Shaikh Hasarat Pasha Treasurer of M.E.C.H. & W. Society, Parbhani for providing infrastructure and necessary facilities. First author is grateful to Dr. R.M. Mulani (Registrar, S.R.T.M. University, Nanded), Dr. M.M.V. Baig (H.O.D., Yeshwant College, Nanded) and Dr. B.M. Kareppa (H.O.D., D.S.M. College, Parbhani) for overall perpetual inspiration, help and support.

References

1. Josephine R.M. (2015) A review on oyster mushroom (*Pleurotus* sp.). *International Journal of Current Research*, 7(1) 11225-11227.

2. Subbiah K.A. and Balan V. (2015) A Comprehensive Review of Tropical Milky White Mushroom (*Calocybe indica* P&C). Mycobiology, 43(3): 184-194.

3. Rathore H., Prasad S., and Sharma S. (2017) Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition*, 5(2): 35-46.

4. Cardwell G., Bornman J.F., James A.P. and Black L.J. (2018) A Review of Mushrooms as a Potential Source of Dietary Vitamin D. *Nutrients*, 10(10) 1498.

5. Dubey R.C. (2001) A Textbook of Biotechnology, S. Chand, Limited.

6. Manjit Singh, Bhuvnesh Vijay, Shwet Kamal and Wakchaure G.C. (2011) Mushrooms Cultivation, Marketing and Consumption. Directorate of Mushroom Research (Indian Council of Agricultural Research) Chambaghat, Solan –173213 (HP).

23. Screening for Phosphate Solubilizing Bacteria (PSB) from Rhizospheric Soil

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Vaishnavi Nagthane

Abstract

In present study, soil samples from rhizospheric niches of Tur (*Cajanus cajan*), Soyabean (*Glycine max*), Neem (*Azedirachta indica*), and Bavachi (*Psoralia corylifolia*) were collected and brought to the laboratory. All rhizospheric soil samples were screened for phosphate solubilizing bacteria on Pikovskaya agar by serial dilution method. Among the soil samples screened, rhizospheric niches from the Soyabean showed highest phosphate solubilizing bacteria, 114 than the other rhizospheric soil samples.The rhizospheric niches of Tur, Neem and Bavachi showed 47, 07, and 02 phosphate solubilizing bacteria, respectively. Out of total 170 Phosphate solubilizing rhizospheric bacteria, SMD6, SMD22, SMD28 and SMD30, presented highest phosphate solubilization index on Pikovskaya Agar.

Key Word: Rhizospheric Soil, Pikovskaya Agar, Phosphate Solubilizing Bacteria.

1.0 Introduction

Phosphorus is an essential macronutrient for growth and development of plants involved in important metabolic pathways like photosynthesis, biological oxidation, nutrient uptake and cell division (Illmer and Schinner 1992, Gupta *et al.,* 2012). Worldwide soils are supplemented with inorganic P as chemical fertilizers to support crop production, but repeated use of fertilizers deteriorates soil quality (Tewari *et al.,*2004). Present scenario is shifting towards a more sustainable agriculture by using Phosphate Solubilizing Bacteria.

Natural solubilization of mineral phosphates is an important mechanism exhibited by different microorganisms, known as phosphate solubilizing microorganisms (PSM). Bacteria are the predominant microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms (Yin, 1988, Paul and Sinha, 2017). Phosphate solubilizing bacteria (PSB)

play an importantrole in biogeochemical phosphorus cycling in both terrestrial and aquatic environments (Das *et al.,* 2007). Application of phosphate solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange (Omar, 1998; Narula *et al.,*2000; Whitelaw,2000).

The present investigation mainly focuses on the isolation of high Phosphate Solubilizing Bacteria from rhizospheric niches of different plants.

2.0 Materials and Methods

2.1 Collection of Soil sample from Rhizospheric Niches

Soil samples were collected from the rhizospheric niches of four crop plants *viz.,* Tur (*Cajanus cajan*), Soyabean (*Glycine max*), Neem (*Azedirachta indica*), and Bavachi (*Psoralia corylifolia*) grown in the farmer fields, near Purna city. For this purpose, the plants were uprooted carefully, shoots were cut off and roots along with rhizosphere soils were brought to the laboratory in polythene bags. The soil samples were processed immediately and stored at 4-8 ºC for the isolation of Phosphate solubilizing microorganisms.

2.2 Isolation of Phosphate Solubilizing Bacteria (PSB)

Phosphate Solubilizing Bacteria (PSB) were isolated from the rhizospheric soil samples by dilution plate technique using Pikovskaya's medium (Pikovskaya 1948) containing tri-calcium phosphate (TCP) (Gupta *et al.,* 2012, Kaur, 2014). Appropriate soil dilutions were plated on Pikovskaya's agar medium by spread plate technique and incubated at 30 ± 1 ºC for 2-3 days. The colonies forming halo zone of clearance (Pikovskaya's medium) around them were counted as P-solubilizers. All the bacterial colonies exhibiting halo zones were selected, purified and maintained on nutrient agar slants for further studies.

2.3 Estimation of phosphate solubilization efficiency

Pure cultures of phosphate solubilizing bacteria were spot inoculated on the plates containing Pikovskaya's medium. The plates were incubated at 28±1°C and halozone around colonies were recorded at regular intervals upto 10 days. The abilities of the isolated phosphate solubilizing bacterium to solubilize TCP on Pikovskaya'sagar media was determined in terms of solubilization index (SI). Phosphate solubilization index was calculated by measuring the colony

diameter and the halo zone diameterand the colony diameter, using the following formula of Edi-Premono *et al.,*(1996).

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{(Colony diameter + Halo zone diameter)}}{\text{Colony diameter}}$$

3.0 Result and Discussion

3.1 Isolation of Phosphate Solubilizing Bacteria (PSB)

In present study, 114 phosphate solubilizing bacteria (PSB) were isolated on Pikovskaya Agar from the Soyabean rhizospheric niches, by using dilution technique, which were far greater than the other rhizospheric niches samples. Similarly, from the rhizospheric niches of Tur, Neem and Bavachi 47, 07, and 02 phosphate solubilizing bacteria were isolated, respectively. Use of Pikovskaya's agar medium for isolation of Phosphate Solubilizing Bacteria (PSB) was a simple way to detect PSB through formation of halo zone on agar plate containing tri-calcium phosphate as a sole Phosphorous source (Kaur, 2014). These rhizospheric isolates were tentatively named as SMD 1 to SMD 170.

