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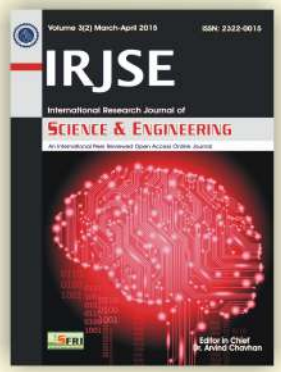

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REVIEW ARTICLES

RESEARCH ARTICLE

Seed surface characteristics and preliminary phytochemical analysis of *Celastrus paniculata* Willd

Ulhe PP

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Thanks to Birbal Sahani Institute of Paleobotany, Lucknow for Scanning Electron Microscopy of seeds photography

ABSTRACT

The ancient medicine was known as herbal medicines. India is considered to be leading exporter of the medicinal plants in the world market. Plants play a very important role in preparation of herbal drugs, medicines etc. Herbal drugs play an important role in health care system. Each and every part of the plant having its specific use. *Celastrus paniculata* Willd. is one of the medicinal plant having its importance in health care system as a indegenous medicinal plant also. Every part is medicinally important but seeds of *Celastrus paniculata* Willd. having its specific use. Here we have studied the seed coat pattern i.e, seed morphology, seed anatomy and preliminary phytochemical analysis of seed powder. In seed morphology, the scanning electron microscopy detect the prominent hard rugae, uniform cell pattern with elevations. The transverse section of seed coat shows ovular section with outer epidermis wavy, palisade tissue compactly arranged in between them. Parenchymatous cells elongated isodiametric with intercellular spaces and tanniferous cells with well-developed endosperm. In preliminary phytochemical analysis the three-extract ethanol, petroleum ether and aqueous extract gives alkaloids, sterols and terpenoids, tannins are detected. Phenolic compounds, mucilage and gums, anthraquinone is absent. This seed coat study is essential for micromorphological observations, seed identification, taxonomic identification in between species to species and secondary metabolites present inside the seeds are well defined by using phytochemical technique for their theruptic efficacy which is beneficial in health care system, economically and in society.

Keywords: *Celastrus paniculata* Willd., seed morphology, SEM (Scanning electron microscopy), seed anatomy, preliminary phytochemical analysis.

INTRODUCTION

Plant served for the purpose of food, fodder, shelter and medicines from the ancient period. The ancient medicine was known as herbal medicines. Plants play a very important role in preparation of drugs, medicines. Various organs of the plant body having its specific use and also medicinally important. So, each and every parameter of the plants like

size, shape, surface, colour, height etc. are very important for the plant study. Many of the traditional medicine are complex mixture of different plants parts that are collected at proper intervals, mixed in specific proportions and administered in definite dozes for required period of time. India being a rich repository of medicinal plants has been a major supply in the world market till 1976. Now a days, India is considered to be leading exporter of the medicinal plants in the world market.

Celastrus paniculatus is a deciduous climbing shrub that can grow very large in size. *Celastrus* is prized by native peoples throughout India for its seeds, which grow in round pods that gradually change from a light yellow to a deep red colour as they mature. Seeds are probably more valuable than any other part of the plant for man and plant (Kozlowski and Gunn 1972). Seeds also constitute an important source in ethnomedicinal formulations and have a tremendous potential for pharmaceutical industry. The seeds are small, oval shaped and grow six per seed pod. It is native to the Indian continent but also grow widely in various countries (Cleversley, 2002).

The whole plants or plant parts contains number of chemical constituents the chief being alkaloids, glycosides, minerals crystals such as calcium oxalate, calcium carbonate, silica as well as tannins, resins, latex, volatile oils etc. seed powder used for various medicinal preperation as drugs. The seeds being a complex organ of multiple origin and metabolically most active that secretes and synthesizes efficient compounds that can be exploited for drug formulations. They are also endowed with definite morphological and structural pattern along with unique ornamentations. The morphological and anatomical seed surface study also important for identification and study of micromorphological characteristics. For detection of seed surface characteristics, chemical constituent, medicinal value this study is very important.

MATERIALS AND METHODS

Sample collection: Seeds of family Celastraceae like *Celastrus paniculata* Willd. were collected from local area. For seed coat study, all the seeds parameters were studied using dissecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM)

To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min. to remove the dust from them. The seed mounted on pin type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of paleobotany, Lucknow.

Seed coat anatomy

For the anatomical observation of seed coat study take the transverse sections of seed coat. Using permanent slide preparation method or double staining method place the section on various alcohol grade like 30%,50%,70%,90% absolute alcohol, xylene, DPX etc. The staining like safranine and light green stain used for staining.

Preliminary phytochemical tests

The preliminary phytochemical analysis is most important for detection of various chemical constituents. Trease and Evans (1989) test were done. Qualitative phytochemical analysis of the crude powder of the seeds of the plant for the identification of phytochemicals like alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, saponine, protein, tannins, amino acids, volatile oil or essential oil. Preliminary phytochemical test was done using different extract.

For Alkaloids: Mayer's reagent, Dragandroff's reagent, Wagner's reagent, Hager's reagent test.
For Carbohydrates: Molisch's test, Benedict's test, Barfoed's test, Fehling's solution test.
For Glycosides: Legal test, H2SO4 test, Borntrager's test, Killer-Killani test.
For Proteins and Amino acids: Millon's reagent, Ninhydrin reagent test, Biuret test.
*For Sterols and Triterpenoids***:** Libermann test, Salkowski test, Noller test.
*For Phenolic compounds***:** FeCl3 test, Zinc-Hydrochloride reduction test.

For Flavonoids : Shinoda test, Zinc-Hydrochloride test, alkaline reagent test.
For Tannins : FeCl3 test, Vanillin-Hydrochloride test, alkaline reagent test, Bromine water test.
For Saponins : Froth forming test.
For Fixed Oils and fats : Spot test
For Mucilage and gums : Ruthenium red test and Water absorption test.
*For Anthraquinone***:** Benzene and 1% NH4 solution test.

RESULTS

Seeds of *Celastrus paniculata* Willd. shows morphological parameters such as seed size ranges 0.36cm-0.18cm (average weight of 15 individual seeds), oval, reddish brown,11.45mg, bilateral, hilum apical, acute/pointed, thick, scaly circular cells, cellular reticulations. seeds are cented. The scanning electron microscopy (SEM) study shows that spermoderm pattern is irregular, at the apical region surface show prominent, hard rugae with elevations uniform cellular pattern giving an apperance of papillate ornamentations.

In anatomical study of seed coat the T.S. of seed coat shows outer epidermis wavy and composed of thick walled cells followed by 3-4 layers of parenchymatous cells. Below this zone thickened compactly arranged cells with tanniferous inclusions can be distinctly marked. This zone is followed by palisade layer composed of elongated cells separated by broad intercellular spaces followed by endosperm. The epidermal cell measures 46.52µm in length and 34.89µm in breadth. The palisade cell measures 58.15µm in length and 11.63µm in breadth.

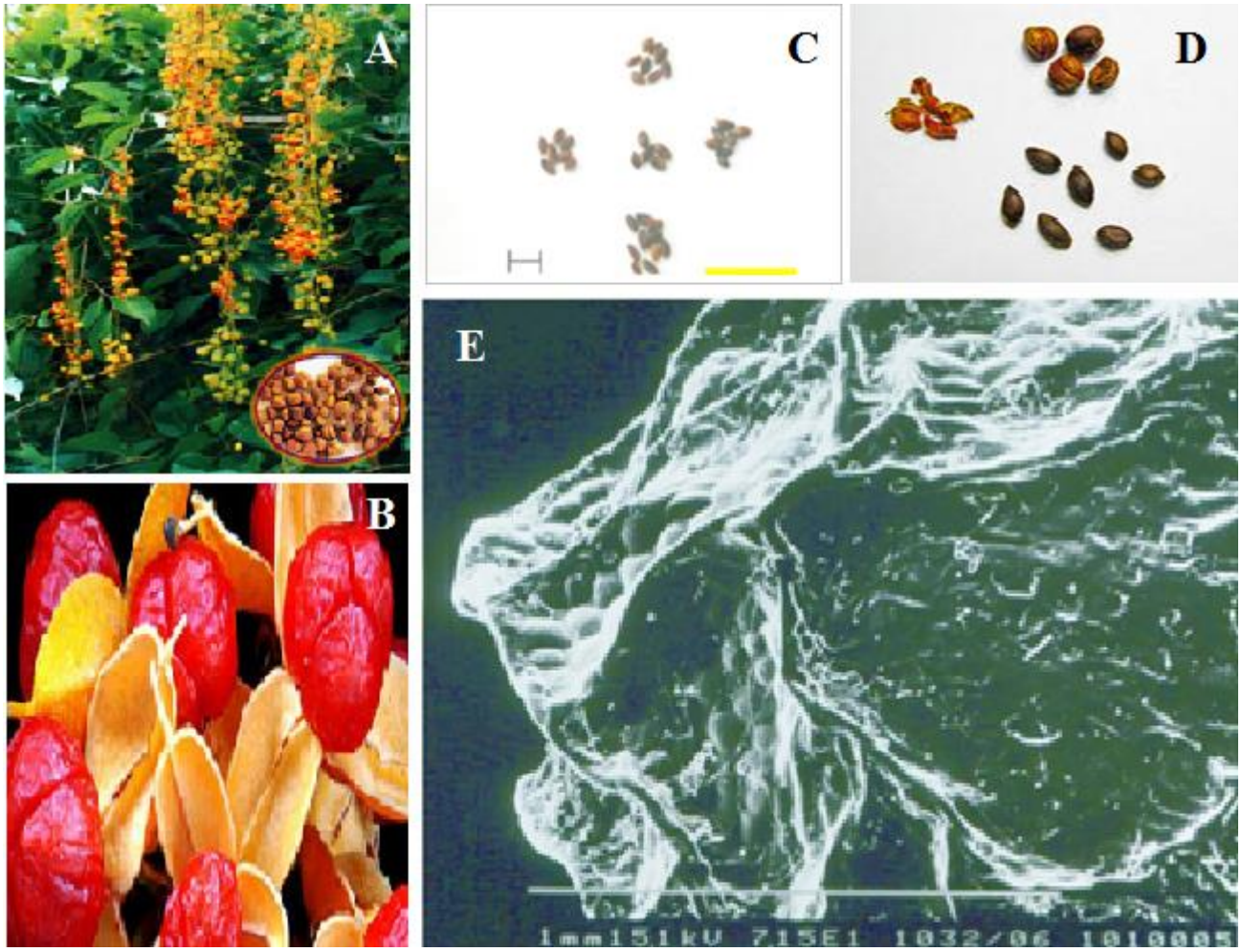

Fig:1: -A –*Celastrus paniculata* Willd. plant with seeds . **B:** *Celastrus paniculata* Willd riped seeds. **C-D:** shows seed samples of *Celastrus paniculata* Willd. seeds are small, oval, **E:** shows whole SEM photograph of seed showing irregular surface with rugae elevations, hard edges, mounded cellular pattern.($7.15x10^1$)

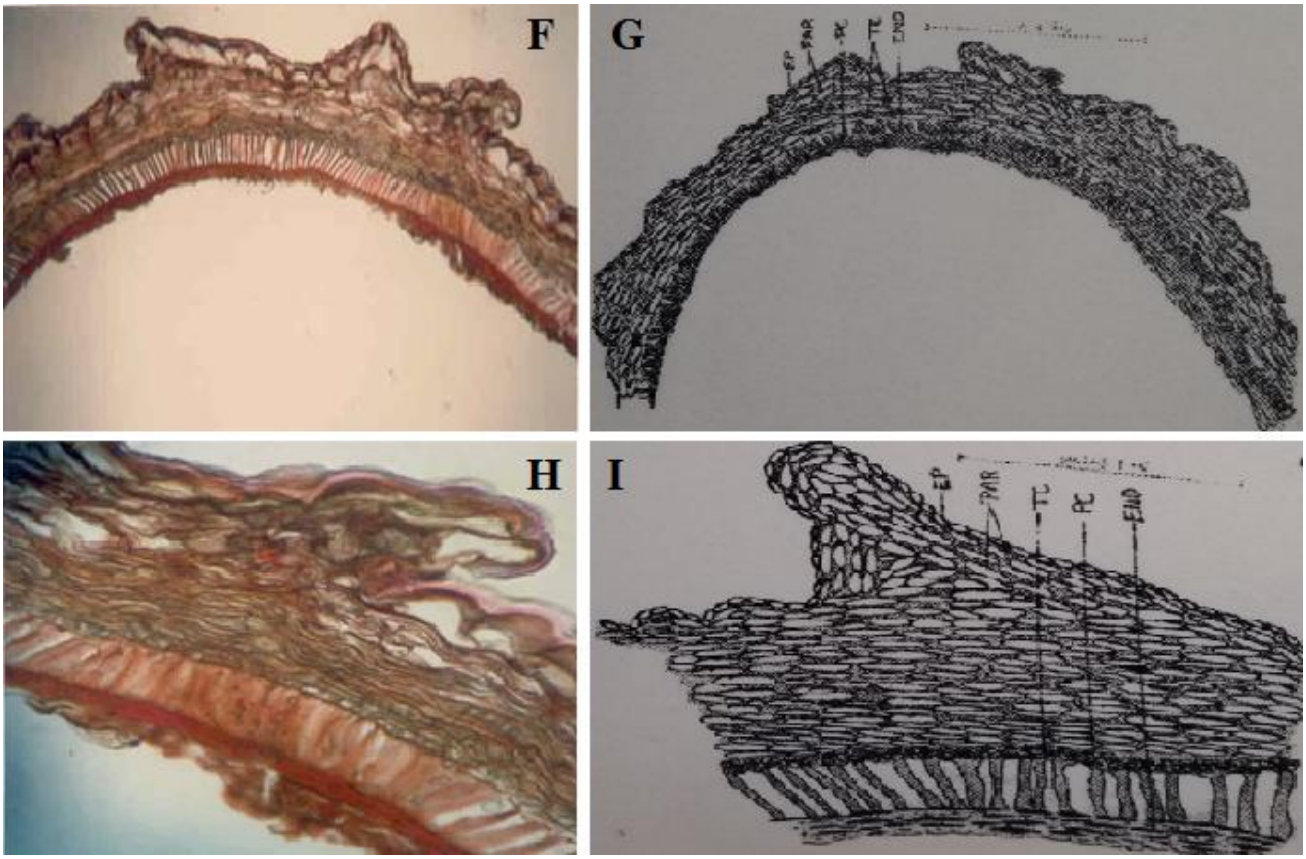

Fig 2 : F: *Celastrus paniculata* Willd.(160x), **G:** *Celastrus paniculata*Willd.(640x) **H**: well ornamented wavy epidermis, parenchymatous cells x 100, **I :** wavy epidermis, isodiametric parenchymatous cells ,palisade and well developed endosperm with tannin cells x 400

Table 1 : Shows preliminary phytochemical observations of seed powdered of *Celastrus paniculata* Willd

Sr no.	Chemical constituents	Ethanol	Petroleum ether	Aqueous extract
01	Alkaloids	+	+	+
02	Carbohydrates	-	+	+
03	Glycosides	-	+	+
04	Protein and amino acids	+	-	-
05	Sterols and terpenoids	+	+	+
06	Phenolic compounds	-	-	-
07	Flavonoids	-	-	+
08	Tannins	+	+	+
09	Saponins	-	+	+
10	Fixed oil and fats	-	+	+
11	Mucilage and gums	-	-	-
12	Anthraquinone	-	-	-

The priliminary phytochemical observations of seed powdered of *Celastrus paniculata* Willd. shows various chemical constituents which is present inside the seeds. The qualitative analysis is important for present study.

For preliminary phytochemical analysis, we use seed powder (using soxhlet apparatus method) which is treated with various chemicals. For this detection, we use three extracts like ethanol, petroleum ether and aqueous extract. All the three extracts detect various chemical constituents present in it. From the above observations, all the three extracts detect the alkaloids, sterols and terpenoids, tannins etc. Carbohydrates, glycosides, saponins, fixed oils and fats are present in petroleum ether and aqueous extract.

Fig. 3: Shows *Celastrus paniculata* Willd. Habit, morphology with inflorescence, fruit, seeds and seed oil.

Phenolic compounds, mucilage and gums, anthroquinone are absent in all the three extracts. Protein and amino acids only in ethanol absent in other extract.

MEDICINAL USES

Seeds having medicinal property. This property is due to presence of secondary metabolites like alkaloids or tannins or phenolics or flavonoids as active constituents. Seed are extensively used against rheumatism, gout, paralysis and leprosy. The seed are bitter, laxative, emetic and tonic (Kapoor 1990). Seeds having good smell.It is also used as alterative stimulant and nervine tonic. Externally the oil is rubefacient (Narayanrao, 2003). Seed oil use as a component of formulation 'Mental syrup' for memory enhancing and mental disorders, improve memory (Kalaskar, 2012).

DISCUSSION

From the above observations, it is clear that the seed coat study and phytochemical analysis of seeds of *Celastrus paniculata* Willd. focus on various aspects related to morphological, anatomical and phytochemical constituents inside the seeds. From all the parts seeds is the highest useful part which is economically, medicinally and other beneficial purposes. The present study helps for the plant identification and taxonomic information. The surface shows uniform cellular pattern with elevations which determine morphological view while anatomical seed coat study gives detailed about cellular pattern well develop wavy, ornamented epidermis, parenchy-matous cells with palisade layers and well-developed endosperm. This study clears the anatomical cellular pattern of seed coat.