These reports support the fact that phosphate solubilizing bacteria can be isolated from rhizospheric niches. In this study, rhizospheric niches of Soyabean showed greater amount of phosphate solubilizing bacteria. Kaur (2014) isolated 1270 bacteria was isolated on Pikovskaya's agar plate by serial dilution method at 10^{-5}dilutions. Out of these 1270 bacterial isolates only 169 bacteria isolates were observed to be formed a halo zone around the colonies.

The role of microorganisms in solubilizing insoluble phosphates in soil and making it available to plant is well known (Kundu and Gaur,1981). Phosphate solubilizing microorganisms include several bacteria, fungi, actinomycetes, yeast and Cyanobacteria (Gerretsin, 1948; Banik and Dey 1982 and lllmer and Schinner 1992). The phosphate solubilizing microorganisms were isolatedfrom different sources such as soil (Gupta *et al.,*1986; Kapoor *et al.,* 1989),rhizosphere (Sardina*et al.,* 1986; Singh and Kapoor, 1994), root nodules (Suranga and Kumar, 1993),compost (Gupta *et al.,* 1993), and rock phosphates (Gaur *et al.,* 1973).

3.2 Estimation of phosphate solubilization efficiency

Qualitative screening of 170 phosphate solubilizing bacterial isolates revealed variations in phosphate solubilization efficiency. In total of 170 phosphate solubilizing bacterial isolates

from different niches, 4 isolates, SMD6, SMD22, SMD28 and SMD30, were found to be fare more than 5 mm zone of solubilization on Pikovskaya's agar plates. The Phosphate solubilization activity of these isolates of PKV agar plates was ranged between 2.0 to 2.6 (Table 4.1). The phosphate solubilization activity of these isolate is shown in photo plate 4.1 the results were found slightly better than Kaur (2014), who stated that 169 phosphate solubilizing bacteria isolated from different rhizospheric niches revealed phosphate solubilization index in range between 1.36 to 3.17.

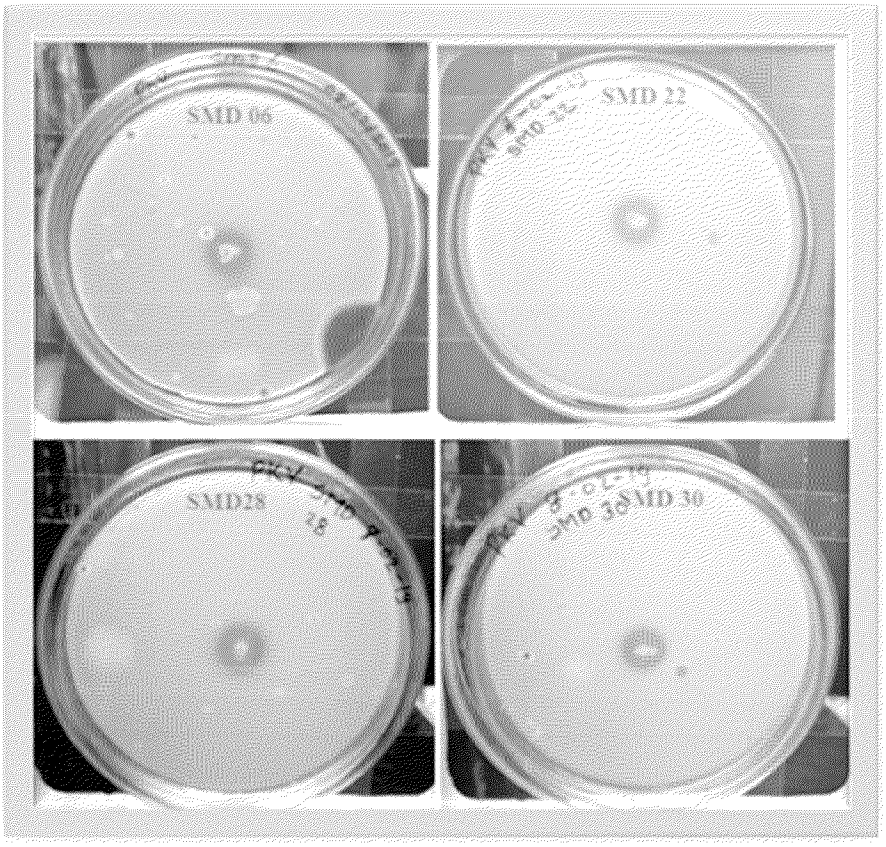

Photo Plate 4.1: Phosphate Solubilization of Isolated Bacteria from Rhizospheric Niches

Table 4.1: Phosphate Solubilization Index of Selected Rhizospheric Isolates

Rhizospheric Isolates	Diameter of Colony + Halo zone (mm)	Diameter of Colony (mm)	Diameter Halo zone (mm)	Phosphate Solubilization Index
SMD 06	10	5	5	2.00
SMD 22	9	4	5	2.25
SMD 28	8	3	5	2.66

SMD 30	8	3	5	2.66

4.0 Acknowledgement

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5.0 Reference

1. R. Yin, (1988). Phosphate-solubilizing microbes in non-irrigated soils in China, *Soils* 20:pp. 243 – 246.
2. Dipak Paul and Sankar Narayan Sinha (2017).Isolation and characterization of phosphate solubilizing bacterium Pseudomonas aeruginosa KUPSB12 with antibacterial potential from river Ganga, India. *Annals of Agrarian Science* 15: pp. 130 – 136.
3. S. Das, P.S. Lyla, and S.A. Khan, (2007). Biogeochemical processes in the continental slope of Bay of Bengal: I. bacterial solubilization of inorganic phosphate, *Rev. Biol. Trop.* 55: 1 – 9.
4. Illmer P, and Schinner F. (1992). Solubilization of inorganic phosphates by microorganisms isolated from forest soil. *Soil Biol Biochem.* 24:389–95.
5. Tewari S. K., Das B., and Mehrotra S. (2004). Cultivation of medicinal plants—tool for rural development. *J Rural Tech.* 3:147–50.
6. Mamta Gupta, Shashi Kiran, Arvind Gulati, Bikram Singh, and Rupinder Tewari (2012). Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of Aloe barbadensis Miller. *Microbiological Research* 167: 358– 363.
7. Omar S. A. (1998). The role of rock-phosphate-solubilizing fungi and vesicular arbuscular–mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. *World J Microb Biot*,14:211–8.
8. Narula N., Kumar V., Behl R. K., Duebel A. A, Gransee A., and Merbach W. (2000) Effect of P solubilizing *Azotobacter chroococcum* on N, P, K uptake in P responsive wheat genotypes grown under greenhouse conditions. J Plant Nutr Soil Sci. 163: 393–8.

9. Whitelaw M. A. (2000) Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv Agron*. 69:99–151.
10. M. Edi-Premono, A. M. Moawad, and P. L.G. Vleck, (1996). Effect of phosphate solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere, *Indones. J. Crop Sci*. 11 13 – 23.
11. Kundu, B.S. and Gaur, A.C. (1981). Effect of single and composite cultures of rock phosphate solubilization. *Haryana Agricultural University Journal of Research*, 11 :559-562.
12. Gurdeep Kaur (2014). A Ph. D. thesis "Studies on Microbial Phosphate Solubilization and Development of Inoculum Formulations". Submitted to Thapar University, Patiala – 147004 (India).
13. Gerretsen, F.C. 1948. The influence of microorganisms on the phosphorus uptake by the plant. *Plant and Soil* 1 : 51-81.
14. Banik, S. and Dey, B.K. 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate solubilizing microorganisms. *Plant and Soil* 69 : 353-364.
15. Gupta, R.D., Bhardwaj, K.K.R., Marwah, B.C. and Tripathi, B.R. 1986. Occurrence of phosphate dissolving bacteria in some soils of north west. Himalayas under varying bio-sequences and climo sequence. *Journal of Indian Society of Soil Science* 34 : 498-504.
16. Kapoor, K.K., Mishra, M.M. and Kukreja, K. 1989. Phosphate solubilization by soil microorganisms. A review. *Indian Journal of Microbiology* 29 : 119-127.
17. Sardina, M.G., Boiardi, J.L. and Erbola, R.J. 1986. Solubilization of phosphorus from low grade minerals by microbial action. *Biotechnology Letters* 8 : 247-252.
18. Singh, S. and Kapoor, K.K. 1994. Solubilization of insoluble phosphates by bacteria isolated from different sources. *Environmental Ecology*, 12 : 51-55.
19. Suranga, S. and Kumar, N. 1993. Phosphate solubilization under varying pH by Rhizobium from the legumes. Journal of Experimental Biology 31 : 855-857.

20. Gupta, R., Shanker, A.B., Saxena, R.K. and Kuhad, R.C. 1993. Solubilization of low grade Indian rock phosphates and inorganic phosphates by Bacillus licheniformis. Folia Microbiologia, 38 : 274-276.
21. Gaur, A.C, Mandan, M. and Ostwal, K.P. 1973. Solubilization of phosphatic compounds by native microflora of rock phosphates. Indian Journal of Experimental Biology 11 : 427- 429.
22. Pikovaskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologiy, 17: 362-370.

24. To Study the DNA and RNA Content in Some Tissues of Freshwater Fish Clarias Batrachus Exposed to Heavy Metal Copper Sulphate

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Abstract

Industries are the major sources of heavy metal pollution and it is released into water and soil. Heavy metals cause several ill effects to aquatic living organisms and environment. The nucleic acid (DNA and RNA) content in different tissues such as brain, liver, kidney and ovary of copper sulphate exposed freshwater fish, *Clarias batrachus* has been studied. The changes in the nucleic acid content of the tissues have been observed. The DNA content in the ovary is higher in comparison to other tissues. The variation of DNA content in control and copper sulphate exposed fish is ovary >liver>brain>kidney. The RNA content also exhibited similar to that of DNA, having higher amount in the ovary and the variation is ovary>liver>brain>kidney. Although the degree of variation between the tissues remains same in both control and experimental groups, the nucleic acid content reduced under copper sulphate exposed freshwater fish, *Clarias batrachus* indicating copper sulphate as a pollutant effect the nucleic acid content in the tissue.

Key Words: DNA, RNA, *Clarias batrachus*, Copper Sulphate.

Introduction

The problem of pollution of the water where the wastes are usually discharged has increased to a great extent in recent years. Aquatic environments are loaded with several types of organic and inorganic pollutants. Huge amounts of agriculture pesticides, used for crop protection, eventually enter into the aquatic system. Similarly, heavy metals, which are released as industrial effluents form the major constituents of aquatic pollution. The presence of excess quantities of these toxic pollutants in water bodies has caused mass mortality of fishes in the past (Wanganeo *et al.*, 1994). Copper sulphate is a fungicide used to control bacterial and fungal

diseases of fruit vegetable, nuts and field crops. Some of the diseases that are controlled by this fungicide include mildew, leaf spots, blights and apple scab. It is also used as an algaecide, an herbicide in irrigation and municipal water treatment system. Copper sulphate is a naturally occurring inorganic salt and copper is an essential trace element in plant and animal nutrition. It is available in dusts wet table powders and fluid concentrates. Copper sulphate is also widely used as an algaecide for controlling phytoplankton in fish ponds and lakes as well as a herbicide used in aquatic weed control since 1982 (Carbonell and Tarazona, 1993). As copper sulphate found to be a pollutant causing deleterious effect on aquatic organisms at different levels, in the present study effect of copper sulphate on nucleic acid content of some important tissues of the freshwater fish, *Clarias batrachus* has been undertaken.

Materials and Methods

Adult and live fish *Clarias batrachus* were collected from the farm Patra and Bhadbhada Bhopal M.P.) brought to the laboratory, cleaned by using 0.1% $KMno_4$ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. Determination of LC50 50 fishes were used for the determination of LC50. The concentration that kills 50 per cent of the fish in 96 h duration (LC50/96h) was determine by Static Bioassay method (Doudoroff *et al.*, 1951) by using the mortality values for 96h the LC50 was determined by plotting the graph. The exposed fish were sacrificed after 96h (LC50) and the tissues such as brain, liver, kidney and ovary were dissected out and processed for determination of DNA/RNA content. In all the cases six observations were made and the results (DATA) were expressed as arithmetic mean with their Standard Deviation, Standard Error and Student't' were made as described by Suedecur (1946) and Fisher (1963). The nucleic acid (DNA and RNA) content of the tissues was estimated by following the Diphenylamine method of Schneider (1940) using DNA as standard. The RNA content of the tissues was also estimated by Orcinol method using RNA as standard.