The preliminary phytochemical analysis essential for study of medicinal property because it contains various secondary metabolites. This study detects various chemical constituents which helps for preperations of drugs. It also included in indigenous herbal medicine. In Ayurveda, this study is most important. Recently, ongoing research on seed oil of *Celastrus paniculata* Willd. pristimerin derived from seed used as a anticancer drugs. It is inhibiting the growth of specific types of cancer cell. (Yang *et al*. 2008) Seed oil is used on various diseases. So extraction, detection and analysis is important.It is a nutrient rich oil that the active compound are found. Such as celastrin, paniculatin and other active alkaloids. It is also used for improving memory, retention and recall, mental activity (Cleversley, 2002).

From the above observations it is clear that the morphological study of seedcoat clear the surface view,the anatomical seedcoat study clear the internal cellular structure. The micromorphological characters helps for taxonomic identification also. The preliminary phytochemical analysis detects various chemical constituents which is use for preparation of various drugs. Seed oil is used on various purposes. So, seeds give potential health benefits. Various brain problems, diseases it is use on large quantity. It is use in medicinal industry in large quantity for preparation of various drugs. In Ayurvedic treatment oil is used as a medicine. So, seeds are in large quantity used and showing their threptic efficacy. Economically and in various pharmaceutical and medicinal industry it is very important and also gives benefits in the society.

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

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RESEARCH ARTICLE

Isolation, Phylogenetics and Growth response of Low Density Polyethylene (LDPE) degrading bacteria

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ABSTRACT

Urbanization, changes in life style and developmental activities have led to tremendous generation of plastic waste across India amounting to 1.5 million tonnes per annum creating the so called "white pollution". Municipal solid waste in India contains 1-4% by weight of plastic waste mainly consisting of low density polyethylene (LDPE) requiring its efficient disposal. In view of this, the current work is centered on bioremediation (biological cleanup) of plastic waste as a method of treatment, which is generally cheaper and more environment friendly than other alternatives such as incineration, chemical treatment or landfills.

Soil samples from various garbage dumping grounds and parks were used for isolation of LDPE degraders by enrichment culture technique using Bushnell & Haas Broth containing increasing concentrations of LDPE (0.2-1.0 %) as sole source of carbon. Eighteen bacterial isolates obtained after five rounds of enrichment were identified using 16s rDNA sequencing. Isolates showing good growth response to LDPE were found to belong to genus *Pseudomonas* and *Arthrobacter*. These five isolates were further evaluated for their ability to degrade LDPE on the basis of growth response to LDPE in liquid media, % weight loss by gravimetric analysis and reduction in molecular weight by GPC.

Keywords: Plastic, LDPE, Polyethylene, bioremediation, phylogenetic.

INTRODUCTION

The word plastic comes from Greek word "plastikos", which means 'able to be moulded into different shapes' (Joel, 1995). The development of plastics in the world started around 1930 with the introduction of polystyrene, polyvinyl chloride, polyethylene, nylons, and with transition from coal-based to petrol-based chemicals. The condensation and addition polymers came into market since 1950s (Datta *et al*., 1998). Originally, plastics were mimicking and replacing natural products like lacquer, shellac, amber, etc. But today, they are largely synthetic

materials made from an extremely inexpensive but non-renewable resource, crude oil (APME, 1999). Thus, plastics have become technologically significant and they have come to replace glass, wood, masonry and other constructional materials, and even metals in many industries (Cain, 1992). Ironically, these same properties are proving to be a major environmental problem when these materials enter into the waste stream. Because plastics are designed to resist degradation, they can become permanent residents in landfills.

Every year more than 140 tonnes of plastic is produced worldwide. As conventional plastics are persistent in environment, improperly disposed plastic materials are significant source of environment pollution, potentially-harming life (Nir *et al*., 1993). Various improper methods of disposal include burying or burning of plastic materials which releases harmful or toxic pollutants into the environment thereby endangering the biosphere. The burning of PVC plastics produce persistent organic pollutants known as 'furans' and 'dioxins'. These pollutants are known to cause adverse effects in humans, including immune and enzyme disorders and chloroacne, and they are classified as possible human carcinogens. Health may be affected by polymer itself, by chemicals added to the plastics to make it more flexible stable and flame retardant or colouring agents (Jayasekara *et al*., 2005). Littering not only threatens wildlife and marine life, but also cause considerable aesthetic nuisance (Yabannavar and Bartha, 1994). They have a direct impact on marine ecosystems and are believed to be responsible for the death of a very large number of birds and fish by ingestion or strangulation (Scott, 1990).

Since, plastics have become an integral part of our everyday life, it is impossible to prevent even in part, the release of these materials into the environment, consequently, it is important to discover the ways to biodegrade these compounds (Cacciari *et al*, 1993). In view of this, most of the current work is centered on bioremediation of plastic waste as a method of treating plastic waste, which is generally cheaper and more environment friendly than other alternatives such as incineration, chemical treatment or landfills. From recent work, it has been concluded that microorganisms capable of degrading polymer components might play a very important role in degradation of plastics (Ishigaki *et al*, 2000).

MATERIALS & METHODS:

(1) Enrichment & Isolation of LDPE degraders:

Materials:

Sterile Busnell and Haas Broth (Bushnell and Haas, 1941) with polyethylene as sole carbon source, Soil samples collected from various garbage dumping grounds and parks (Gorai Dumping Ground, Deonar Dumping Ground, Borivli National Park, Mahim National Park, Bombay Port Trust garden), Sterile nutrient agar.

Method:

5g samples of soils were collected from various garbage dumping grounds and parks showing visible littering of plastic and were inoculated in 100 mL of sterile Busnell and Haas Broth containing increasing concentrations of polyethylene (0.2-1.0 %) as sole source of carbon (Hadad *et al.,* 2005). Five successive rounds of enrichment were carried out by incubating the flasks at 28±2^{o}C for one month under shaking conditions at 120 rpm. Individual isolates were obtained by streaking the enriched medium on sterile Nutrient agar after 48 h incubation at 28±2^{o}C

(2) Identification of the isolated bacterial strains by 16s rRNA:

Materials:

Nucleotide BLAST (NCBI site),
Clustal omega program on the internet (http://www.ebi.ac.uk/Tools/msa/clustalo/)

Methods:

Total genomic DNA for 16S rDNA amplification was isolated from cells grown to the late exponential phase. Amplification of the 5' end of the 16S rRNA gene was performed with universal primers and the 16s rDNA sequences were obtained. The 16s rDNA sequences thus obtained were aligned against known deposited 16S rDNA sequences using nucleotide BLAST on NCBI website. Determination of possible phylogenetic relationship between isolates was done by constructing a Phylogenetic tree using Clustal omega program.

(3) Assessment of polymer degradation potential of bacterial isolates:

Materials:

18 h old isolates grown on Nutrient agar, Sterile Busnell and Haas Broth containing 1g of polyethylene, Gooch crucible, GPC.

Method:

100mL of **s**terile Busnell and Haas Broth containing 1g of polyethylene was inoculated with 1% of 18 h old isolates grown on Nutrient agar (10^{11} cells/mL). The flasks were incubated at room temperature (28 ± 2^0C) under shaking conditions (120 rpm) for 4 weeks.

(a) Growth Response: The growth response of the isolates was monitored turbidimetrically at the end of every week for four weeks.

(b) Weight loss analysis: At the end of 4 weeks of incubation period, the loss in weight of polyethylene was estimated gravimetrically by weight loss analysis using Gooch crucible (Mathur *et al.,* 2011).

The percent weight loss (mass reduction) was computed with the following formula:

$$\% \text{ weight loss} = \frac{(W1-W2)}{W1} \times 100$$

Where, W1 is the pre-incubation weight of LDPE and W2 is the post-incubation weight of LDPE.

(c) Determination of Molecular weight by Gel Permeation Chromatography:

Mn (Number-average molecular weight), Mw (Weight-average molecular weight), Mz (Z-average molecular weight) and PD (Polydispersity) of LDPE samples were determined using Gel Permeation Chromatography.

RESULTS AND DISCUSSION

(1) Isolation: A total of 18 Isolates (named PE – 1 to PE – 18) were obtained. Five isolates that showed better growth response to LDPE as compared to other isolates were studied further.

(2) (a) Identification of the isolated bacterial strains by 16s rRNA:

Isolate	16s rDNA sequence	Description
PE-2	GGGGCACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCACACCTAGTGCC CAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTC CTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGC GCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCC ACTGCAGAACCGGAGTTGAGCCCCGGTCTTTCACAGCAGACGCGACAAACCGCCTACGAGC TCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCA CGTAGTTAGCCGGCGCTTCTTCTGCAAGTACCGTCACCCCCAAAGAGGGCTTCTTCCCTAC TGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTT CGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC CAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCC ACCAACAAGCTGATAGGCCGCGAGTCCATCCAAAACCACAAAAAGCTTTCCACCCCCCACC ATGCGATGAGGAGTCATATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAG GGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTAATCCCCGGCGCAAGCACCGGATC ATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCTGAG	*Arthrobacter* sp. AD1
PE-8	AGGCGGTCGACTTAATGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTC GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGC ACCTCAGTGTCAGTATTAGCCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCT ACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCATACTCTAGCTCGCCAGTT TTGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACG CGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTG GCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTTAC TGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCAT GGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCT GGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCC TTGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGG TCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACG TTGTCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCATGG AGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTATGCCTGCCGCCAGCGTTCAATCTG A	*Pseudomonas stutzeri strain AT11*

PE-15	TGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGGC GTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTA TCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTA CACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTCGCCAGTTTTGGATGCAGTTCCCA GGTTGAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAG TAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGG TGCTTATTCTGTCGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAAC TTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTT CGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTC CAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCT CACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCT TTCTCCCCGTAGGACGTATGCGGGATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCA GGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGA	*Pseudomonas taiwanensis strain YLCu18*
PE-16	ACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTT TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTG TCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTC ACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTTGCCAGTTTTGGATGCA GTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTAACAAACCACCTACGCGCGCTTTA CGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGT TAGCCGGTGCTTATTCTGTCGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCC TCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATC AGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTC TCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCC ATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTC CCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCA CTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCAAGGAGCAAGCTCC CGTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGC	*Pseudomonas putida strain BM38*
PE-18	GGGGCACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCACACCTAGTGCC CAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTC CTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGC GCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCC ACTGCAGAACCGGAGTTGAGCCCCGGTCTTTCACAGCAGACGCGACAAACCGCCTACGAGC TCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCA CGTAGTTAGCCGGCGCTTCTTCTGCAAGTACCGTCACCCCCAAAGAGGGCTTCTTCCCTAC TGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTT CGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC CAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCC ACCAACAAGCTGATAGGCCGCGAGTCCATCCAAAACCACAAAAAGCTTTCCACCCCCCACC ATGCGATGAGGAGTCATATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAG GGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTAATCCCCGGCGCAAGCACCGGATC ATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCTGAG	*Arthrobacter sp. AD1*

(2) (b) Construction of Phylogenetic tree:

Key
PE – 2 *Arthrobacter sp. AD1*
PE – 8 *Pseudomonas stutzeri strain AT11*
PE– 15 *Pseudomonas taiwanensis strain YLCu18*
PE – 16 *Pseudomonas putida strain BM38*
PE – 18 *Arthrobacter sp. AD1*

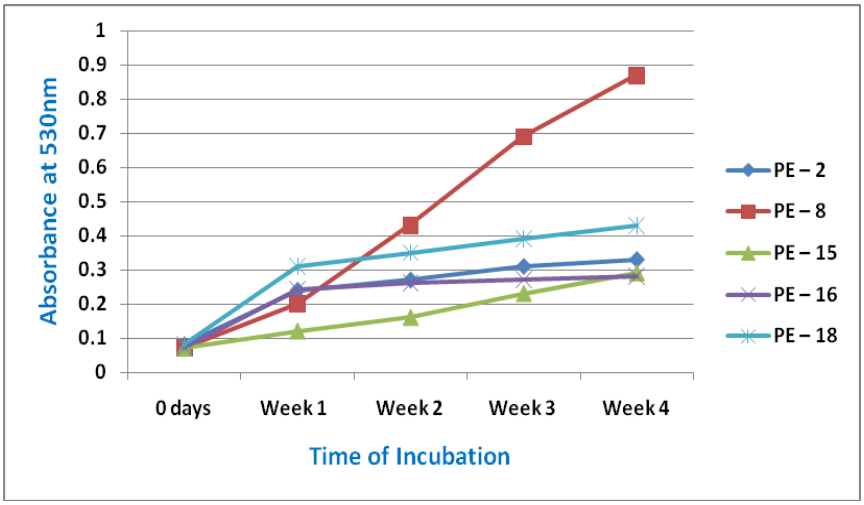
The Phylogenetic tree shows that:

- PE-2 and PE-18 are identical and cluster together.
- PE-2, PE-18 and PE-8 have diverged from a common ancestor.
- PE-15 and PE-16 form a separate clade and cluster together.

(3) Assessment of polymer degradation potential of bacterial isolates:

Table 1: Growth Response of bacterial isolates to polyethylene

Isolate	16s rDNA Identification	Absorbance at 530nm				
		0 days	Week 1	Week 2	Week 3	Week 4
PE – 2	*Arthrobacter sp. AD1*	0.08	0.24	0.27	0.31	0.33
PE – 8	*Pseudomonas stutzeri strain AT11*	0.07	0.20	0.43	0.69	0.87
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	0.07	0.12	0.16	0.23	0.29
PE – 16	*Pseudomonas putida strain BM38*	0.07	0.24	0.26	0.27	0.28
PE – 18	*Arthrobacter sp. AD1*	0.08	0.31	0.35	0.39	0.43



- The above graph shows that the isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows the best growth response to polyethylene.
- The isolate to show growth response second to PE-8 is PE-18 (*Arthrobacter sp. AD1*).

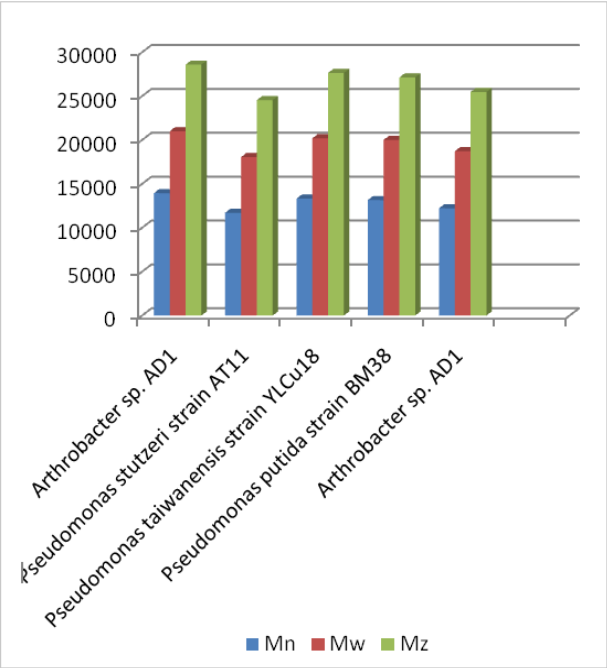
Table 2: Weight loss analysis:

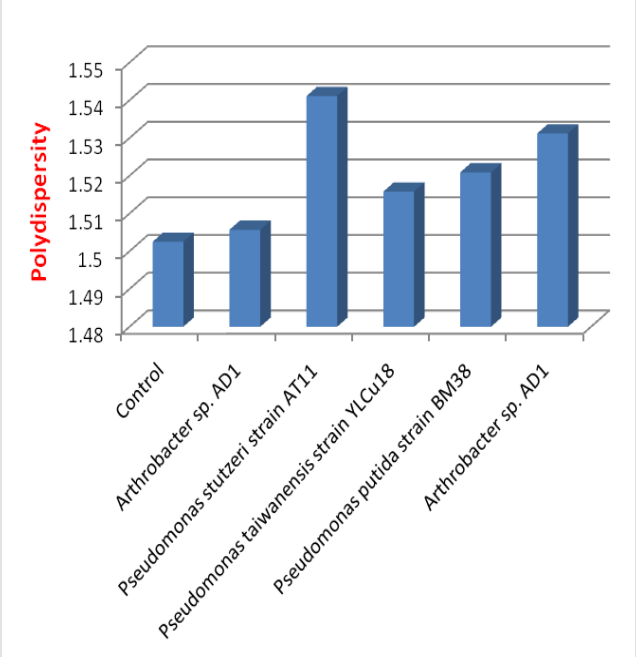
Isolate	16s rDNA Identification	W1 (Pre-incubation weight of Polyethylene in G)	W2 (Post-incubation weight of Polyethylene in G)	% Weight Loss of Polyethylene $\frac{(W1-W2)}{W1} \times 100$
PE – 2	*Arthrobacter sp. AD1*	1.000	0.999	0.100 %
PE – 8	*Pseudomonas stutzeri strain AT11*	1.000	0.980	2.000 %
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	1.000	0.999	0.100 %
PE – 16	*Pseudomonas putida strain BM38*	1.000	0.999	0.100 %
PE – 18	*Arthrobacter sp. AD1*	1.000	0.990	1.000 %

The isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows maximum degradation of LDPE, that is 2.00% reduction in LDPE weight in 4 weeks of incubation. The second best isolate to is PE-18 (*Arthrobacter sp. AD1*) which shows 1.00% reduction in LDPE weight.

Table 3: Determination of Molecular weight by GPC:

Isolate		Mn	Mw	Mz	PD
Control	-	14334	21537	29209	1.5025
PE – 2	*Arthrobacter sp. AD1*	13952	21008	28589	1.5057
PE – 8	*Pseudomonas stutzeri strain AT11*	11715	18054	24553	1.5411
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	13320	20191	27651	1.5158
PE – 16	*Pseudomonas putida strain BM38*	13154	20007	27133	1.5209
PE – 18	*Arthrobacter sp. AD1*	12227	18722	25477	1.5312





The isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows maximum decrease in Mw and increase in PD, followed by PE-18 (*Arthrobacter sp. AD1*).

Table 4: Showing five isolates studied for biodegradation of LDPE.

Isolate	% Weight loss of LDPE after 4 weeks of incubation	Mw of LDPE after 4 weeks of incubation
Arthrobacter sp. AD1	0.100 %	21008
Pseudomonas stutzeri strain AT11	2.000 %	18054
Pseudomonas taiwanensis strain YLCu18	0.100 %	20191
Pseudomonas putida strain BM38	0.100 %	20007
Arthrobacter sp. AD1	1.000 %	18722

DISCUSSION AND CONCLUSION:

The compiled results tabulated above show *Pseudomonas stutzeri strain AT11* is the best isolate amongst the five isolates studied for biodegradation of LDPE. This isolate shows maximum growth response to LDPE, maximum % weight loss of LDPE, & maximum reduction in Mw. The results obtained are consistent with the fact that *Pseudomonas stutzeri* has been known to be involved in polymer degradation by production of depolymerases (Sharma, 2004 and Ghosh et al, 2013) and serine hydrolases (Shimao, 2001). The data obtained from the current research is encouraging and eventually will help us device an effective method for Biodegradation of LDPE.

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

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RESEARCH ARTICLE

Investigation of environmental parameters affecting feather degradation and keratinase production by *Stenotrophomonas maltophilia* K279a

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ABSTRACT

Feathers represent a sizable portion of waste generated by poultry processing industries. This waste can be utilized for production of feather hydrolysate which can be used as animal feed. The present study investigated effect of various environmental parameters on chicken feather degradation and keratinase production by keratinolytic isolate *Stenotrophomonas maltophilia* K279a. *Stenotrophomonas maltophilia* can utilize feathers as a sole source of carbon, nitrogen and energy. Feather degradation and keratinase activity gradually increased and reached an optimum level on the 6th day. Maximum feather degradation, keratinase activity and bacterial growth were observed under shaker condition. The bacterium was able to grow at pH 5.0 to 9.0 with optimum feather degradation (73.34 %) and keratinolytic activity (44.30 units/ml) observed at pH 7.5. Optimum feather degradation (75.6 %) and keratinolytic activity (45.62 units/ml) was observed at 37 ºC. 2 % Inoculum volume and 1% feather concentration was found to be optimum for feather degradation and keratinase production. Amongst various co-carbon sources investigated, feather degradation and keratinase activity increased in presence of 1 % glucose, glycerol, maltose and lactose. Similarly, amongst co-nitrogen sources; 0.1 % concentration of peptone, tryptone, yeast extract and ammonium sulphate had positive effect on feather degradation and keratinase production. Presence of reducing agent, sodium sulphite (0.1 %) in growth media caused significant increase in feather degradation. Feather degradation and keratinase production were not affected by the presence of surface tension reducers in growth media. Results of this study can help in designing media with appropriate nutrients and incubation conditions, which can lead to increased feather degradation under submerged condition.

Keywords: Keratinolytic activity, *Stenotrophomonas maltophilia*, feather degradation.

INTRODUCTION

Amongst various agriculture segments in India, poultry is considered to be one of the fastest growing segments, growing at a rate of 8 % to 10 %. Thus in the coming years, there will be significant increase in generation of poultry waste which, if not handled properly, can lead to environmental pollution and health hazard. One of the major constitutions of poultry waste is feathers. Feathers are almost pure keratin protein and hence can be used as a cheap alternative for production of protein rich animal feed. Current methods to convert feathers into animal feed include physical and chemical processing. However, these processes require significant amount of energy. Further, these processes also cause destruction of certain essential amino acids, reducing nutritional value of the feed. Chemicals used in feather processing are responsible for environmental pollution (Jin-Ha Jeong, 2010). Biodegradation of feathers by keratinolytic microorganisms is seen as a potential alternative to reduce energy consumption, improve nutritional value and reduce environmental pollution.

Unlike most proteins which are easily degraded by common proteolytic enzymes like papain, pepsin and trypsin, feather keratin protein is not degraded by these enzymes. Feather protein keratin is stabilized by disulphide bonds, hydrogen bonds and hydrophobic interactions. Keratinolytic proteases produced by certain microorganisms play an important role in feather degradation in nature. A variety of bacteria, actinomycetes and fungi are known to produce keratinolytic enzymes. Amongst bacteria, keratinolytic activity has been widely documented in Gram-positive bacteria *Bacillus* spp. and *Streptomyces* spp. Amongst Gram-negative bacteria, keratinolytic activity has been reported in *Xanthomonas maltophilia*, *Vibrio* sp., *Stenotrophomonas* sp. D-1, and *Chryseobacterium* sp. strain kr6 (Gupta and Ramnani 2006). These microorganisms can be exploited for biodegrading of keratinaceous waste, which can be converted into valuable product such as animal feed, nitrogen rich organic fertilizer, etc. Keratinase enzyme produced by these organisms is a valuable resource for food industry, detergent producing industry, leather industry, cosmetic industry, etc (Saber et al., 2010). Thus, it is important and necessary to determine conditions which can lead to increased feather degradation and improved enzyme production for such applications.

Recently, we have isolated and characterized a novel feather degrading Gram-negative bacterium, *Stenotrophomonas maltophilia* K279a. The organism can grow by utilizing feather as the sole source of carbon, nitrogen and energy. The aim of the present study was to investigate the effect of various environmental parameters on chicken feather degradation and keratinase production by keratinolytic isolate *Stenotrophomonas maltophilia* K279a.

MATERIALS AND METHODS

Microorganism and growth medium: *Stenotrophomonas maltophilia* K279a used in this study was isolated from a poultry waste dumping site in Mumbai. Feathers were procured from a local poultry shop. They were washed thoroughly with tap water followed by final washing with distilled water. The feathers were dried at 40 ºC and cut into pieces of 1 – 2 cms. Feather basal media (FBM) used for growing the isolate had the following composition: NaCl, 0.5 gm; K_2HPO_4, 0.3 gm; KH_2PO_4, 0.4 gm; Na_2SO_3, 0.5 gm and Feathers, 10 gm; pH 7.5; D/W, 1000 ml. The medium was sterilized by autoclaving.

Feather degradation (FD):
Feather degrading potential of the organism was tested by inoculating 1 ml of freshly prepared suspension (approximately 10^8 cells/ml) in 100 ml of sterile FBM broth in 250 ml Erlenmeyer flask. The medium contained whole feathers as a sole source of carbon, nitrogen and energy. The incubation was carried out at 37ºC for 6 days. Percentage feather degradation was estimated by gravimetric method (Suntornsuk and Suntornsuk, 2003).

Keratinase Activity (KA):
Keratinase activity was determined by the Sigma Aldrich method with minor modification (Shah and Vaidya, 2017).

Effect of static and shaker conditions on FD:
For this, two sterile FBM flasks were inoculated with *Stenotrophomonas maltophilia* K279a. One flask was incubated in shaker condition (90 rpm) and other flask at static condition. The incubation was carried out at 37ºC for 6 days. % FD and KA was estimated at the end of 6th day by the method described above. Increase in cell number was measured by pour plate technique and reported as CFU/ml.

Effect of rotation speed on FD:
The effect of rotation speed (rpm) on FD and keratinase production was studied by incubating the inoculated FBM broth at different rpm (90, 120, 150 & 180) at 37 ºC for 6 days. % FD and KA was estimated at the end of 6th day by the method described above.

Time course of FD:
The effect of incubation period on FD and keratinase production was studied by incubating the inoculated FBM broth at 90 rpm and 37 ºC for 3, 4, 5, 6 and 7 days. Un-inoculated FBM medium was kept as control. % FD, KA and increase in cell number was estimated by the method described above.

Effect of initial medium pH on FD:
The test isolate was inoculated in 100 ml of sterile FBM flasks having pH values of 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0 and incubated at 90 rpm and 37 ºC for 6 days. Un-inoculated FBM flasks having the same pH values were kept as controls. After incubation, % FD and KA were estimated by the method described above.

Effect of incubation temperature on FD:
FBM flasks having pH 7.5 were inoculated with the test isolate. The flasks were incubated on incubator-shaker maintained at following temperatures viz. 27˚C, 32˚C, 37˚C and 42˚C for 6 days. Un-inoculated FBM flasks maintained at the same temperatures were kept as controls. % FD and KA were estimated by the method described above.

Effect of initial feather concentration on FD:
Sterile FBM flasks with 0.5%, 1%, 1.5% and 2% feathers were inoculated with the test isolate. Un-inoculated FBM flasks having same concentration of feathers were used as controls. The flasks were incubated on incubator-shaker (90 rpm) at 37 ºC for 6 days. % FD and KA were estimated by the method described above.

Effect of inoculum volume on FD:
Sterile FBM with 1% feathers was inoculated with 1 %, 2 % and 3% of freshly prepared suspension (approximately 10^8 cells/ml) of the test isolate. Un-inoculated FBM was kept as control. The flask was incubated on incubator-shaker (90 rpm) at 37 ºC for 6 days. % FD and KA was estimated by the method described above.

Effect of various co-carbon and co-nitrogen sources on FD:
Sterile FBM was supplemented with 1% co-carbon sources such as Dextrose, Sucrose, Fructose, Galactose, Lactose, Maltose, Mannitol, Glycerol, Xylose and Starch and 0.1% co-nitrogen sources such as Peptone, Yeast Extract, Tryptone, Meat Extract, Beef Extract, Ammonium Sulphate, Sodium Nitrate, Ammonium Chloride and Ammonium Nitrate. The flask was inoculated with the test isolate. Un-inoculated FBM was kept as control. The flask was incubated on incubator-shaker (90 rpm) at 37 ºC for 6 days. % FD and KA was estimated by the method described above.

Effect of reducing agents and detergents on FD:
Sterile FBM was supplemented with 0.1% reducing agents such as Sodium Sulphite, Di-thiothreitol and β-mercaptoethanol and 0.1 % detergents such as Tween 80, SDS and Triton X 100. The flasks were inoculated with the test isolate. Un-inoculated FBM flasks were kept as controls. The flasks were incubated on incubator-shaker (90 rpm) at 37 ºC for 6 days. % FD and KA was estimated by the method described above.

Statistical analysis:
All the experiments were carried out in triplicates. The results presented are mean ± standard deviation.

RESULTS AND DISCUSSION

Stenotrophomonas maltophilia K279a is able to grow on feather basal medium containing feathers as a sole source of carbon, nitrogen and energy (figure 1). The organism, when grown in FBM under submerged condition at 37 ºC for 6 days, caused significant degradation of feathers (76.61%). Keratinase activity in the culture free supernatants was 47.91 Units/ml. Media pH gradually increased from 7.5 at Day 1 to 8.5 at Day 6. Alkalinization of culture media is one of the characteristic features of keratin degradation and is due to release of alkaline products from breakdown of keratin into peptides and amino acids, which further undergo deamination to release amines and ammonia (Nereida et. al., 2009). Kaul and Sumbali (1997) proposed that fungi having strong keratinolytic ability rendered the culture medium more alkaline than those that were less keratinolytic.

Maximum feather degradation, keratinase activity and bacterial growth were observed under shaker condition as compared to static condition (Figure 2).

Figure 1: A Residual feathers after treatment with *Stenotrophomonas maltophilia* K279a; B – Standard Proteinase K graph using Keratin Azure k 8500 as a substrate.

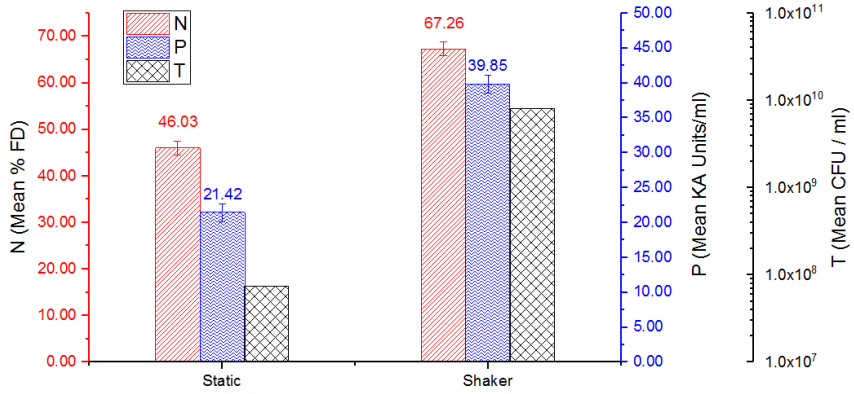

Figure 2: Effect of static and shaker conditions on cell number, FD and KA.

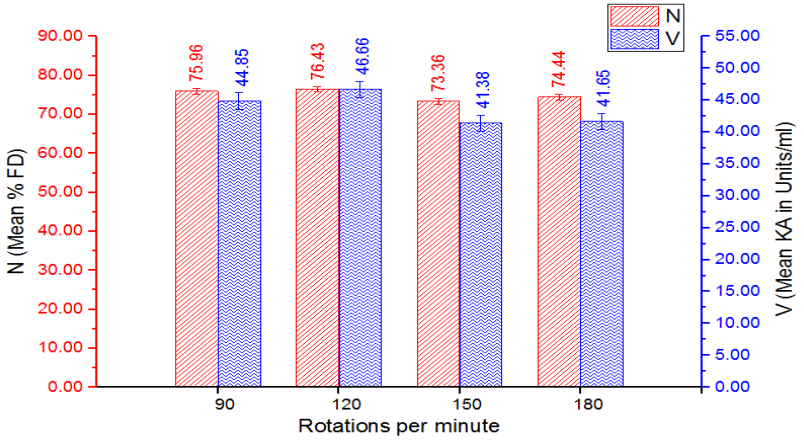

Figure 3: Effect of rotation speed on FD and KA.

The rotation speed of 120 rpm resulted in maximum feather degradation and keratinase production. However, no significant difference between feather degradation and keratinase activity was observed at 90 rpm and 120 rpm. There was negligible reduction in feather degradation and keratinase production at 150 and 180 rpm (Figure 3). In *Bacillus* sp. FK 46, maximum increase in cell number, feather degradation and keratinase production were reported at 120 rpm as compared to lower rpm (Suntornsuk and Suntornsuk, 2003).

The time course of feather degradation and keratinolytic activity by *Stenotrophomonas maltophilia* K279a culture grown in a feather basal medium is shown in Figure 4. The maximum keratinolytic activity was about 45.83 Units/ml after 6 days of cultivation and decreased to 41.44 Units/ml on 7th day. Similarly, maximum feather degradation of around 77 % was observed on 6th day and remained constant on 7th day.

The time course of keratinase production and feather degradation followed similar pattern. Feather degradation was accompanied by change in media pH

from 7.5 to 8.3 at the end of 6th day, indicating proteolysis and release of alkaline products in the medium. Cell number (Cfu/ml) gradually increased to maximum on 5th day (9.51 X 10^9) and decreased thereafter. Geun-Tae Parka and Hong-Joo Son (2009) reported complete degradation of chicken feathers by *B. megaterium* F7-1 after 7 days of incubation at 30 ºC. Similarly, Williams *et al*. (1990) demonstrated that *Bacillus licheniformis* PWD-1 degraded chicken feather completely at 50 ºC in10 days.