Results and Discussion

The nucleic acid content in different tissues such as brain, liver, kidney and ovary of both control and copper sulphate exposed fish *Clarias batrachus* has been studied. The following results were observed in both control and copper sulphate exposed fish, ovary contain large amount of DNA in comparison to other tissues (Table 1). The degree of DNA content in control

and copper sulphate exposed fish, ovary >liver>brain>kidney. The RNA content also exhibited similar to that of DNA having higher amount in the ovary. The degree of RNA content in control and copper sulphate exposed fish (Table.1) ovary >liver>brain>kidney.

Table 1: Showing DNA and RNA content (mg/g) in different tissues of the freshwater fish, *Clarias batrachus* on exposure to Copper sulphate.

	Brain		Liver		Kidney	
Gonads	**DNA**	**RNA**	**DNA**	**RNA**	**DNA**	**RNA**
Control	**133.75±0.81**	**49.33± 0.25**	**145± 0.80**	**51.5±0.31**	**87.91± 0.40**	**49± 0.13**
170± 0.79	**58± 0.81**					
CuSo4	**135.20±0.79**	**46.76± 0.49**	**137.5± 0.86**	**46.76±0.42**	**85.41± 0.76**	**45.95± 0.29**
142.5± 0.86	**55.51± 0.31**					
exposed						

DNA/RNA ratio in different tissues: - Control:-brain has 2:1, liver 2:1, ovary 2:1 and only in kidney it is 1:1. Copper sulphate (CuSO4):- brain has 2:1, liver 2:1, ovary 2:1 and only in kidney it is 1:1.Total RNA content comprised of m, r and t RNA are variable. Miglavs and Jobling (1989) The RNA/DNA ratio indicates the protein synthetic potential of a cell and it is an index of fish growth. Wilder and Stanley (1989) reported the fall of RNA/DNA ratio of salmonid fishes by the treatment of carbaryl. Significant loss of metachromasia has been observed in mercury treated fishes after 9 and 30 days of exposure and moderate loss was found after 22 days. The effects of sub-lethal concentrations (3% and 15 % v/v) of untreated nickel chrome electroplating effluent on the reproduction of female *Clarias batrachus* were studied during preparatory, prespawning and spawning phases of reproductive cycle. Both GSI and HSI of the exposed fish were lower as compared to control fish in all the phases. An irregular pattern of deposition of macromolecules (DNA, RNA and Proteins) in liver as well as in ovaries indicated that exposed fish were not cycling properly due to stress. Alterations in the contents of macromolecules were greater in treatment T2 (15 % v/v) than T1 (3 % v/v) during all the phases (Kaur and Kaur, 2005). The present study clearly indicates that a short term exposure to copper sulphate, the DNA and RNA content of tissues get reduced in the freshwater fish *Clarias batrachus* indicating copper sulphate as pollutant effect the nucleic acid content in the tissues.

Conclusion

After the above discussion it had been concluded that copper sulphate causes deleterious effects on fishes and much altars the DNA and RNA contents of certain tissues. In sub lethal concentration it may not be fatal for an individual organism but it does affect the growth rate and reproduction resulting in disturbance to whole community and tropic levels of food chains, ultimately the ecosystem.

References

1. Carbonell, G. and Tarazona, J. V. 1993. A proposed method to diagnose acute copper poisoning in cultured rainbow trout (*Oncorhynchus mykiss*). Sci. Total Environ. 2: 1329-1334.
2. Doudoroff, P., Aderson, B. G., Burduck, G. E., Galtsaff, P. S., Hart, W. B., Pattrick, R., Stronge, E. R., Surber, E. W. and Van horn. 1951. Bio-assay for the evaluation of acute toxicity of industrial wastes to fish. Sew. Ind. Wastes. 23: 1310-1397.
3. Fisher, R. 1963. Statistical methods for research worker 6thEd., Edinburgh.
4. Kaur, A. and Kaur, K. 2005. Biochemical response of *Chinna punctatus* to induced stress during different phases of the reproduce Cycle. Indian J. Fish. 52 (3):258-292.
5. Miglavs, I. and Jobling, M. 1989. Effect of feeding regime on food consumption, growth rates and tissue nucleic acids in juvenial Artic charr (*Salvenius alpinus*) with particular respect to compensatory growth. J. Fish. Bio. 34: 947-957.
6. Schneider 1940. From the McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison.
7. Shukla, V., Rathi, P. and Sastry K. V. 2002. Effect of Cadmium individually and in combination with other metals on the nutritive value of freshwater fish *channa punctatus* Suedecor, C. W. 1946. Statistical methods lowa state college press Amesiowa.
8. Wanganeo, A., Pani, S., Nandan, M. J. and Suresh, I. V. 1994. Incidence in fish mortality in lower lake of Bopal. Giobios, 21: 145-146.

25. Diversity of Dragonflies and Damselflies (Order: Odonata, Class: Insecta) Around the Morna Dam, Patur, Dist-Akola (India)

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Abstract

Dragonflies and Damselflies (Odonates) are one of the most common insects flying over forests, fields, meadows, ponds and rivers. Area around the Morna Dam, Patur, Dist-Akola (M.S.) was selected for the present study. The objectives of the present investigation were to explore the Diversity of Dragonflies and Damselflies (Class Insecta, phylum Arthopoda) of the present region. Total 33 species of dragonflies and damselflies belonging to four families namely Lebellulidae, coenogrionidae, gomphidae and Platycenemididae were recorded. In our study the most abundant family was Lebellulidae which represents 17 species followed by coenogrionidae with 13 species, while family gomphidae and Platycenemididae were least abundant and represents two and one species respectively. We also observed that the present area was rich in diversity of dragonflies and damselflies. This diversity of odonates (Dragonflies and Damselflies) may be due to good natural habitats and lack of pollution in the desired area.