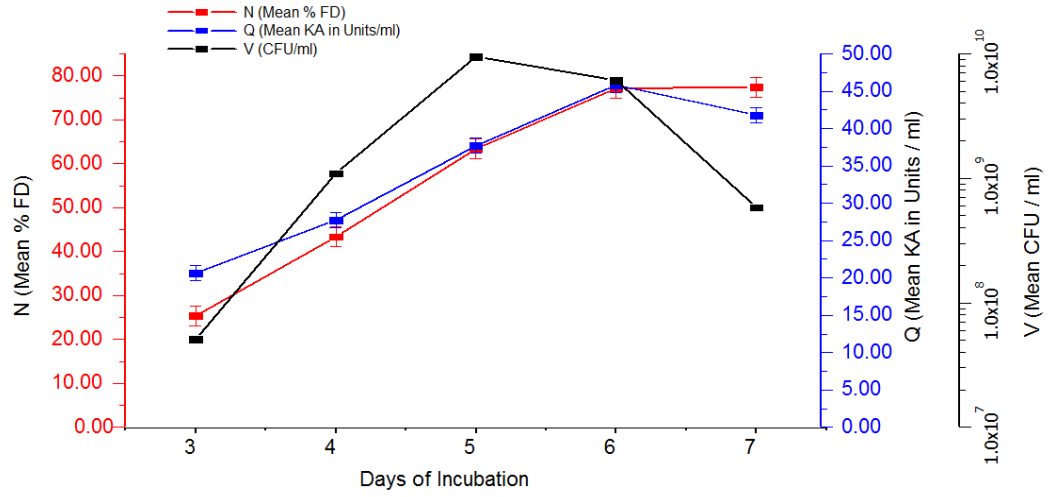

Figure 4: Time course of change in cell number, feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

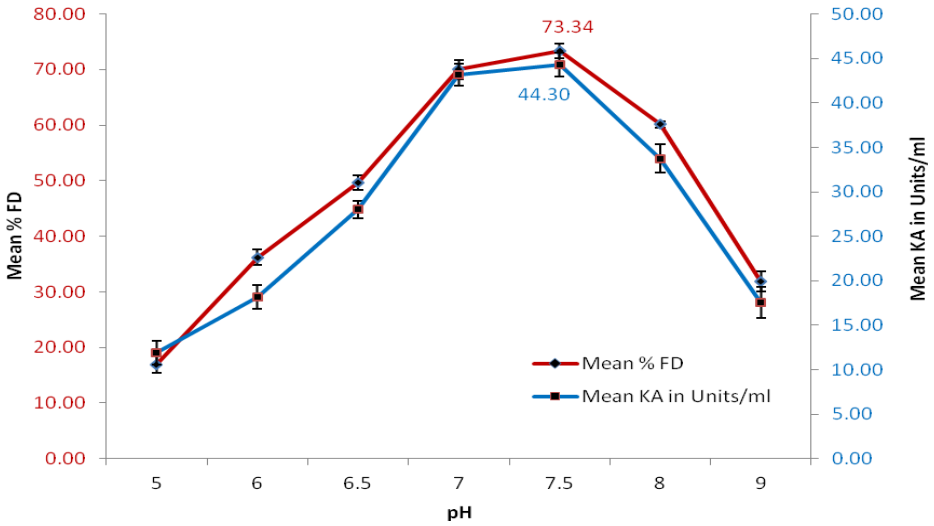

Figure 5: Effect of initial media pH on feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

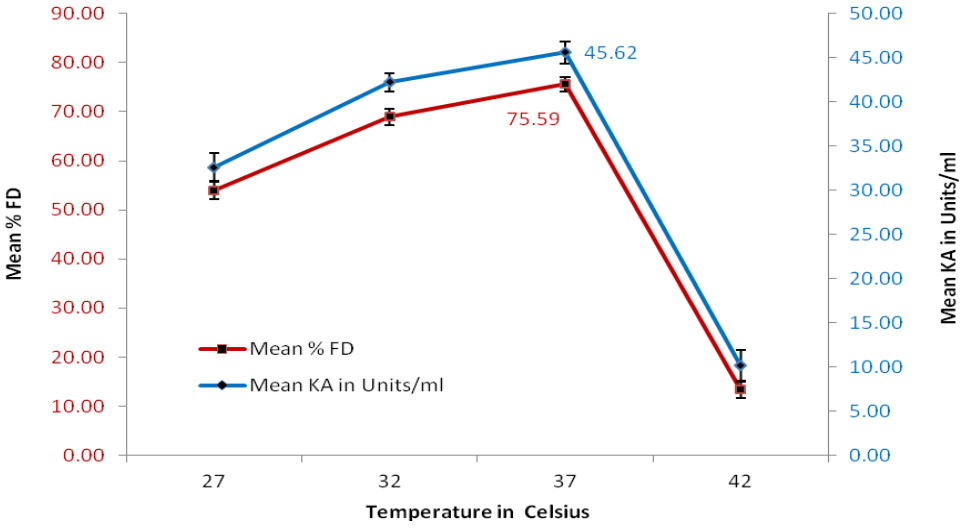

Figure 6: Effect of incubation temperature on feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

The optimum pH for feather degradation and keratinolytic enzyme production was determined by growing *Stenotrophomonas maltophilia* K279a at pH 5.0 – 9.0 and 37 ºC for 6 days. As shown in Figure 5, maximum feather degradation (73.34 %) and keratinolytic activity (44.30 Units/ml) were observed at pH 7.5. The enzyme activity was considerably lower in the pH range of 5.0 – 6.5, while it was higher in pH range 7.0 to 8.0. The organism was able to grow over a wide range of pH 5.0 – 9.0. In *B. licheniformis* keratinase production was optimum in neutral conditions i.e. in pH range of 7.0 –7.5, and for *B. subtilis* it was best in an alkaline condition (pH 8.0–8.5) (Wang and Shih, 1999). *Stenotrophomonas maltophilia* DHHJ exhibited maximum production of keratinase at pH 7.5 (Zhang et. al., 2009). Keratinolytic enzyme production in *Stenotrophomonas maltophilia* R13 was observed in pH range of 4.0–11.0, with an optimum production at pH 7.0. (Jin-Ha Jeonga et. al., 2010).

The effect of incubation temperature on Feather degradation and keratinase production is shown in figure 6. Maximum feather degradation of 75.6 % and keratinase activity of 45.62 units / ml were observed at 37 ºC after 6 days of incubation in shaker condition. Brigitte Bockle *et al.* (1995) reported that *Streptomyces pactum* DSM40530 partially degraded native chicken feathers at 50 ºC. For *Stenotrophomonas maltophilia* DHHJ, maximum keratinase production was reported at 40 ºC. Similarly, in *Stenotrophomonas maltophilia* R13, optimum temperature for the enzyme production was 30 ºC. Keratinase production in *Chryseobacterium* strain kr6 and *Vibrio* strain kr2 and was optimum at 25 ºC and 30 ºC respectively (Sangali and Brandelli, 2000; Riffel *et al.*, 2003).

S. maltophilia was grown in the medium containing 0.5 % to 2 % feather concentrations. The maximal feather degradation was observed at 1 % feather concentration. However, 1 % and 1.5 % feather concentration showed maximum keratinase production (Figure 7). Further, inoculum volume of 2 % (v/v) resulted in maximum feather degradation and keratinase enzyme production (Figure 8). For, *Bacillus subtilis* KD-N2, inoculum volume of 2 % was found to be optimum for keratinase production (Cai C., and Zheng X., 2009).

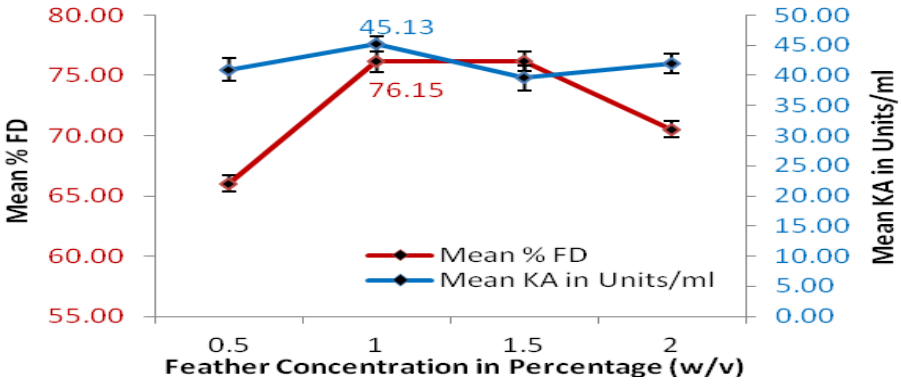

Figure 7: Effect of feather concentration on feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

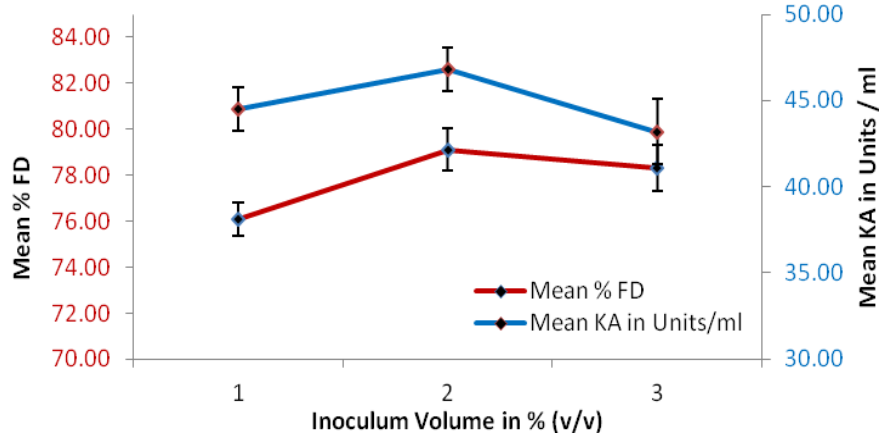

Figure 8: Effect of inoculum volume on feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

Table 1: Effect of various carbon, nitrogen, reducing agent and surface tension reducers on feather degradation and keratinolytic enzyme production by *Stenotrophomonas maltophilia* K279a.

	% FD	% increase or decrease in FD	KA (Units/ml)	% increase or decrease in KA
Co-carbon (1 %)				
Basal FBM	76.61 ± 1.92	0.00	47.91 ± 1.58	0.00
Fructose	74.85 ± 1.50	-2.29	41.93 ± 1.26	- 12.48
Galactose	73.67 ± 1.71	-3.84	40.40 ± 1.08	- 15.67
Glucose	85.13 ± 1.15	11.12	58.76 ± 1.99	22.65
Glycerol	79.11 ± 1.12	3.26	54.66 ± 1.67	14.09
Lactose	78.87 ± 1.21	2.95	49.30 ± 1.94	2.91
Maltose	78.90 ± 1.22	2.99	50.97 ± 1.26	6.39
Mannitol	74.53 ± 0.79	-2.71	42.91 ± 2.66	- 10.44
Starch	73.97 ± 0.99	-3.45	41.83 ± 1.99	- 12.69
Sucrose	73.36 ± 2.26	-4.25	43.18 ± 1.07	-9.86
Xylose	71.76 ± 1.83	-6.33	41.79 ± 1.19	- 12.77
Co-Nitrogen (0.1 %)				
Basal FBM	75.72 ± 1.52	0.00	46.80 ± 2.40	0.00
Meat Extract	72.43 ± 0.66	-4.34	41.38 ± 0.32	- 11.59
Peptone	79.66 ± 0.83	5.21	54.45 ± 1.62	16.35
Tryptone	78.85 ± 0.70	4.14	51.81 ± 1.89	10.70
Yeast Extract	78.65 ± 0.77	3.87	52.99 ± 1.15	13.22
Ammonium Chloride	72.33 ± 1.42	-4.47	42.07 ± 1.85	- 10.10
Ammonium phosphate	74.14 ± 0.77	-2.08	44.09 ± 1.27	- 5.79
Ammonium Sulphate	78.48 ± 0.62	3.65	52.71 ± 0.55	12.63
Sodium Nitrate	76.36 ± 1.07	0.85	48.96 ± 1.67	4.61
Reducing agents (0.1 %)				
Basal FBM	74.12 ± 0.91	0.00	44.16 ± 0.75	0.00
β-mercaptoethanol	72.47 ± 0.54	- 2.23	42.00 ± 1.69	- 4.89
Di-thiothretol	73.31 ± 1.16	- 1.09	45.13 ± 1.82	2.20
Sodium Sulphite	86.96 ± 0.27	17.32	60.71 ± 1.48	37.48
Detergents (0.1%)				
SDS	73.21 ± 0.77	- 1.23	44.71 ± 1.48	1.26
Tween 80	70.05 ± 0.67	- 5.49	40.40 ± 1.37	- 8.51
Triton X 100	73.21 ± 1.39	- 1.23	42.70 ± 1.66	- 3.31

The influence of the addition of various co-carbon and co-nitrogen sources, reducing agents and surface tension reducers to the feather basal salt medium is shown in table 1. Addition of co-carbon sources such as glucose and glycerol resulted in significant increase in keratinase production, whereas presence of maltose

resulted in slight increase in enzyme production. In case of *B. megaterium* F7-1, presence of fructose, galactose, and glucose in growth medium resulted in slight increase in keratinase enzyme production (Geun-Tae and Hong-Joo, 2009). In *S. maltophilia* R13, increased keratinase activity was observed for various co-carbon sources, of which glucose promoted the greatest degree of keratinolytic enzyme production and cell growth (Jin-Ha Jeong et al., 2010). Conversely, In *Stenotrophomonas sp*. D-1 keratinase production is partially inhibited by glucose (Yamamura et. al., 2002). Similarly, in *Streptomyces* MS2, glucose and starch showed negative effect on keratinase production (Mabrouk, 2008). Amongst co-nitrogen sources, peptone, tryptone, yeast extract and ammonium sulphate caused significant increase in keratinase production. In *Stenotrophomonas sp*. D-1 keratinase production increased in presence of yeast extract. Similarly, keratinolytic enzyme production by *S. maltophilia* R13 increased in presence of polypeptone and decreased in presence of yeast extract (Jin-Ha Jeong et al., 2010). In *B. megaterium* F7-1 keratinolytic enzyme production is positively influenced by tryptone and yeast extract. Usually, the effects of co-carbon and co-nitrogen sources on keratinase production vary according to the species and carbon or nitrogen source and their concentration (Cai and Zheng, 2009). Thus, it is necessary to optimize composition of culture media on a case-by-case basis to improve keratinase production. Increased enzyme production in presence of some co-carbon and co-nitrogen sources can be due to increased cell growth. Presence of sodium sulphite in the culture medium caused significant increase in feather degradation and keratinase production. Feathers are highly stable to degradation due to large number of di-sulphide bonds in keratin protein. Presence of sodium sulphite in culture medium may be responsible for reduction of di-sulphide bonds in keratin protein resulting in increased feather degradation. Presence of surface tension reducers in medium did not cause any significant difference in feather degradation and keratinase production.

CONCLUSION

In this work, we have investigated various environmental parameters that affect feather degradation and keratinase production by keratinolytic isolate *Stenotrophomonas maltophilia* K279a. *Stenotrophomonas maltophilia* shows maximum feather degradation and keratinase production in shaker condition at 37 ºC and pH 7.5. Addition of glucose, glycerol, maltose, peptone, tryptone, yeast extract, ammonium sulphate and sodium sulphite in the growth medium had a positive effect on feather degradation and keratinase production. The results of this study can help in determining incubation conditions and designing appropriate fermentation medium with feathers and for keratinase and feather hydrolysate production.

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

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RESEARCH ARTICLE

Isolation and Characterization of Cadmium Resistant Bacteria From Industrial waste water and Soil

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ABSTRACT

Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of metals to the environment. Heavy metals such as cadmium are not readily absorbed or captured by microorganisms and become threat to an aquatic and environmental system. In Present study bacterial strains were isolated from soil and water samples taken from the metal contaminated industrial area and Cadmium resistance of the isolates were investigated. Seven cadmium resistant bacteria were isolated and identified as *Staphylococcus spp.(S1,W1),Bacillus spp.(S3,W3),Pseudomonas spp.(S2,W2),* two remains unidentified on the basis of morphological and biochemical characteristics.Minimum inhibitory concentration (MIC) and antibiotic resistance pattern of the isolates was also studied. *Bacillus spp.*)W3) and *Staphylococcus spp*. (S1) were found to have high resistance pattern against Cadmium (550 µg/ml). It was observed that the metal resistance bacteria exhibited high resistance pattern towards a group of antibiotics.

Key words: Cadmium resistance, Minimum inhibitory concentration (MIC), antibiotics.

INTRODUCTION

Toxic heavy metal like Hg, Cr, Cu, Zn, Pb, Cd are well known for their toxicities, mutagenic and carcinogenic impact on human beings and other living system especially those metals classified under priority list of pollutants (Mustapha and Halimoon 2015). Natural resources including plants and microorganism are extensively explored to combat metal ion pollution (El-Deeb, 2009). Cadmium which are major contaminants found into the environments and extremely poisonous to humans, animals, plants and microbes which can damage cell membrane alter particularity of enzyme and destroy the structure of DNA (Marzan *et al*., 2017). Cd and almost all of its compound are water soluble and hence easily gain entry in human food chain, no physiological role of cadmium in human cellular metabolism has been reported so far and it is extremely toxic in very minute quantity. It has also been reported to cause osteoporosis and fractures, anemia, eosinophilia, anosmia, apoptosis, diabetes mellitus,

oncogenes, activation and chronic pulmonary problem (Khan *et al.,* 2016). Cd is highly toxic non-essential as these are not needed for the survival of organism and are toxic in low concentrations. Cd is non-biodegradable heavy metal with half-life of 20 years. (Demirezen, 2006). The threat of heavy metals pollution to human and wild life has led to increased interest in developing system that can remove or neutralize heavy toxic metals in soil and waste water (Valls *et al*., 2002). The cadmium cycle between river, water and sediments, the vegetation in the area and the metal levels in the blood and urine of residents in several studies have proven the direct and indirect impact of environmental pollution on human health. (Kafilzadeh et al., 2013). Cadmium particularly accumulates in renal, lung, pancreas, and liver and damages those (Nishijo *et al.,* 2006). The presence of high levels of heavy metals in the environment has an inhibitory effect on most microorganisms. However, microorganisms have evolved their resistance mechanisms that lead to the selection of resistant variables that can tolerate metal toxicity (Nasrazadani *et al.,* 201l). various conventional ways are used for removal of cadmium from waste water like electrochemical treatment, chemical precipi tation, ion exchange, reverse osmosis, membrane technology, phytoremediation, oxidation and reduc-tion are very expensive and not environmen-tally acceptable (Abbas *et al*., 2014). Cadmium is one of the most toxic pollutants of the surface soil layer released into the environment by mining and smelting activities atmospheric deposition from metallurgical incinera-tion of plastics and batteries (Abbas et al. (2014). No treatment for cadmium toxicity has been approved so far. Several chemicals and physical methods are used to remove cadmium from industrial effluent prior to release the effluent into environment but all these methods are expensive and less effective. Bacteria remove heavy metal ions including Cd^{2+} from the environment either by metabolism independent absorption on their cell walls or metabolism depen-dent intracellular accumulation. Hyper accumulation of Cd^{2+} has been reported to disturb the cell physio-logy by reactive oxygen species (ROS) production and disruption of bacterial respiratory proteins.

The aim of this study was isolation and identification of cadmium resistant bacteria, determination of the resistance spectrum by measuring the minimum inhibitory concentration and to study antibiogram of the isolated bacteria.

MATERIALS AND METHODS

Collection of Sample

The experiment was conducted using the industrial waste water and soil samples collected from the MIDC area, Jalna.Water samples were collected in sterile bottle and soil samples were collected in zip bags, brought to the labarotory and stored at 4°C for further study.

Isolation and identification of cadmium resistant bacteria

The cadmium metal was isolated from samples by inoculating the metal and the metal sample using concentration of 50μg/liter. Isolation was achieved by serial dilution method. The waste water and soilsample was serially diluted in which 9ml of sterile saline in 6 test tubes then 1ml of sample was added to the 1st test tube to have 10^{-1} repeated up to 10^{-6} then 0.1ml of the higher dilution was spreaded on the surface of the agar plates and incubated at 37°C for 24hrs. Bacterial strains were isolated from soil and effluent of metal industries. Biochemical and morphological characters of the predominant bacterial genera were studied and finally characterized and identified by standard identification methods (Holt *et al.,* 1994).

Minimum inhibitory concentration (MIC)

All the Seven isolates were checked for metal tolerance. The initial concentration used was 50µg/ml and thereby gradual increasing the concentration of heavy metal each time on nutrient agar (NA) plates until the strains failed to give colonies on the plate. The growth of cultures on last concentration was transferred to the higher concentration by streaking on the plate. The lowest concentration that prevented bacterial growth was considered the MIC.

Antibiogram of the bacterial isolates

Isolated heavy metal resistant isolates were tested for antibiotic sensitivity and resistance according to Kirby -Bauer disc diffusion method (Bauer *et al.,* 1996). Anti-biotic disc containing penicillin (10units), azithromy-cin (15mcg), vancomycin (30mcg), cefazolin (30mcg), Clindamycin (2mcg), Erythromycin (15mcg), tecopla-nin (30mcg) was used. The culture was spread on nutrient agar plates. The antibiotics disc were placed on plates and incubated at 37 ^{0}C for 24 hours. (Nath *et al.,* 2012). After incubation, the organisms were classified as sensitive or resistant to an antibiotic

according to the diameter of inhibition zone given in standard antibiotic disc chart.

RESULTS AND DISCUSSION

The seven isolates of cadmium resistant bacteria were isolated from the industrial waste water and soil from MIDC area of Jalna. They were identified as *Pseudomonas spp., Bacillus spp.,* and *Staphylococcus spp.*

Minimum inhibitory concentration

The seven isolates were further referred for MIC count the bacteria showing minimum inhibitory concentration for heavy metals ranging from 50µg/ml- 600µg/ml. The detailed information is given in table 1.

Table 1: Minimum inhibitory concentration (S = soil sample, W= water sample)

Bacterial Isolate	Strain Name	Source	MIC
Staphylococcus spp.	S1	Soil	550µg/ml
	W1	Water	350µg/ml
Bacillus spp.	S3	Soil	450µg/ml
	W3	Water	550µg/ml
Pseudomonas spp.	S2	Soil	350µg/ml
Unidentified	W2	Water	500µg/ml
	W4	Water	500 µg/ml

The minimum inhibitory concentration was shown highest in W3 strain (550µg/ml) and the lowest were found in W1 and S2 (350µg/ml)

Table 2: Antibiogram of cadmium resistant bacteria

Bacterial Isolate	Strain Name	Sensitive	Resistant
Staphylococcus spp.	*S1*	Azithromycin,Vancomycin, Clindamycin, Cloxacillin.	Cefazolin, Penicillin, Erythromycin, Teicoplalin.
	W1	Teicoplalin, Cloxacillin.	Azithromycin, Vancomycin, Clindamycin, Cefazolin, Erythromycin, Penicillin.
Bacillus spp.	S3	Nil	Azithromycin, Vancomycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.
	W3	Teicoplalin, Vancomycin.	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Cloxacillin,
seudomonas spp.	S2	Teicoplalin, Vamcomycin, Erythromycin, Cloxacilln	Azithromycin, Clindamycin, Cefazolin, Penicillin.
Unidentified	W2	Vancomycin	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.
	W4	Vancomycin	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.

Antibiogram of cadmium resistance bacteria

The bacterial isolates were tested for the antibiotic sensitivity. The predominant isolates that are tolerant to cadmium were found to be multi drug resistant (S1, S2, S3, W1, W2, W3, and W4). Some strains were resistant and some were sensitive to some antibiotic results are given in table 2.

The antibiotics such as Azithromycin, Clindamycin, Cefazolin, Erythromycin, and Penicillin were resistant to the isolated strains cadmium resistant bacteria whereas the antibiotics Teicoplalin, Vancomycin, Cloxacillin were sensitive to some of the isolated strain. Multiple tolerance occurs only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria.

The result of this study indicates that bacteria isolated were *Staphylococcus spp., Bacillus spp., Pseudomonas spp.* As these species shows high tolerance to cadmium and are good candidates for the treatment and elimination of cadmium polluted rivers. Heavy metal resistant microorganisms play an important role in the bioremediation of heavy metal contaminated soils (Ray and Ray, 2009). Cd is considered as one of the most toxic heavy metals and they can appear either in water or soil of any polluted site because of their high mobility, especially in agricultural fields, thus greatly threatens human health via food chains (Goris *et al.,* 2001). Heavy metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and possibly be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget *et al*., 2005).

The concentration of a toxic metal that affects the growth and survival of different microorganisms varied greatly. It is clearly indicated that domestic waste and industrial waste are responsible for the development of bacterial resistance along with the risk of human health and environment. These bacteria helps to formulate bioremediation agent to detoxifying tannery effluent at industrial waste water and soil. Metals are of special concern for the environment and agro-ecosystems, for they appear to be particularly dangerous because of their high toxicity patterns, their extremely long residence time in ecosystems and their ubiquitous distribution worldwide, as pointed out before. As many researchers have mentioned, common physical and chemical methods used to remove heavy metals from waste water samples are expensive and time consuming (Nasrazadani *et al*., 2011). Heavy metal exerts their toxic effect on microorganism through various mechanism and metal tolerant bacteria could survive in these habits and possibly isolated and selected for the potential application in the bioremediation of contaminated sites. Thus, from the present study it can be concluded that the application of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

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

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RESEARCH ARTICLE

Soil Trap Culture of strawberry associated AM fungi from Melghat (M.S.) India

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ABSTRACT

Present world demands the production of high quality food in a most sustainable way causing least damage possible to the ecosystem. Today, fertilizers and pesticides are being used at high levels in the intensive production of plants. A cheap and non-destructive means of achieving high productivity rests on the establishment of a viable low input farming system. However, in order to implement such a plan, we must develop plant systems that can efficiently scavenge and utilize soil nutrients present at low levels. The symbiotic arbuscular mycorrhizal fungi (AMF) have a major impact on the functioning and stability of any ecosystem. An attempt has been made in the present research to isolate and identify AM fungi of strawberry fields in the Melghat area of Maharashtra, India. The rhizosphere soil was then used for soil trap culture of dominant AMF species. Various practices like use of waste substrates along with the traditional substrate (soil-sand mixture) are being tried for mass culture of AM fungi these days. Addition of any substrate into soil provides minerals in addition to beneficial elements that ultimately enhance the growth of AM fungi as well as plant.

Since strawberry is the most economically competent crop of the area, the focus has been to isolate, characterize and identify its most dominant AM fungal species to prepare trap culture. The two varieties of Strawberry (*Fragaria* Sp.) namely winter down and tissue culture were collected for the study. Assessment of rhizosphere soil samples and roots was carried out. All the samples showed presence of AMF propagules. The genus *Glomus* was found to be the most frequent morpho-taxonomically identified AMF. The preparation of trap cultures is the only viable technique to increase spore number and to recover intact, fresh and healthy spores which may be used in future for initiation of monospecific cultures. The research finds its extension in the industrial produce on large scale by mass multiplication of these native AM fungal strains and its wider application in the farming. The findings of the present study are significant in upbringing of the local tribal community using nature's own resource.

Keywords: AM fungi, strawberry, Melghat forest area.

INTRODUCTION

Arbuscular Mycorrhizal fungi (AMF) are natural and integral part of healthy soil ecosystems which contribute to efficient utilization of soil resources namely nutrients and water. AM fungi belonging to the phylum *Glomeromycota* are geographically ubiquitous occur over a broad ecological range including associated agriculture, horticulture, pasture grasses, tropical plants and cereals. Mycorrhizal plants are known to alter nutritional status, altered photosynthetic rates, regulating substances and altered patterns of root exudation due to change in membrane permeability. Further, AM fungi play siginificant role in fruit production by transporting slowly mobile nutrients is specially P, Mn, Zn, Fe, and Cu from bulk of soil beyond the depletion zone surrounding active roots. AMF are said to reduce the impact of environmental stress. Thus, mycorrhiza can be considered as biological agents in maintaining the quality and sustainability of fast degrading environment and managing these symbiotic organisms in agriculture and flori-horticultural systems of world for the benefits of society and soil welfare.

Melghat is among one of the nine tiger reserves of India. It is located at 21 26' 45" to 770 11' 55" E in northern part of Amravati district of Maharashtra in India. The inhabitants are mainly tribal, largely of the Korku tribe. There is a deep connection between the tribal economy and minor forest produce. To protect the biodiversity of melghat forest there is ban to collect forest produce from this area so directly effect on their self-reliance. So, there is urgent need to develop new agriculture technology to sustainable livelihood of farmers of this region.

The strawberry, *Fragaria*, is one of the most popular berry fruits in the world and it offers a wide range of health benefits. Strawberries are an excellent source of vitamins C and K as well as providing a good dose of fibre, folic acid, manganese and potassium. They contain powerful antioxidants and are thought to protect against inflammation, cancer and heart disease. Therefore the objectives of this study are to determine the diversity of dominant AM fungi associated with strawberry plant to develop soil trap culture.

MATERIALS AND METHODS

Site selection and sampling

Amravati is a district in the state Maharashtra, India. It is the seventh most popular metropolitan area in Maharashtra. At the northern extreme of the Amravati district on the border of Madhya Pradesh, lies the Melghat in the South-western Satpura mountain ranges. Melghat means "meeting of the ghats". Motha is a small village near the Chikhaladara from which samples of strawberry plants were collected. Two varieties of strawberry namely tissue culture (Kamaroja) and Winter dawn variety samples were collected in the month of December 2015 along with their roots and rhizosphere soil in triplicates. The plants were carefully uprooted and roots were washed under tap water. A part of fresh root system of each variety of strawberry was cut and after careful rinsing with tap water, the root samples were stored in FAA. The rhizosphere soils from each plant was dug out up to the depth of 15-20 cm and about 500 gm soil sample was collected in polythene bags. These soil samples were brought to the laboratory and after shade drying the soil was stored in clean polythene bag. Each bag was labeled with sampling site number, name of the host and sampling date, etc. The composite sample was used for physico-chemical analysis by standard methods and the individual samples were used for isolation of AMF spores to find out the most dominant AM fungal spores for soil trap culture. physico-chemical analysis of soil samples was done by standard methods Jackson ML (1967).

Processing of Roots

The preserved root samples were used for the further analysis by the process given by Phillips and Hayman (1970). The AM percent root colonization was calculated by using the Grid line intersect method (Giovannetti and Mosse, 1980). The isolation of AM spores was carried out by following method of Gerdemann and Nicolson (1963). The method given by Gaur and Adholeya (1994) was used for counting AMF spores.

The isolated spores were given a thorough microscopic examination to record their morpho-taxonomic features. The AM fungi were identified by using the manual of Schenck and Perez (1990). Soil trap culture were prepared by the method by Rodrigues and Muthukumar,2009.

RESULTS AND DISCUSSION

As far as the current agricultural practices and socio-economic growth of target area is concerned this project is a unique attempt for Melghat region to explore belowground interactions, focusing on the studies of Arbuscular Mycorrhizal fungi associated with strawberry plants. On this account, Melghat soils attracted the attention for detailed studies of AMF and utilization of these in future for the welfare of mankind. Keeping this view in mind the present study was made and the following results were obtained.

physico-chemical parameters of rhizosphere soil samples-

The pH of soil samples tested was moderately alkaline (7.68) in nature. Electrical Conductivity (Ec) was in normal range 0.65 ds/m. Review suggested that nutritionally deficient soils especially p-deficient harbours more AMF. Available P of soil samples is low as compared to the normal range(2.95Kg/ha). Potassium was found to be very high (390.78 Kg/ha). From the field of strawberry, the Organic Carbon was very low (0.19%). The major role in plant growth is also played by micronutrients in soil particularly as per previous research work the Cu and Zn uptake is enhanced by mycorrhizal association. In the present investigation, it was found to be 2.00ppm and 2.01ppm respectively (Table 1).

The % colonization and spore count

The whole root system and rhizosphere soils of all the plants of study area were collected and screened for their mycorrhizal status. The young feeder roots are primary sites for initiation and infection of AMF. Hence all those were collected and stored separately and assessed to know the percent root colonization. The observations are recorded in Table 2.

All the plant roots showed the typical inter and intracellular coenocytic mycelium, Hyphal coils, H-shaped connections, Arbuscules and Vesicles which are the characteristic features and thus confirmation of AM colonization.

The variation in number of spore propagules in each rhizospheric soil and site were observed. Density of AM fungal spores or the resting spores of AM fungi in the rhizosphere soils of both the host plants from all the sites were studied and the results are tabulated in Table-II. The total number of spores in per 100g of all samples of rhizosphere soils found in the range of 1210 to 1558. The highest spore density was observed in the rhizosphere soil of Kamaroza at S2.

Table 1: Correlation between AMF colonization, Spore count and edaphic factors

	pH	EC	Organic C	N	P	K	Cu	Zn	% Col	Spore
pH	1									
EC	-0.3546152	1								
Organic C	-0.8356752	0.01572	1							
N	-0.2060736	-0.2084	-0.00585	1						
P	-0.5292297	0.451344	0.605094	-0.02172	1					
K	-0.0099562	0.211047	0.299936	-0.3438	0.669718	1				
Cu	-0.3760053	-0.28757	0.512941	0.084242	-0.09246	0.222814	1			
Zn	0.30621716	-0.52368	-0.41361	0.209987	-0.89818	-0.89449	-0.3542	1		
%Col	0.74924162	-0.2575	-0.41961	-0.16519	0.075592	0.601924	-0.17556	-0.32363	1	
Spore	0.53562858	-.15937	-0.19505	-0.13956	0.40043	0.637173	-0.38182	-0.49638	0.902148	1

Table 2: AMF % colonization and spore count

Sr. No.	Variety	% colonization	Spore/ 100g
1.	Kamaroza		
	S1.	64%	1499
	S2.	58%	1558
	S3.	54%	1210
2.	Winterdawn		
	S1	56%	1308
	S2	60%	1498
	S3	58%	1411

Table 3: AM fungal species isolated from rhizosphere soil of strawberry

Sr. No.	Variety	AMF species
1.	Kamaroza	
	1.	*Glomus leptotichum*
	2.	*Glomus botryoides*
	3.	*Glomusfecundiosporum*
	4.	*Glomus fistulosum*
	5.	*Glomus australe*
	6.	*Glomus deserticola*
	7.	*Glomus dimorphicum*
2.	Winterdawn	
	1.	*Glomus albidum*
	2.	*Glomus tortuosum*
	3.	*Glomus reticulatum*
	4.	*Glomus etunicatum*
	5.	*Glomus fasciculatum*
	6.	*Glomus aggregatum*
	7.	*Glomus ambisporum*

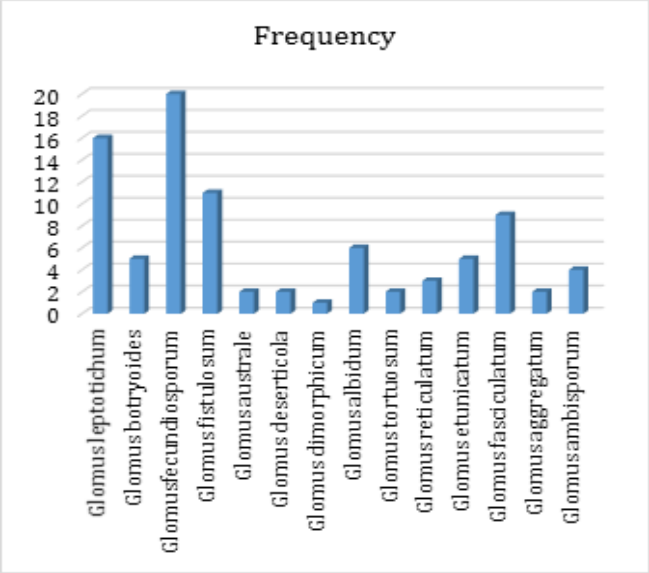

Fig. Frequency of AM fungal species isolated from rhizosphere soil of strawberry

Strawberry Field at Motha (Melghat)

Soil Trap Culture

Identification of AMF Spores

A total of fourteen AMF species of *Glomus* were isolated during the course of this investigation from the sites considered for studies from the field of Strawberry in two varieties. The data is recorded in the Table-III (Graph- I). Some of the interesting observations, which were made during this study, were of spore syndrome. Development of many small spores inside the dead spore of other species is called spore syndrome. Such spores were observed by the author in the present study. Most of the spore syndrome were of *Glomus* species. Formation of spore

syndrome could be a protective mechanism from the parasitic attack by soil microorganism or predatory larvae. The present study is the first report on inventory of AMF species associated with Strawberry from Melghat forest of Maharashtra for its soil trap culture. Similarly Norman et al. (1996) observed colonization rates of 55.4%-70.8% in strawberry plants when inoculated with *Glomus fasciculatum and Glomus etunicatum*.