Key words: *Dragonflies and Damselflies, Diversity, Abundance, Morna Dam, insects*

Introduction

Dragonflies and Damselflies of order Odonata belongs to one of the most primordial creatures in world with fossil records dating back to the Permian era 230-280 million years ago with 5740 species and subspecies from 654 genera in 32 families throughout the world (Andrew, *et al* 2008; Batzer, *et al* 1996). They are regarded as indicator of ecosystem health (Hart, 2014) and thus diversity of dragonflies *and Damselflies* in an area shows the state of ecosystem of that area.There is no widespread account of Indian odonates after (Hodgkin and Watson, 1958) published during 1930's. Large numbers of endemic *Dragonflies and Damselflies (*odonates) are being threatened due to huge scale habitat destruction. Huge scale habitat alterations such as sand mining, damming channel diversion

and pollution are critically threatening the survival of these species (Johansson and Suhling, 2004).

2. Material and Methods

2.1 Study area: The present Morana Dam is situated at 20°41 N, 76°99 E, the hilly region with good forest cover of Maharashtra state and only seven km away from the Patur tahsil. The area is rich in dense forest with minimum interference of human beings.

Figure 1: Satellite image of Morana Dam, Dist-Akola, India

2.2 Sampling of Odonates

Sampling of *Dragonflies and Damselflies* (odonates) was carried out from July 2017 to June 2018 in different study sites selected around Morana Dam. Sampling was carried out by direct searching methods following Sutherland (Sutherland, 1996) at a fifteen days interval during the period of 10:00 to 16:00 hours, because *Dragonflies and Damselflies* are found mostly active during the studied time due to their reliance on sunlight directly to regulate their body temperature (Das, *et al*, 2013). Odonates were photographed and identified using the keys of Andrew, *et al*. (2008) and Subramanian (2005).

3. Results and Discussion

A total of 33 species under 4 families were recorded during the entire study period (Table 1). On the basis of total number of species Libellulidae was the most dominant family with 17 species followed by coenogrionidae with 13 species. The list dominant families were gomphidae which represents 02 species and Platycnemididae with only one species.

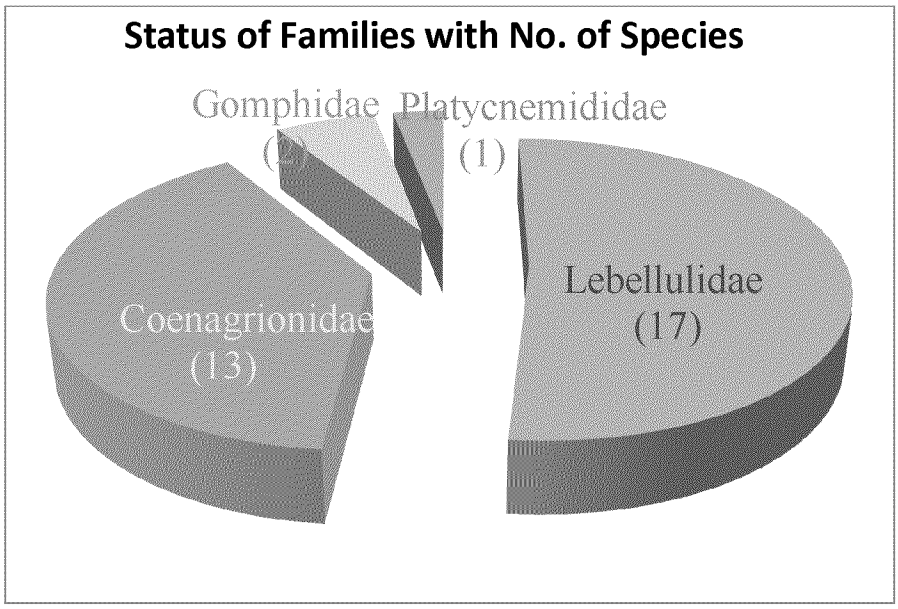

Figure 2: Different Families of *Dragonflies and Damselflies with number of species.*

On the basis of total number of species recorded, Lebellulidae was the most dominant family constituting 56.10% of the total number of recorded species. Family Coenagrionidae was the second most dominant family and represents 42.90% of the total species of Odonates recorded during the study period. Gomphidae and Platycnemididae were comprised by a two and one species and constituting 6.60% and 3.30% of the total number of species of the Odonates, respectively. Similarity, 37 species of Odonates from eight families has been reported from Corbett Tiger Reserve (Singh and Prasad, 1977 and 1979; Khanna, 2008). Likewise, 6 species from 4 families of Odonates have been represented from Nanda Devi Biosphere Reserve, India. The most dominant family was Platycnemididae with 3 species, followed by Aeshnidae, Cordulegasteridae and Synlestidae (1 each) (Kumar, 1997). On the other hand, in Maharastra India, 46 species of Odonata have reported from Mula and Mutha river basins (Kulkarni and Subramanian, 2013).

Table 1: Diversity of the Dragonflies and Damselflies from Morana Dam, Dist-Akola, India

SR. No.	FAMILY	COMMON NAME	SCIENTIFIC NAME
1.	**Lebellulidae**	TRUMPET TAIL	*Acisoma panorpoides* (Rambur, 1842)
2.		DITCH JEWEL	*Brachythemis contaminata*