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

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RESEARCH ARTICLE

In Vitro shoots multiplication through callus culture of *Gloriosa superba* L., a threatened medicinal plant of Melghat Tiger Reserve, Maharashtra, India

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ABSTRACT

Melghat Tiger Reserve is situated in the mountainous region of Satpuras from Dharni and Chikhaldara Tahsils of Amravati District of Maharashtra State. *Gloriosa superba* is belonging to family liliaceae commonly known as Kal-lavi (Glory lily) useful in the treatment of parasitical affection of skin and scorpion bite. The callus is initiated and regenerated from nodal region on Murashige and Skoog medium supplemented with BAP (4 mg/l) + NAA (2 mg/l). Highest number of multiplied shoots obtained from callus cultured on MS medium supplemented with BAP (2 mg/l) without in combination with another growth regulator. The protocol established here is very much useful in the conservation of this threatened medicinal plant.

Keywords: Melghat, *In vitro*, multiplication, threatened, *Gloriosa superba*.

INTRODUCTION

Melghat Tiger Reserve is situated in the mountainous region of Satpuras from Dharni and Chikhaldara Tahsils of Amravati District of Maharashtra State. It is dry deciduous type of forest and consist of total 715 species belonging to 424 gnera are reported from Melghat, out of which 169 species are medicinal.

Gloriosa superba is belonging to family Liliaceae commonly known as 'Glory lily' (Fig a). It is widely distributed in the tropical and subtropical parts of India, Ceylon, Tropical Africa, Malacca (Cook, 1958; Hooker, 1894) up to an altitude of 2000 m. The plant grows in sandy-loam soil in the mixed deciduous forest all over India. It is branched herbaceous climber with showy, large flowers and borne solitary or in corymbose inflorescence. The fruit is loculicidal capsule with 3 lobes consisting of near about 30 – 150 rounded spongy seeds (Narain, 1977). The seeds are a rich source of colchicine and gloriosine (Farooqi *et al*., 1993). Colchicine is used in the treatment of cancer, gout and plant breeding work for inducing polyploidy. Various parts such as leaf, rhizome and seed of the

plants are claimed as useful parts by tribes of India. It is being used in 29 diseases like leprosy, lice, wound along with ulcers and sores, rheumatism, snakebite, scorpion bite, gout, abdominal pain; and useful as abortifacient, anthelmintic, etc. (Bhide and Acharya, 2012).

Colchicine is main alkaloid isolated from this plant and its practical use is more as in industries. This has led to exploitation of this plant all over the world. Once a very common plant on the bordering low hills of Satpura, Melghat forest of Amravati District facing the plains and in Pohra hills. However, plant population of this plant has decreased considerably in the last 10 years due to clearing of supporting vegetation (Dhore, 1986). If the present rate of demand for rhizome continues, the pressure of shortage from the interior forest areas will increase as soon as the availability from the easily accessible area diminishes. Poor seed germination, susceptibility towards many pests, and excessive collection in habitats for medicinal purposes have pushed this taxon to endanger.

Tissue culture studies can play an important role in the *in vitro* conservation of this threatened plant. A major objective of current plant cell and tissue culture work is more efficient exploitation of specific properties of plant genotypes (Johnson, 2002). Different workers all over the world have studied tissue culture studies of *G. superba* viz., Finnie and Staden, (1989); Somani *et al*., (1989); Samarajeewa *et al*., (1993); Hassan and Roy, (2005); Sivakumar and Krishnamurthy, (2000); Ade and Rai, (2011); Khandel *et al*., (2011); Venkatachalam *et al*., (2012); Yadav *et al*., (2012).

The objective of the present work is to frame tissue culture experiments, to induction, proliferation of callus and multiplication of shoots.

MATERIALS AND METHODS

Tubers were collected from Melghat Tiger Reserve core and buffer area forest of Amravati District. They were established and maintained in Botanical garden. Sprouted tuber buds and young shoots were collected, defoliated and washed in running tap water for about 15 min continuously. Tuber, shoot tips and nodal explants were soaked common soap solution for 10 min. After decanting soap solution it was washed with sterilized distilled water. Explants were surface sterilized with 70% alcohol for 30 seconds followed by 0.1% mercuric chloride for 8 min. In the intermediate of this sterilization process rinses of distilled water were given to the plant materials. Then they were cut into proper size and inoculated on Murashige and Skoog (1962) growth medium supplemented with different concentrations of growth regulators viz, (BAP) 6-Benzylamnopurine, (NAA) α-Naphthalene acetic acid, (IAA) Indol-3-acetic acid, (2,4-D) 2,4-Dichlorophenoxy acetic acid, (Kn) Kinetin (Himedia, Bombay); also consisting of sucrose and Agar. The pH was adjusted to 5.8 before autoclaving. All the culture vessels and media were sterilized at 121°C for 20 to 30 min. All the cultures were incubated at 16±1 Hr light and 8±1 Hr dark photoperiod and temperature of 20±2°. The cultures which show good results were sub-cultured into fresh medium. The data was analyzed statistically by using standard formula of standard deviation (SD) and standard error (SE).

RESULTS AND DISCUSSION

Mass propagation of plant species *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture (Hassan, 2005). The callus started initiating from nodal explants after 6th week of culture on MS medium supplemented with BAP (4 mg/l) + NAA (2 mg/l). It was first white in colour (Fig b) but later on tuberous in texture and yellowish white in colour and hard. This callus was used further for regeneration of shoots. Related results were obtained by Roy and Pal (1995) in *Costus speciosus* where brown hard callus was proliferated on Schenk and Hildebrandt medium augmented with BA (0.5 m/l) in combination with NAA (0.5 mg/l); again Ahmad *et al*., (2007) in *Podophyllum hexandrum* obtained yellowish callus on MS medium with uniform concentration of BAP and NAA (1.5 mg/l). It was again confirmed that MS medium and B5 medium were supplemented with various concentrations and a combination of Auxin, Cytokinin, and organic acids was used, 98% of callus induction occurred in non-dormant corm bud explants of *G. superba* (Rishi, 2011).

Highest number of multiplied shoots obtained from callus cultured on MS medium supplemented with BAP (2 mg/l) without in combination with another growth regulator (Table 1).

Table1: Effect of growth regulators on shoots response of callus in *G. superba*

Cytokinin (mg/l)		Auxin (mg/l)	% of shoot	Length of shoot (cm)	Multiplication of shoot
BAP	Kn	NAA	initiation	Mean ± SE	Mean ± SE
2	-	-	100	2.6 ±0.18	5.3 ±0.29
4	-	3	80	3.75 ±0.16	2.63 ±0.18
4	-	2.5	90	4.44 ±0.19	4.67 ±0.18
-	3	1.5	70	1.71 ±0.17	-

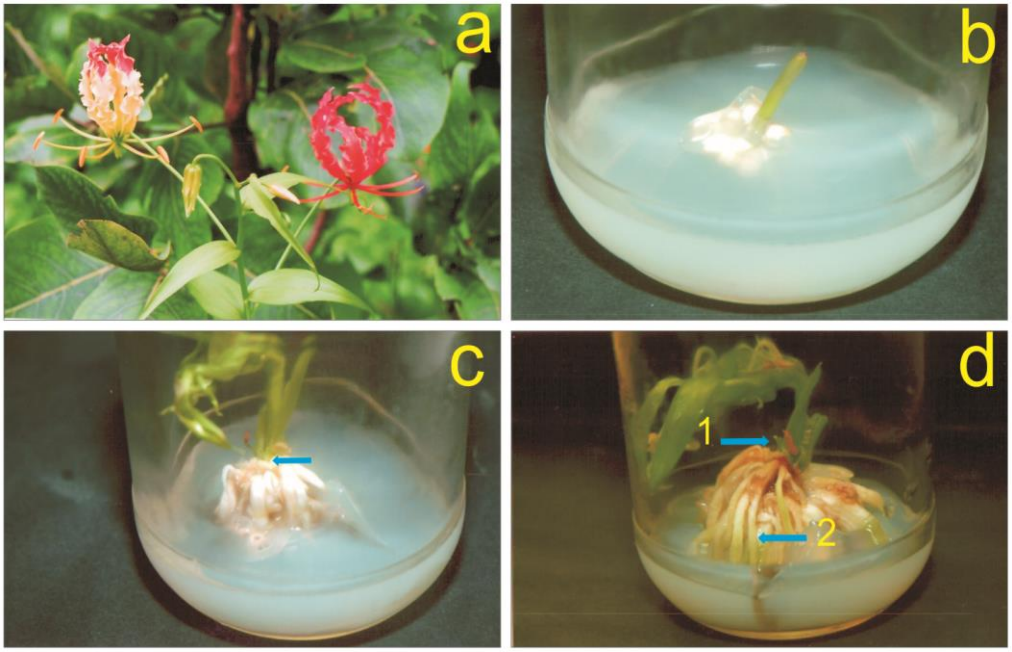

Figure- 1: *G. superba* (a) A flowering twig, (b) Initiation of callus, (c) shoot emergence, (d 1) multiplied shoots, (d 2) roots

MS medium containing BAP 4 mg/l + NAA (3 mg/l) regenerated 80 % shoots (Fig c and d1), showing average shoot number i.e., 2.63 ± 0.18, shoot length 3.75 ± 0.16. In another combination, MS medium supplemented with BAP (4 mg/l) + NAA (2.5 mg/l) showed 90% initiation with maximum shoot number recorded 4.67 ±0.18 having soot length 4.44 ± 0.19 cm. From the above two combinations, best results was obtained in second combination. Frequency of shoot number and shoot length declined markedly at higher concentration of NAA than the other growth regulator. Hence it was important to note that BAP was responsible for shoot initiation and growth. Previous results of Benmoussa *et al*., (1996) in *Asparagus densiflorus* are in accordance with present findings; 6BA promoted more shoots per callus than Kn with low concentration of NAA; the number of shoot buds decreased sharply in the presence of higher doses of cytokinin. There is another example where BAP along with low concentration NAA initiated shoot differentiated from callus; however, the process was retarded at higher concentration of BAP in the cultivars of *Cucurbita pepo* (*Pal et al*., 2007). Moreover, in another combination of Kn (3 mg/l) + NAA (1.5 mg/l) shows very low percentage of shoot initiation and growth 1.71 ± 0.17 cm without multiplying. Samarajeewa (1993) reported that Kinetin was less effective than BAP for shoot multiplication which supports the view of present author. The multiplied shoots showed extensive rooting without transfer in fresh medium (Fig d2).

In words of Singh *et al*., (2013), that *G. superba* will conserved by tissue culture technique; not only we cultivate the good quality of plant but also enhance the valuable component of plant and reduce the over harvesting of plant from its natural habitat.

CONCLUSION

G. superba is occurred as a threatened category medicinal plant in Melghat Tiger Reserve,

Maharashtra. Present report of shoot regeneration and multiplication through callus culture can provide wide prospect for further research and study for *in vitro* conservation of this threatened and medicinally useful herb.

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

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RESEARCH ARTICLE

Antibacterial activity of spice essential oils against uropathogenic bacteria

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ABSTRACT

The medicinal values of many spices and condiments used in Indian cooking have been known from centuries. The aim of this study is to evaluate the antibacterial activity of spice essential oils which use in kitchen on uropathogenic bacteria. Urinary tract infection is second most common infection next to Respiratory tract in our human body. The indiscriminate use of antimicrobial drugs has led to the resistance in uropathogens globally. In present scenario, alternative and safe therapy is urgently needed. Essential oils of ajwain (*Trachyspermum ammi*), cardamom (*Amomum subulactum*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), black pepper (*Pipera nigrum*) and turmeric (*Curcuma longa*) were used in this study. Their antibacterial activity against various uropathogenic bacteria viz. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* was determined by disc diffusion method. The minimal inhibitory concentrations (MICs) of oils were determined by broth macrodilution method. Among all oils ajwain showed highest inhibitory activity with zone of inhibition ranging from 40 to 35 mm diameter against all uropathogenic bacteria. Turmeric was found to be ineffective against all uropathogenic bacteria. Cardamom and clove also showed strong activity for all uropthogens. Activity of black pepper and cinnamon oil was found to be moderate against uropathogens.

Keywords: Spice, Spice essential oils, Uropathogens, Antibacterial activity.

INTRODUCTION

Urinary Tract Infections (UTI) is the second most common infection in humans (Tabiban *et al*., 2008). Different sex and age group of people are affected by urinary tract infection. Sometimes, the UTI is symptomatic or asymptomatic and complicated or uncomplicated. Usually UTI infection is confirmed by significant bacteriuria. i.e., 10^5 organisms/ml (Anandraj *et al.* 2015). UTI refers to the presence of clinical signs and symptoms arising from the genitourinary tract associated with the presence of one or more microorganisms. UTIs are usually localized to the bladder, kidneys or prostate. *Escherichia coli* is the predominant uropathogen

responsible for roughly 80% of all UTI cases, followed by *Staphylococcus*, *Klebsiella*, *Enterobacter*, *Proteus* and *Enterococci* species (Ronald, 2003). Isolation, characterization, early detection and antibiotic therapy are also very important for Urinary tract infection. Current management of UTI's are usually empirical, without the use of urine culture or susceptibility testing to guide therapy (Manjunath *et al*., 2011). Today many broad-spectrum antibiotics are used to treat many urinary tract diseases pose serious threat of drug resistance and hypersensitivity reactions. The success of chemotherapy lies in the continuous search for new drugs to couter the challenge posed by resistant strains (Gibbons, 1992). The present investigation evaluates the antibacterial effects of essential oils of various spices against urinary tract pathogens.

MATERIALS AND METHODS

The following standard cultures were procured from microbial Type culture collection (MTCC), & American Type Culture Collection (ATCC)
Escherichia coli ATCC 14948
Klebsiella pneumoniae MTCC 4030
Pseudomonas aeruginosa MTCC 4676
Staphylococcus aureus ATCC 33591
The standard cultures were maintained on the culture media as recommended by MTCC and ATCC

Essential Oils

In the present study, seven genuine essential oils of, clove, ginger, turmeric, ajwain, black pepper, cardamom & cinnamon were obtained from different commercial sources. The oils were stored in glass bottles at 4^{o} C under refrigeration.

Antibacterial assay of spice essential oils

In the present work, essential oils of seven different spices were used. The test organisms were grown in nutrient broth. The fresh bacterial cultures were compared with 0.5 Mc Farland turbidity standard, which is equivalent to approximately $1x10^{6}$ CFU/ml were used. Then the test organisms were inoculated on the Muller-Hinton agar plates. Sterile swabs were used for inoculation purpose. The sterile discs were placed on agar surface. 20 µl essential oil of each spice was added on the sterile discs. These plates were incubated at 37^{o} C for 24 hours. The zone of inhibition was observed and recorded.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of six essential oils namely ajwain, cardamom, clove, cinnamon, black Pepper and ginger oil were determined by Broth Macrodilution Sensitivity testing method. This test was performed in round bottom sterile glass tubes (12x75 mm) using Muller Hinton Broth supplemented with 0.15% agar. Serial two fold dilutions of stock solution of each spice essential oils were prepared in tubes over the range of 0.02-10µl/ml with a final oil concentration range 0.01-5µl/ml.

A working inoculum suspension of $1x10^{4}$ CFU/ml was prepared by diluting the stock inoculums. The inoculums were added in each tube & one tube kept as a control i.e. inoculum was not added in this tube. The tubes were then incubated at 37^{o} C for 24 hrs and observed for the presence or absence of visible growth. (Kamble 2006)

RESULTS AND DISCUSSION

The antibacterial activities of seven spice essential oils were screened against four organisms by disc diffusion method. Essential oils showed antibacterial activity in varying magnitudes. Ajwain oil, cardamom oil, cinnamon oil, black pepper, clove oil, & ginger oil showed inhibitory activity against all uropathogens (Table 1).

Out of these seven spice essential oils, ajwain oil was found to be most effective with > 30mm diameter of inhibition zone against all uropathogenic bacteria. This result is in agreement with the report of Hassanshahian et al. (2014) reported that essential oil of ajwain was found to be very effective against *E. coli, K. pneumoniae & S. aureus*.