			(Fabricius, 1793)
3.		GRANITE GHOST	*Bradinopyga geminata* (Rambur, 1842)
4.		RUDDY MARSH SKIMMER	*Crocothemis servilia* (Drury, 1770)
5.		GROUND SKIMMER	*Diplacodes trivialis* (Rambur, 1842)
6.		BROWN-BACKED RED MARSH HAWK	*Orthetrum chrysis* (Selys, 1891)
7.		BLUE MARSH HAWK	*Orthetrum glaucum* (Brauer, 1865)
8.		CRIMSON-TAILED MARSH HAWK	*Orthetrum pruinosum* (Burmeister,1839)
9.		GREEN MARSH HAWK	*Orthetrum Sabina* (Drury, 1770)
10.		WANDERING GLIDER	*Pantala flavescens* (Fabricius, 1798)
11.		YELLOW-TAILED ASHY SKIMMER	*Potamarcha congener* (Rambur, 1842)
12.		COMMON PICTURE WING	*Rhyothemis variegata* (Linnaeus, 1763)
13.		CORAL-TAILED CLOUD WING	*Tholymis tillarga* (Fabricius, 1798)
14.		CORAL MARSH TROTTER	*Tramea virginia* (Rambur, 1842)
15.		CRIMSON MARSH GLIDER	*Trithemis aurora* (Burmeister, 1839)
16.		BLACK STREAM GLIDER	*Trithemis festiva* (Rambur, 1842)
17.		LONG-LEGGED MARSH GLIDER	*Trithemis pallidinervis* (Kirby , 1889)
18.	**Gomphidae**	COMMON CLUBTAIL	*Ictinogomphus rapax* (Rambur, 1842)
19.		COMMON HOOKTAIL	*Paragomphus lineatus* (Selys, 1850)
20.	**Coenagrionidae**	PALE SLENDER DARTLET	*Aciagrion pallidum* (Selys, 1891)
21.		PRUINOSED DARTLET	*Agriocnemis femina* (Brauer, 1868)
22.		WHITE DARTLET	*Agriocnemis pieris* (Ladlaw, 1919)
23.		PIGMY DARTLET	*Agriocnemis pygmaea* (Rambur, 1842)
24.		SPLENDID SCARLET	*Agriocnemis splendidissima*

			(Laidlaw, 1919)
25.		TINY HOODED DARTLET	*Agriocnemis species*
26.		COROMANDEL MARSH DART	*Ceriagrion coromandelianum* (Fabricius, 1798)
27.		GOLDEN DARTLET	*Ischnura aurora* (Brauer, 1865)
28.		SENEGAL GOLDEN DARTLET	*Ischnura senegalensis* (Rambur, 1842)
29.		TREE-LINED DART	*Pseudagrion decorum* (Rambur, 1842)
30.		BLUE GRASS DARTLET	*Pseudagrion microcephalum* (Rambur, 1842)
31.		SAFFRON-FACED BLUE DART	*Pseudagrion rubriceps* (Selys, 1876)
32.		PIXIE DARTLET	*Rhodischnura nursei* (Morton, 1907)
33.	**Platycnemididae**	YELLOW BUSH DART	*Copera marginipes* (Rambur, 1842)

4. Conclusions

Thirty three species of Odonata under four different families were observed around the Morana Dam. High diversity of Dragonflies and Damselflies were observed in the study area. This high diversity in the limited area may be due to good water quality of Morana Dam with good environmental conditions and surrounding dense forest area with minimum human beings interactions.

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5. References

- **Andrew, R. J., K. A. Subramaniam and A.D. Tiple, 2008**. Common odonates of central India. Proceedings of the 18th International Symposium of Odonatology, November 5-9, 2008, Hislop College, Nagpur, India, pp: 1-3.
- **Batzer, D.P. and S.A. Wissinger, 1996.** Ecology of insect communities in nontidal wetlands. Ann. Rev. Entomol., 41: 75-100.

- **Das S. K., P. K Sahoo., N. Dash., S. Marathe., S. Mahato., A. Dashahare *et al.* 2013**. Odonates of three selected tiger reserves of Madhya Pradesh, Central India. Check List; 9(3):528-532.
- **Hart L. A., M. B. Bowker., W. Tarboton., C.T. Downs 2014.** Species composition, distribution and habitat types of Odonata in the Simangaliso Wetland Park, KwaZulu-Natal, South Africa and the associated conservation implications. PLoS ONE; 9(3):e92588.
- **Hodgkin, E. P. and J. A. L. Watson, 1958.** Breeding of dragonflies in temporary waters. Nature, 181: 1015-1016.
- **Johansson, F. and F. Suhling, 2004.** Behaviour and growth of dragonfly larvae along a permanent to temporary water habitat gradient. Ecol. Entomol., 29: 196-202.
- **Khanna V. 2008.** Zoological Survey of India, Fauna of Corbett Tiger Reserve, Conservation Area Series; 35:195- 203.
- **Kulkarni A. S., K.A. Subramanian 2013.** Habitat and seasonal distribution of Odonata (Insecta) of Mula and Mutha river basins, Maharastra, India. Journal of Threatened Taxa.; 5(7):4084-4095.
- **Kumar A. 1997.** Odonates. Fauna of conservation areas: Zoological Survey of India. Fauna of Nanda Devi Biosphere Reserve; 9:45-47.
- **Singh A. and M. Prasad 1977.** Odonata (Insecta) of Corbett National Park (Uttar Pradesh, India). Journal of Bombay Natural History Society.; 73:419-421. 19.
- **Singh A. and M. Prasad 1979.** Odonata in: Fauna of Corbett National Park by Lamba BS, Bhatnagar RK. Cheetal; 21:43-44. 20.
- **Subramanian, K. A. 2005.** Dragonflies and Damselflies of Peninsular India: A Field Guide. Indian Academy of Science, Bangalore, India, Pages: 118.
- **Sutherland W. J. 1996.** Ecological Census Techniques. Cambridge: Cambridge University Press, 450.

26. Study of Physio-Chemical Parameters of Waghur Dam, Tal. Jalgaon, Maharashtra

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Abstract

Water samples from Waghur dam were collected and analyse for various Physio-chemical parameters, during September 2017 to August 2018. The various parameters such as Atmospheric Temperature, Water temperature, pH, Transparency, Turbidity, DO, C02, BOD, total dissolved solids, alkalinity and hardness of water etc were tested. The results revealed that there was significant seasonal variation in some Physio-chemical parameters and most of the parameters were in the normal range and indicated better quality of dam water.

Keywards: Physio-chemical parameter of Waghur dam, dissolved oxygen, water transparency

Introduction

Water is the one of the unavoidable compound to the ecosystem. Quality of the water described by its physical, chemical and microbial characteristics. But some correlation were possible among these parameters and the significant one would be useful the indicate quality of water (Usharani et.al., 2010).

Now a day, due to increased human population and manmade activities such as rapid industrialization and indiscriminate and heavy use of chemical fertilizers in agriculture are causing remarkable and varied pollution in aquatic environment leading to deterioration of water quality and depletion of aquatic biota (N. Subhas et.al., 2016). So it is necessary that the quality of drinking water should be checked at regular time interval because due to use of contaminated drinking water peoples suffers from a variety of water born diseases. The physio- chemical parameters of water and the dependence of all life processes of these factors make it desirable to take water as an environment (Premlata et.al., 2009).