Cardamom oil showed strong inhibitory activity with 33mm, 30 mm, 27mm & 23 mm inhibition zone diameter for *E. coli, K. pneumoniae*, *P. aeruginosa* & *S. aureus,* respectively. Kumar et al. (2010) reported that the antimicrobial property of cardamom is due to having wide verity of secondary metabolized such as tannins, alkaloids & flavonoids. Ritender et al . (2013) concluded that, the essential oil of cardamom (*Ammomum subulatum*) is useful for treatment of infection caused by *S. aureus P. aeruginosa & E.coli.*

Table 1. Antibacterial activity of undiluted essential oils of spices against uropathogens.
Zone of Inhibition in mm

Organisms	Essential Oils						
	Ajwain	Cardamom	Clove	Cinnamon	Black Pepper	Ginger	Turmeric
E.coli	40	33	25	26	16	12	No Zone
K. pneumoniae	42	30	22	8	20	13	No Zone
P. aeruginosa	41	27	22	18	15	12	No Zone
S. aureus	35	23	23	24	18	13	No Zone

Table 2. Minimum inhibitory concentrations (MICs) of essential oils against uropthogens

Organisms	MICs of essential oils (µl /ml)					
	Ajwain	Cardamom	Clove	Cinnamon	Black pepper	Ginger
E. coli	0.62	1.25	1.25	1.25	2.5	5.0
K. pneumoniae	0.62	1.25	1.25	2.5	2.5	5.0
P.aeruginosa	0.62	2.5	1.25	2.5	2.5	5.0
S. aureus	1.25	2.5	1.25	1.5	2.5	5.0

Aneja & Joshi (2010) studied in importance of clove & clove oil & indicated that clove oil can be used as an antimicrobial agent. Present investigation supports the findings of Aneja & Joshi. In the present work clove oil showed inhibitory activity for all uropathogens. The essential oil of cinnamon was found to be inhibitory for *E.coli , S. aureus & P. aeruginosa* & showed little inhibition for *K. pneumoniae.* Poppachan et al .(2007) evaluated the antimicrobial activity of six indian spices extracts namely clove, cinnamon & mustard, garlic, ginger & mint against *E .coil S .aureus* and *B. cereus* & reported that the extract of clove, cinnamon & mustard had good inhibitory activity. Ginger oil showed inhibition ranging from 12 to 13 mm diameter zone of inhibition. This result is in agreement with Nader *et al.* (2009) who reported ginger essential oil to be moderately effective against bacteria. In present studies turmeric oil was found to be ineffective against all uropathogens. Jelena et al. (2016) also reported the ineffectivity of turmeric oil against *S. aureus, E. coli* and *L. monocitogen*.

In the present investigation MICs of six spice essential oils was determined by broth macro dilution method. *E. coli, K. pneumoniae*, & *P. aeruginosa* were inhibited by Ajwain oil at concentration 0.62 µl/ml. Ajwain oil inhibited *S. aureus* at concentration of 1.25 µl/ml. Omidpanah et al. also (2016) reported that, essential oil of ajwain inhibited the growth of *E. coli* at concentration of 0.5 µl/ml and *S. aureus* at concentration 1 µl/ml The cardamom oil inhibited *E. coli*, *K. pneuononiae*, *P. aeruginosa* & *S. aureus* at concentration 1.25, 1.25, 2.5 & 2.5 µl/ml respectively. Cinnamon oil inhibited *E. coli* at concentration of 1.25 µl/ml, *K. pneumoniae* & *P. aeruginosa* at 2.5 µl/ml & *S. aureus* at 1.5 µl/ml. All the bacteria were inhibited by the black pepper and ginger oil at 2.5 µl/ml & 5.0 µl/ml concentration respectively. MICs for all bacterial test cutures are shown in Table 2.

CONCLUSION

Present study concluds that the spice essential oils have antimicrobial property against uropathogens. By the view of therapeutic management spice and herbs are of low cost and biodegradable. Therefore spice essential oils may be the natural and safe way rather than use of expensive and nonbiodegradable antibiotics for treatment of UTI.

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

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RESEARCH ARTICLE

Isolation and identification of bacterial flora from bat guano and its study on bioremediation of industrial waste

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ABSTRACT

Preliminary investigation were carried to identify the bacteria from bat guano. Morphological and biochemical test were performed and the identification of bacteria was made. Guano is rich in existing organic fertilizers with a better balance of essential N-P-K, wealth of microorganism and much higher level of organic matter .Various bacteria present in guano have proved to be efficient for breakdown of industrial effluents i.e. by bioremediation process of microbes which clean up toxic substances from the industrial waste. Recent research indicates that different bacteria confined in guano may actually provide important medicinal materials. The present work is an attempt to study the effect of bat guano on bioremediation of industrial waste effluents from Mahananda dairy and Coca cola bottling plants. The results showed that with a period of 15 days there was a remarkable reduction in the Chemical Oxygen Demand (COD) values up to 50%-70%, thus stabilizing the industrial effluents. Besides this, values of various physico-chemical parameters were notably found to reduce suggesting that industrial effluents can be effectively treated by bat guano.

Keywords: Bat guano, Bacteria, Bioremediation, Industrial effluents.

INTRODUCTION

The word guano originated from the Quichua language of the Inca civilization and means “the dropping of sea birds”. It describes both bat and sea bird manure. Bat guano provides a considerable supply of richest fertilizer. Recent research indicates that different bacteria confined in guano may actually provide important medicinal material. A marvelous symbiosis exists between the microorganisms and bats. Countless microbes are regularly excreted along with waste products and together with soil organisms they constitute the microbial population of a bat guano deposit. Their main function is to accelerate the process of breaking down of organic matter in guano.

Bioremediation is a spontaneous or managed process in which biological (especially micro biological) catalyst act on pollutants and thereby

remove environmental contamination Bioremediation technology have become famous in early 1980's for site cleanup. Various bacteria present in guano have proved to be efficient for breakdown of industrial waste i.e by bioremediation process of microbes which clean up toxic substances from the industrial waste. By considering this aspect present study was carried out to assess the effect of bat guano as a bioremediator. Bat guano is a safe and environment friendly alternative to harmful chemicals. Kelehar (1998) suggested that bat guano is 100% organic and natural. According to Alper (1983), bioremediation is at least six times cheaper than confinement.

In the present investigation, an attempt is made to isolate the bacterial flora from the bat guano, by morphological, biochemical studies and its effect as a bioremediator was observed on industrial waste water effluents namely Local dairy industry and soft drink (Coca cola) bottling plant at Nagpur.

MATERIALS AND METHODS

Bat guano samples were collected from the roosting sites of bats from the Urban area of Amravati City. The dropping was collected from a clean site with negligible contamination of soil manually by using gloves and placed in sterile polythene bags. Bat guano samples were brought to the laboratory, weighted and immediately process for serial dilution and culturing of the bacteria. Isolation of bacteria was performed by making serial dilution.

Morphological and biochemical test were also performed. Morphological characteristics such as shape and size were determined under light microscope and using gram staining. The bacterial isolates were biochemically characterized by sugar fermentation test and IMVIC test. Identification of bacteria was made by MTTCC Chandigarh.

The sterile sampling bottles were used to collect the industrial effluents sample from Mahananda dairy and Coca cola bottling plants. Dissolve Oxygen was fixed in situ immediately. The samples were brought to the laboratory and various parameters like pH, Dissolved Oxygen (DO), Total suspended solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), sulphate, chlorine and oil and grease were analyzed. Statistical analysis of the COD reduction results was carried out using two-way ANOVA test by utilizing SPSS utility package.

RESULTS AND DISCUSSION

In the present investigation, moist bat guano samples green and yellow were collected from roosting sites of the bat colonies and were brought the laboratory on cultured on nutrient agar plates at 37^{o}C.After 24 hrs a luxuriant growth as obtained from the guano samples. Seven morphologically different colonies three each from bat guano were obtained (Table 1).

All colonies obtained were transfer to nutrient agar slants in order to get pure isolates of each colonies and heavy growth was obtained in pure nutrient agar slants (Fig.1). The isolated colonies were streaked on selective and differential media to enhance the growth of desired organism. The media used are Bismuth sulphite agar (BSA)(Fig.2), Deoxycholate agar (DCA) Mac Conkey (MaC) and Eosin methylene blue agar (EMB).

Table.1. Colony morphology of bacteria observed on nutrient agar plated from serial dilution 10^{4}

Medium	Colony		Growth	Colony Morphology				
				Size	**Shape**	**Colour**	**Edges**	**Opacity**
	Yellow moist guano	**C-I**	Present	Medium	Circular	Red	Entire	Opaque
		C-II	Present	Medium	Circular	White	Entire	Opaque
		C-III	Present	Small	Circular	Yellow	Entire	Opaque
	Green moist guano	**C-I**	Present	Small	Circular	Red	Entire	Opaque
		C-II	Present	Large	Circular	White	Entire	Transparent
		C-III	Present	Medium	Circular	Yellow	Erose	Opaque

Fig.1.

Fig.2.

Fig.1. Nutrient agar slant showing colonies of bat guano
Fig.2. Isolated colonies on BSA

Table 4 : Physicochemical parameters analysed before and after treatment with bat guano of locally dairy waste sample.

Parameters	Raw effluents without treatment(mg/L)	After treatment(mg/L)
pH	8.5	7.8
Dissolved Oxygen(DO)	Nil	5.0
Oil Grease	18.3	9.8
Total Suspended Solids(TSS)	280	65
Biological Oxygen Demand(BOD)	1400	500
Chemical Oxygen Demand (COD)	257	590
Chlorides	2650	590
Sulphates	39.0	19.9
Total Dissolved Solids (TDS)	34.8	42.2

Table 5 : Physicochemical parameters analysed before and after treatment with bat guano of soft drink bottling plant sample

Parameters	Raw effluents without treatment(mg/L)	After treatment(mg/L)
pH	9.8	7.4
Dissolved Oxygen(DO)	Nil	4.2
Oil Grease	21.6	8.2
Total Suspended Solids(TSS)	210	42
Biological Oxygen Demand(BOD)	280	32
Chemical Oxygen Demand (COD)	1200	390
Chlorides	29.8	19.24
Sulphates	31.2	26.5
Total Dissolved Solids (TDS)	1567	380

The isolated colonies from nutrient agar slants were used to study gram reaction. Isolates from yellow moist guano showed that presence of organism which were short rods gram -ve and few were short and long rods gram +ve .In addition green moist guano showed presence of gram -ve short rod and short rod coccobacilli, cocci in bunches which were gram +ve. Identification of bacteria was made by MTCC, Chandigarh. ***Serratia marcescencs*** and ***Bacillus pantothenticus are the*** two bacteria which were identified. *Serratia marcescencs* are rod shaped gram –ve bacteria and *Bacillus pantothenticus* are rod shaped gram +ve bacteria.The result obtained from the present study indicate that bat guano contain several different bacterial group i.e gram positive and negative . Literature reveals that not much work has been carried out on the present topic. There have been several made to examine the type of bacteria that can be isolated from faecal material of different animal species and human (Gilliland *et al*.,1975); (Mitsuoka, 1974) and (Zammi,1974). Faecal bacteria are isolated and identified from Swine by Salantro *et al*. (1977).

Gram positive ***Bifidobacterium suis*** and ***Lactobacillus acidophilus*** have been identified from swine faeces by Gavani *et al*. (2006).

From chicken faeces five strains of *Lactobacillus thermotolerant* lactic acid bacteria were isolated. These strains were characterized taxonomically by Niamsup *et al.* (2003). They were heterofermentive lactobacilli that produce DL-lactic acid. *Paenibacillus favisporus* sp.nov.a xylon degrading microorganism ,a sporulated bacterium was isolated from recent and old cow dung and rectal samples by Veazquez *et al.* (2004).

In the present study, analysis of bat guano for its main constituents were performed by the standard methods. The values were compared with the known values of the constituents of the bat guano. It was found that the Nitrogen and Phosphorus were comparatively very high whereas Potassium values analyzed were extremely low. The organic matter was found to be substantial in amount.

Certain physico-chemical parameters of two industrial effluents were analyzed in the present investigation and the results are given in Table.4 and 5.

After treating the industrial effluents with guano sample at different concentration, the COD reduction values obtained are given in Table.6 and 7. Table 6.1 shows the percentage data calculated from the values of Table 6. Table 7.1 shows the percentage data calculated from the values of Table 7.

The result indicates that the minimum dosage of sample innoculum for reducing the COD value for the dairy waste found to be 10mL which reduced the COD level from 2570mg/L to 590mg/L.

Table 6 : Results of COD reduction values of the dairy waste industrial effluents by treating with different concentration concentrations of bat guano samples

Conc.of bat guano	**COD reduction values mg/L**				**Total**
	1st day	7 th	15 th	21th	
2mL	2650	2253	1850	1800	**8553**
4mL	2700	2295	1755	1720	**8470**
6mL	2700	2095	690	690	**6175**
8mL	2600	2210	610	610	**6120**
10mL	2570	2185	590	590	**5963**

Table 6.1 : Percentage data of COD reduction values (dairy waste)

	Percentage data			
Conc of bat guano	**1st day**	**7 th**	**15 th**	**21th**
2mL	30.98	26.34	21.63	21.05
4mL	31.88	27.10	20.72	20.31
6mL	43.72	33.93	11.17	11.17
8mL	42.48	36.11	11.44	9.97
10 mL	43.10	36.64	10.36	9.89

Table 7 : Results of COD reduction values of the soft drink industrial effluents by treating with different concentration concentrations of bat guano samples

Conc.of bat guano	**COD reduction values mg/L**				**Total**
	1st day	7 th	15 th	21th	
2mL	1200	1020	395	390	3005
4mL	1250	1063	350	350	3013
6mL	1250	1013	373	365	3001
8mL	1250	1089	386	380	3105
10mL	1250	1056	352	350	3008

Table 7.1:Percentage data of COD reduction values (Soft drink waste)

	Percentage data			
Conc of bat guano	**1st day**	**7 th**	**15 th**	**21th**
2mL	39.93	33.94	13.14	12.98
4mL	41.49	35.28	11.62	11.62
6mL	41.65	33.76	12.43	12.16
8mL	40.26	35.07	12.43	12.24
10 mL	41.56	35.11	11.70	11.64

Table8:Significance by 2-way ANOVA test fort the effect of bat guano on the reduction of COD values of the local dairy waste effluent.

Source of Variation	SS	Df	MSS	F tab	F cal	Result
Concentration of Sample in ml	1771828	4	442956.9	3.922105	3.25916	Significant
Days	9238495	3	3079498	27.26702	3.4903	Significant
Error	1355263	12	112938.6			
Total	12365585	19				

Table 9:Significance by 2-way ANOVA test fort the effect of bat guano on the reduction of COD values of the soft drink waste effluent

Source of Variation	SS	Df	MSS	F tab	F cal	Result
Concentration of Sample in ml	1949.8	4	487.45	1.845802	3.25916	Significant
Days	3095137	3	1031712	1790.183	3.4903	Significant
Error	6915.8	12	576.3167			
Total	3104003	19				

Where SS:Sum of squares ,df: Degree of freedom, MSS: Mean sum of square, F tab: F tabulated , Fcal :F calculated.

The minimum dosage for reducing the COD value for soft drink was found to be 2ml, where it reduced the COD level from 1200mg/L to 390 mg/L. Though the optimization of COD values was obtained on 15 th day of the experiment, the values remained more or less stabilized till 21st day. The similar study was performed to assess the effect of bat guano as a soil cleanser against fungi for bioremediation of lake soil (Pawar and Deshmukh,2004). Biodegradation of lignocellosic waste by *Aspergillus terreus* was reported first time by Emitiazi *et al.* (2001). Literature reveals a reduction in the COD value of lignocellulosic waste by 40 -80 after treating with the fungus *Aspergillus terreus.* The presence of bat guano brings about biodegradation of toxic compounds in favourable environmental condition (Coyane,1999).

Two-way analysis of variance (ANOVA) test performed for COD values in dairy waste water effluent sample showed that there is a significant difference between concentration of samples and between the days on which the effluents was treated with bat guano sample, since Fcal >Ftab at 5% level of significance (Table 8). Whereas, for soft drink the test was found to be insignificant as there was not significant difference between the treatment values (Table 9).

CONCLUSION

High values of Nitrogen, Phosphorus, Potassium and organic matter in the bat guano analysis reveals that, bat guano can become a good manure and can be used as a fertilizer. An attempt should be made to identify further the bacterial flora of bat guano so that it can be used as soil cleaner. From the optimization experiment, it can be concluded that less concentrations of bat guano seed can reduce the chemical oxygen demand (COD) levels significantly and stabilize the industrial waste water. The reduced

values of the physiochemical parameters of diary waste and Coca cola bottling plant by using bat guano suggest that industrial waste can be effectively treated by bat guano.