The present investigation involves the analysis of water quality in terms of selected physio- Chemical parameters of Waghur Dam, Dist. Jalgaon, Maharahstra. This Dam was constructed in 2007, the reservoir is located on 75° 19* North latitude and 18°48' East longitude. The length of dam is about 1112.85 mts. and 18.10 mts. Height with storage capacity is about 12.80 mcub meters. This dam is source of drinking and irrigation water for nearly about 42 villages and Jalgaon Corporation under the canal irrigation. Dam water getting polluted due to domestic and agricultural waste leading to notable changes in the water quality and to quantify the changes, present investigation was carried out from September 2017 to August 2018.

In India many researchers have done studies on physio-chemical and biological characteristics of some rivers and water reservoirs (Ram Sharma, 2012, Pandey et. al., 1993; Mohammad Musaddiq et. al., 2002; Kodarkar, 2006; Pawar and Pulle 2015; Jakher and Rawat, 2003; Patil et.al., 2014 and Shubhachandra et. al., 2013 etc).

Table 1. Physio-chemical analysis of surface water of Waghur dam at station A (Raipur) and B (Neri) from September 2017 to August 2018.

Months	Station	Parameters										
		Air Temp in °C	Water Temp in °C	p	Transparency	Turbidity	Dissolved oxygen	Carbon dioxide	BOD	Total Dissolved Solids	Total Alkalinity	Total Hardness
Sept. 2017	A	29	27	6.	28	238	6.9	5.0	11.0	284	45	128
	B	28	27	6.	28	240	6.9	5.2	10.9	285	45	128
Oct.	A	27	26	6.	34	242	6.7	6.1	9.9	269	52	118

2017	B	27	26	6.	34	243	7.0	6.2	10.9	270	52	120
Nov.	A	23	21	6.	45	236	7.0	5.9	9.7	236	57	113
2017	B	23	21	7.	46	236	7.1	5.8	9.9	238	57	117
Dec.	A	21	20	7.	54	228	7.9	5.4	8.0	207	65	118
2017	B	21	20	7.	54	228	7.9	5.1	7.9	208	58	119
Jan.	A	20	20	6.	58	226	7.2	4.9	7.4	198	74.5	148
2018	B	20	20	7.	58	226	7.2	5.0	7.3	199	75	148
Feb.	A	21	21	7.	60	238	7.1	4.2	12.5	294	66	151
2018	B	22	21	7.	60	238	7.1	4.3	11.5	295	66	151
Mar	A	27	24	7.	61	242	7.4	4.6	23.0	298	56	153
2018	B	27	24	7.	62	243	7.4	4.9	24.0	298	57.5	152
April	A	34	30	7.	68	290	7.6	5.7	24.2	287	60	156
2018	B	34	30	7.	70	289	7.6	5.8	25.0	283	61	152
May	A	36	34	7.	78	275	6.5	6.0	26.0	288	63	158
2018	B	36	34	7.	78	258	6.6	6.1	26.0	288	65	156
June	A	37	35	7.	63	254	7.1	6.1	24.0	278	56	170
2018	B	37	35	7.	60	247	7.2	6.3	25.0	278	63	170
July	A	26	27	6.	58	250	7.8	5.2	26.0	276	41	171
2018	B	26	27	6.	52	250	7.8	5.2	26.0	276	42	172
Aug.	A	27	25	7.	45	248	7.1	5.3	21.0	275	43	174

2018	B	27	25	7.	40	248	7.2	5.1	23. 0	273	43	175

Material and methods

The surface water samples from Waghur dam were collected from two sampling stations A (Raipur) and B (Neri) in the hours at 10.00am to 11.00pm regularly for every month during the study period in clean black two litres plastic cans and immediately transported to the laboratory for the estimation of various physico-chemical parameters. The parameters like water temperature, pH, transparency were recorded at project sites, where as Turbidity, DO, TDS, BOD, Total Hardness and Total Alkalinity were estimated in the laboratory. Turbidity of water were estimated by through Jackson candle turbid meter, while other parameters were estimated in the laboratory by using standard methods as prescribed by Trivedy and Goel (2017), APHA (1998), Kodarkar (2006) and Gupta P.K. (2007).

Result and Discussion

The seasonal variation in Physio-chemical parameter were found as shown in table number 1. The atmosphere temperature was recorded between 20°c to 37°c. It was maximum in the May and June month. The atmospheric temperature is important and plays an important role in the environment, while water temperature was recorded 20°c to 35°c. The water temperature was maximum in May and June month. Water temperature is the most important factor which influences the chemical, bio-chemical and biological characteristics of the water body. The water temperature was observed by Pawar, Pulle (2015) and Dey , Kallol (2015).

pH was slightly alkaline the value being around 7 or greater than 7 at both station, maximum 7.8 and minimum 6.7 at sampling station. The factors like air temperature bring about changes the pH of water. Most of bio-chemical and chemical reactions are influenced by the pH, it is great practical importance. The reduced rate of photosynthetic activities reduces the assimilation of carbon dioxide and bicarbonates which ultimately responsible for increase in pH, the low oxygen values coincided with high temperature during the summer months.

Transparency values in present study were ranged from 28 to 78cm. The water Transparency value was maximum in summer and minimum in monsoon. Transparency is a physical variable significant to primary production and also depends on the micro-organism present in water bodies and suspended organic and inorganic matter present in water. In one of

the study (Sharma 2012) observed the minimum value during August while maximum value was recorded during the month March.

The turbidity of water ranges between 226 to 290 NTU. The turbidity was recorded maximum level in the monsoon whereas minimum value was recorded 231 NTU in the month of January. Monsoon generally causes high turbulence and mixing of water leading to an increasing the concentration of suspended particulate matter (SPM). Study of similar lines recorded turbidity range between from 230 to 289 mg/lit.

The dissolved oxygen was varied from 6.5 to 7.9 mg/lit. The DO was maximum 7.9 mg/lit. in the month of December whereas minimum was recorded 6.5 mg/lit. in the month of May. The DO is one of the most important factors in any aquatic ecosystem. The main source of DO is from dissolution from atmosphere and the photosynthesis. Several worked also observed similar trend to results in fresh water lakes (Chavan et. al.,2004) and (Trivedy et. al.2017) have reported DO range between 2.3 to 10.8 mg/lit.

The values of free carbon dioxide were ranges from 4.2 to 6.3 mg/lit. The free CO_2 was maximum in month of June and minimum in the month of February. The values of free carbon dioxide were inversely proportional to dissolved oxygen at the sampling station. This may be depends upon plants aquatic animals present in water body as well as alkalinity and hardness of water. The free carbon dioxide values were extremely high and high values of free carbon dioxide may result from breakdown of organic matter.

The BOD were varied from 7.3 to 26.0 mg/lit. It was maximum in summer and monsoon months and minimum during winter season. BOD has been used as measure of the amount of organic material in an aquatic solution which supports the growth of micro organic. Pawar et., al. (2015) were observed the high BOD values from the samples exposed to municipal water at near sewage dam. In other study (Nisar S. et., al. 2004) were recorded values varied from 0.72 to 3.02 mg/lit.

The total dissolved solids ranged from 200 to 298 mg/lit. in the sampling station and it was found maximum in the month of March and minimum in January. In other study TDS from Udaipur lake range found to be from 202 to 724 mg/lit (Gupta et., al. 2001). The high level of TDS in drinking water causes laxative effects. Mohmmad Musaddiq et.al. (2002) observed values of total suspended solids ranged within 45 to 152 mg/lit. of surface water in Akola city. Khobragade et.al. (2003), observed the values of TDS was in the range of 9100 to 2500 mg/lit.

Total alkalinity of the dam was varied from 41 to 75 mg/lit. Its value was found maximum in winter season and minimum in monsoon season. The low alkalinity might be due to high pH. The high pH may be due to the hydroxide carbonates and bicarbonates, photosynthetic activity of aquatic plant which reduces alkalinity value. High alkalinity may be due to the addition of waste from organic matter. Narasinha Rao et.al. (2001) found the alkalinity values varied from 90 to 265 mg/ lit. in sewage fed fish culture pond at Nambur. In one of the study Sakare P. et.al. (2003) found the alkalinity values from 672 to 1023 mg/lit. in Jamda in minor wetland in Chalisgaon town, in Maharshtra.

The total hardness values ranged from 113 to 175 mg/lit. Total Hardness was found maximum during month of in monsoon season and minimum in winter. The total hardness is the total soluble magnesium and calcium salts present in the water expressed as its CaCO3 equivalent. Total hardness are also includes the sulphate, chlorides of calcium and magnesium. In most natural water the predominant ions are those of bicarbonates associated mainly with calcium to lesser degree with magnesium and still less with sodium and potassium, sulphate and chlorides of calcium and magnesium bicarbonates of per magnet caused by soluble calcium and magnesium carbonates and salts of inorganic acid. In other study Mishra and Tripathi (2001) reported high values of 295 mg/lit.

References

- **APHA** (1998), Standard methods for the examination of waste water American Public Health Association, Washington D.C 874.
- **Chavan,** Sawant, Hiware and Tat (2004). "Study on water quality of Manjara project reservoir in Beed District", *J. Aquatic Biology*, Vol. 19 (2), pp 73-76.
- **Dey, Kallol,** Mohapatra, S. C., Misra, Bidyabati, (2015), "Assessment of water quality parameters of the river Brahmani at Rourkela", *Journal of Industrial Pollution Control*, 21(2), 265-270.
- **Gupta and** Suruchi, *Asian J. Chem.*, 2001 13 (3), 16-17
- **Khobragade P. N.** and K. Shama (2003). "Water Quality analysis of river Cauvery in Akola City". *Journal of Environ. Biol.* Vol. 20(4): pp 347-352.
- **Kodarkar M. S.** (2006), "Methodology for water analysis, Physio-chemical, Biological and Microbiological", *Indian Association of Aquatic Biologist Hyderabad*; pub. 2: pp50-55.

- **Mohammad Musaddiq** and Anil K. Fokmare (2002), "Determination of water quality index of surface water source of Akola city (M.S.)", J. of Aquatic Biology, Vol. 18(2), pp.27-31.
- **Mishra P.K. ,**Tripathi M. K. (2001), "Physico-chemical and biological characteristics of Bardon Lake". *Journal of Water Quality and Toxicity* Vol. 4(1) pp 35-39.
- **NarasinhaRao ,**Jaya Raghu (2001),"Hydrological studies in the inshore waters of Bay of Bengal". *Jourol*. 26; pp 58-65.
- **N. Shubhas Chandra** Meitei, P.M. Patil and Bhosale A.B. (2016), "Physio-chemical analysis of Puma river of paternity", *J. Aquatic Biology,* Vol. 19(1), pp. 103-105.
- **Nisar Shaikh and Yeragi** S.G. (2004), "Some Physio- chemical aspects of Tansa river of Thane district (M.S.)", J. Aquatic Biology, Vol. 19 (1), pp. 99- **102**.
- **Patil S. P.**, J.M. Patwari and R.G.Auti (2014, "Ground water quality from Pravara area, district Ahmednagar (M.S.)", *J. Aquatic Biology*, Vol. 18(2),pp.85-86.
- **Pawar S.K.** and J.S. Pulle (2015), "Studies of physio- chemical parameters in Pethwadaj Dam, Nanded district (M.S.)
- **Premlata, Vikal**, (2009), "Multivariant analysis of drinking water quality parameters of lake Pichhola in Udaipur, India", *Biological Forum- An International Journal*, 1(2), pp 97-102.
- **Sakare P.,** Joshi P. (2003), "Seasonal variation in physical aspects of Pollution in Nalda river", *Journal of Environ. And Ecotoxicology* Vol. 21 (2) 93-96.
- **Ram Sharma** (2012), "Studies on physio-chemical and biological characteristics of rivers and reservoirs in Uttar Pradesh" ,*Water Quality Journal* Vol. 16 pp.11-16.
- **Trivedy R.K.** and P.K. Goel (2017), Chemical and biological methods for water pollution studies, Environmental Publication, Karad, Maharashtra.
- **Usharani K, K Umarani, P.M Ayyasamy, K Shanthi,(2010)**, "Physico-Chemical and Bacteriological Characteristics of Noyyal River and Ground Water Quality of Perur, India*", Journ. of Appl. Sci. and Envn. Management* Vol.14(2) pp 128-135.