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

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RESEARCH ARTICLE

In vitro direct multiple shoots regeneration through mature seeds of Pigeon pea (*Cajanus cajan*)

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ABSTRACT

Our research work has been mainly focused on development of regeneration protocol for *In Vitro* direct multiple shoots regeneration in pigeon pea (*Cajanus Cajan)* by using explants as a pre soaked mature seeds of *Cajuns cajan* (Cv. Maruti). This protocol includes washing of overnight pre soaked mature seeds of pigeon pea with laboline and rinsed with tap water and then agitated with bavistin fungicide for 5-6 min. After that surface sterilization of explants by 0.1% of Hgcl2 followed by 70% ethanol. Surface sterilized explants were inoculated on MS media supplemented with different hormonal concentrations of BAP (3mg/l, 5mg/l, 7mg/l, 8mg/l) in combination with NAA (0.5mg/l) and IAA (1.5mg/l). The media MS+BAP (7mg/l) gives best results of multiple shoots induction with standard deviation of (3.1 ± 1.3901) after 25 days of inoculation and (3.2 ± 1.4349) after 50 days of inoculation. We got maximum 7-8 no of multiple shoots on this standardized media by comparing with the control media (MS+ without hormone).We standardize this media i.e. MS +BAP (7mg/l)by comparing with the another two, first is MS+BAP (7mg/l) +IAA (1.5mg/l) which gives standard deviation of (2 ± 0.8968) after 50 days of inoculation and second is MS+BAP (7mg/l) +NAA (0.5mg/l) which gives standard deviation of (1.8 ± 0.8071) after 50 days of inoculation. We also overcome the problem of phenolic compounds secretions by replacing the solidifying agent in our media i.e. agar by clarigel (2.5%). This research paper deals with the progress made on direct multiple shoot induction from mature seeds of pigeon pea, which helps in fulfillment of all the future research threats.

Key words- *Cajanus Cajan,* Multiple shoots regeneration, MS Media, BAP, *In Vitro* culture.

INTRODUCTION

Pigeon pea belongs to the family fabaceae which is an important high-protein grain legume of the semi-arid tropics and caters to the protein requirement of a population in Indian sub-continent. The planting of pigeon pea also replenish soil nutrient and controls soil erosion (ICRISAT

1998). Due to its high nutritive value and special protein content, it can be utilized to combat malnutrition in children. But there is a low per capita consumption of 40g as against the requirement of 80 to 100g due to the shortage in production. The main constraints leading to low yield in pigeon pea include factors such as lack of tolerance to pests, diseases and stress, heavy dependence on rainfall (Lal and Chandra, 1987). Improvement of pigeon pea cultivars possessing resistance to pests and diseases, tolerance to abiotic stresses and low allergic proteins in seeds is therefore desirable for improvement in yield of pigeon pea. The best option currently available for control of insect and pest are through use of chemical insecticides that are expensive and not affordable for most farmers in India. At this stage, development of biotechnological approaches like cell and tissue culture techniques in pigeon pea would be of help to overcome some of these constraints by allowing wide hybridization for inducing genetic variability for yield as well as resistance to biotic and abiotic stresses. Due to high demand in market pigeon pea becomes commercial target for micropropogation and tissue culture, and it can be used for large scale production of pigeon pea. The technique of tissue culture provides reliable system for the rapid multiplication of genetically uniform disease free plant.

MATERIALS AND METHODS

Plant material and explants preparation-

The required experimental plant material of pigeon pea (matured seeds of Cv. Maruti) was taken from the plants available at farm of Shri. Shivaji College of Agril Biotechnology Amravati. These mature seeds (Healthy & uniform) were used as explants. These seeds were thoroughly washed under tap water and soaked for overnight. Then these soaked seeds of pigeon pea washed properly with laboline followed by mixing with bavistin solution (fungicide) to avoid future fungal contamination, for 5-6 min and wash properly with distill water. After that firstly these explants treated with 0.1% mercuric chloride for 4-5 min and followed by two washings of distill water. Secondly it's treated with 70% alcohol for 2 min followed by 2-3 washings of distill water. Then the explants were inoculated on MS media supplemented with different hormonal concentration i.e. BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) without any other hormonal combination, then BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with IAA (1.5mg/l) and NAA (0.5mg/l) and then placed under standard growth conditions i.e. 16 hours light and 8 hours dark photoperiod at 25±2ºc temperature.

Culture Medium and Conditions-

Sterilized glassware was used for media preparation. The MS media was prepared with 3% (w/v) sucrose supplemented with different hormonal concentration i.e. Firstly only BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) without any hormonal combination, secondly BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with IAA (1.5mg/l) and BAP (3mg/l, 5mg/l, 7mg/l, and 8mg/l) in combination with NAA (0.5mg/l). All media were adjusted to pH of 5.8 prior to the addition of clarigel (2.5%) and autoclaved at 121ºc temperature, 15 lbs pressure for 20 minutes. After preparation of growth media the surface sterile explants were inoculated on it under aseptic conditions. Inoculated bottles were placed in growth room (culture room) under standard growth conditions i.e., 25±2ºc temperature, 16 hours light and 8 hours dark photoperiod, 2000-3000 Lux light intensity provided by cool white fluorescent light.

We get direct multiple shoots from explants of *Cajanus cajan* (mature seeds of Cv. Maruti) after 15 days of inoculation. These multiple shoots with explant were sub cultured on new fresh but same multiple shoot regenerating media after every 15 days for its proper maintenance by future experimental point of view.

RESULTS AND DISCUSSION

Surface sterilized seeds culture directly on MS media showed 75-80% seed germination and development of single shoot, and then explants with single shoot sub cultured on fresh multiple shoot regeneration medium. And the multiple shoot regeneration was observed directly from the mature seeds of *Cajanus cajan* after 2-3 weeks in the regeneration medium (MS) of various concentrations of growth hormones. Successful regeneration of legume species has been greatly aided by species- specific determination of critical parameters, such as explants source, genotype and media concentrations (Geetha et al., 1998). We obtained healthy multiple shoots of *Cajanus cajan* (Cv. Maruti) along with greenish color.

Table 1: Effect of different concentration of BAP on multiple shoot generation in *Cajanus cajan:*

Sr. No.	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without growth regulator	No Response	No Response
1.	MS + BAP (3mg/l)	1.4 ± 0.6278	1.5 ± 0.6726
2.	MS + BAP (5mg/l)	1.6 ± 0.7174	2.0 ± 0.8968
3.	MS + BAP (7mg/l)	3.1 ± 1.3901	3.2 ± 1.4349
4.	MS + BAP (8mg/l)	Swelling of seeds	Swelling of seeds

Table 2: Effect of different concentration of BAP in combination with IAA on multiple shoots generation in *Cajanus cajan*:

Sr. No.	Concentration of hormone (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without growth hormone.	No Response	No Response
1.	MS + BAP(3mg/l)+IAA(1.5mg/l)	0.8 ± 0.3587	1.0 ± 0.4484
2.	MS + BAP(5mg/l)+IAA(1.5mg/l)	1.6 ± 0.7174	1.8 ± 0.8071
3.	MS + BAP(7mg/l)+IAA(1.5mg/l)	1.8 ± 0.8071	2.0 ± 0.8968
4.	MS + BAP(8mg/l)+IAA(1.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587

Table 3: Effect of different concentration of BAP and NAA on multiple shoot generation in *Cajanus cajan*

Sr. No.	Concentration of hormones (mg/l)	Multiple shoot induction observed after 25 days.	Multiple shoot induction observed after 50 days.
Control	MS + without Growth Regulator	No Response	No Response
1.	MS + BAP(3mg/l)+NAA(0.5mg/l)	0.6 ± 0.2690	0.8 ± 0.3587
2.	MS + BAP(5mg/l)+NAA(0.5mg/l)	1.2 ± 0.5351	No Response
3.	MS + BAP(7mg/l)+NAA(0.5mg/l)	1.6 ± 0.7174	1.8 ± 0.8071
4.	MS + BAP(8mg/l)+NAA(0.5mg/l)	Swelling of seed	Swelling of seed

Fig. 1. Inoculation of explants (mature seed) on the MS media under aseptic condition

Fig. 2. The best result of multiple shoots obtained at the media concentration of (7mg/l BAP) from the treatments given in table 1

Fig. 3. The best result of multiple shoots obtained at media concentration of (7mg/l BAP ± 1.5mg/l IAA) from the treatments given in table 2.

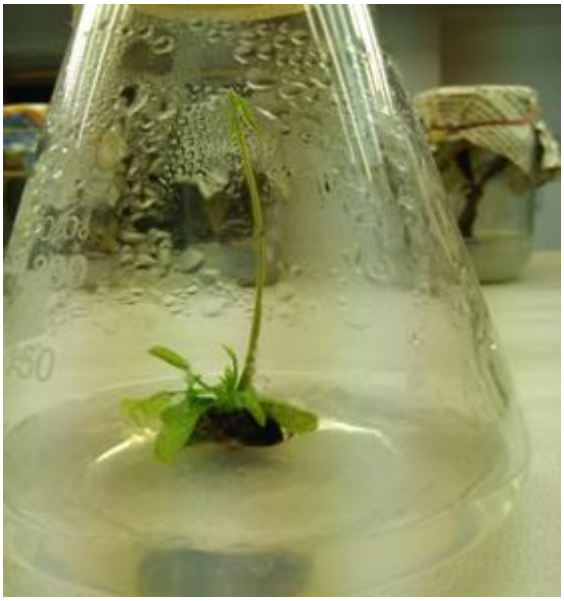

Fig. 4. The best result of multiple shoots obtained at the media concentration of (7mg/l BAP + 0.5mg/l NAA) from the treatments given in table 3.

As given in table 1 mature seeds of pigeon pea were inoculated on MS media supplemented with 4 different concentrations of BAP (3mg/l, 5mg/l, 7mg/l and 8mg/l) from which the best results of multiple shoot (7-8) regeneration were obtained on media supplemented with 7mg/l BAP which comes out as the best standardized media for multiple shoot regeneration.

We obtained our standardized media (7mg/l BAP) by inoculating our explants on three different media with three different hormonal combinations. First is only BAP without any other hormonal combination (as given in table 1), second is BAP + IAA (1.5mg/l) (as given in table 2) and lastly BAP + NAA (0.5mg/l) (as given in table 3). All the results obtained in above three tables were compared with the explants which inoculated on control media (MS + without any hormonal combination). From these all three different hormonal combinations we tried in our experiment the best results of multiple shoots were obtained on the MS media which is supplemented with only BAP.

CONCLUSION

From our present study, the combination of MS + BAP (7mg/l) showed higher multiple shoots formation (S.D. 3.2 ± 1.4349), observed after 50 days. We try our level best to form the plant regeneration protocol for *Cajanus cajan* which act as a pre-requisite for the exploitation of various biotechnological techniques. A remarkable progress can be made in *Cajanus cajan* improvement through combination of conventional and biotechnological approaches. This protocol can be of help in aspect of other biotechnological experiments.

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

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RESEARCH ARTICLE

Studies on diversity and activity of *PSB* isolated from citrus field soil

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ABSTRACT

Phosphorous plays an important role in plant in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate. Vidharbha is called as California of India for citrus as citrus is the main crop of this region of Maharashtra. The present study will focus on diversity and activity of PSB in citrus field as compare to cash crops. A total of 60 soil sample were collected from various villages of Katol tehsil (Vidharbha). These samples were subjected to isolation and identification of PSB. In present study total of 30 isolates each, was isolate obtained from normal crop and citrus field. The results showed that out of 30 isolates from various crop fields, 2 isolates (PSBV 14 and 22) showed highest phosphate solubilizing (28 mm). Whereas out of 30 strains from citrus field, 1 isolates (PSBC 16) showed highest zone of solubilization up to 25 mm. besides this strains PSBC22, 24 and 25 given 24 mm zone of phosphate solubilization. It was found that 17 isolated strains were given zone of Phosphate Solubilization more than 20mm, as compare to citrus field, in citrus field only 11 isolates given zone of Phosphate Solubilization in this range. Bacillus spp., Pseudomonas spp, and Enterobacter spp, were identified from the efficient phosphate solubilizers in both fields. This study concluded that the phosphate solubilization activity was higher in normal Crop field as compare to citrus field. The most efficient Strains of PSB (PSBV14), (PSBV22), (PSBV12) isolated from Normal Crop field. In Normal Crop field the crops rotation taken place which may leads the Phosphate Solubilization of Bound Phosphate.

Key words : Phosphate Solubilization, Citrus field, Diversity, PSB)

INTRODUCTION

Phosphorous is essential for growth and productivity of plant. It plays an important role in plant in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate. Phosphorous deficiency result in the leaves turning brown accompanied by small leaves, weak stem and slow development. In most soil phosphors is available primarily as certain

cation precipitate or poorly soluble organic compound or is bound to particle. The main inorganic phosphate in the soil is iron, aluminum and calcium phosphates (Baby , 2001).

Phosphorous is an essential nutrient for plant, but is often not available due to it fixation in soil Phosphate Solubilizing Bacteria (PSB) solubilized insoluble phosphate and make its available to the plant (Bhattacharya and Jain, 2000). Indian soil on an average contain 0.05% phosphorous that Constituent 0.2% of plant dry weight. Even applied phosphorous combine with metal ion PSB are required for its release (Bagyaraj and Varma, 1995).

Iron and aluminum phosphates are the major phosphate compounds in acidic soil. Whereas calcium phosphate in neutral to alkaline soil, phosphorus is the second vital nutrient next to nitrogen required for growth of microorganism and plants. But most of phosphors are not available to plants. Only 1-2% phosphors is supplied to above ground parts of the plants. Therefore, to meet out the phosphors demand of plant, exogenous sources of phosphorus is applied to plant as chemical fertilizers.

PSB secrete organic acids and enzyme that act on insoluble phosphate and convert into soluble form thus providing phosphorous to plant. PSB also produce amino acid, vitamin, and growth promoting substances (Bagyaraj and Varma, 1995, Schachtman *et al.,* 1998) which promote plants growth. Increased growth and yield of oats, coffee, tea, banana, mustard, maize, rice, sorghum, barley chickpea, soyabeans, groundnut, sugar beet cabbage,and tomato to the extent of 10-20 have been reported by using of PSB (Ponmungan and Gopy, 2006).

There are several phosphate Solubilizing microorganism (PSM) present soil, for example the species of *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Aspergillus, Penicillium, Fusarium* etc. They can utilize tri-calcium phosphate, apatite, rock phosphate, $FeSo_4$ sole phosphate source present in the medium. The indication of utilization is that they produce clearing zone around each colony.

They secret organic acid such as acetic acid, lactic acid, succinic acid, propionic acid, formic acid etc. Consequently bound form phosphates are solubilized and charge molecule of phosphorus is absorbed by the plant. Therefore, the PSM save 30-50 kg/ha of supper phosphate and increase crop yield up to 200-500 kg/ha. Phosphorus was probably discovered around 1669 by a German Alchemist, H. Brandt in Hamburg. He derived the word phosphorus from its property in a Greek terminology "phos" meaning "light"and" phorus" meaning "bringing". It exist in two allotropic forms yellow (white) and red (brown) froms (Goeland Pathode, 2004).

In small quantity as aluminum andiron phosphate and largely in the form of rock phosphate (Ca_3 (Po_4)2 it is also found in soil as insoluble phosphates, soluble phosphates, organic phosphate and residual phosphates (Goeland Pathode, 2004, Dardarwal, 1992). Assimilation of phosphate from organic compound by plants and microorganism take place through the enzyme "phosphate" which is present in wide variety of soil microorganism plant can absorb phosphate only in soluble from. The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil. "A large fraction of soil microbes can dissolve insoluble inorganic phosphate present in the soil and make them available to the plants" (Bhattacharya and Jain, 2000; Richardson, 2001).

The medium used to estimate the population density of phosphate solubilizers show a clear zone around the colonies indicating phosphate solubilization. Phosphorus the "Master key" element in crop production is next only to nitrogen as a major plant nutrient due to chemical fixation of phosphate it remain largely unavailable to growing plant. In alkaline soil, the predominal form of fixed phosphate is tri calcium phosphate carried out by majority of bacteria and fungi (Gaur, 1990).

Bromfield (1959) found dissolution of rock phosphate by fungi mainly by the secretion of organic acids. Yin (1988) observed the solubilization of hydroxyapatite and rock phosphate by a variety of Gram negative and positive bacteria, fungi and Actinomycetes under liquid medium conditions. Gaur (1974) found that TCP and hydroxyapatite were easily Solubilizers than RP by different groups of bacteria normally, TCP is solubilized with an equal case by fungi and bacteria but fungi solubilized RP in more amount than bacteria.

Vidharbha is called as California of India for citrus as citrus is the main crop of this region of Maharashtra in

Vidharbha around 100,000 hectare of land is under cultivation of citrus that is lemon, orange, sweet lemon. In our country 48% of citrus is produce in Vidharbha such a large area under cultivation lion's share of around 50% in country productivity. Citrus of Vidharbha attract academic interest and motivate to research.

The present study was focused on the assessment of PSB diversity and its Solubilizing activity in citrus field as compare to cash crops. This study will help in improving phosphate intake of Citrus crop which will lead to increase productivity, The Study also aimed to assess the efficiency of Phosphate Solubilization of isolates in various normal crop field and citrus field.

MATERIALS AND METHODS

Isolation of Phosphate Solubilizing Bacteria (PSB)

Collection of soil sample

Soil sample were collected from different villages of Katol, tehsil for the isolation of phosphate Solubilizing Bacteria the samples were air dried under shade and used for isolation and identification of organisms. A total of 60 soil sample were collected from various villages of Katol tehsil. These samples were used for isolation and identification of PSB. The samples were processed to analyze within same day. The samples were collected by using standard procedure. Mix the sample and fill the sample bag make sure that all the cores are thoroughly mixed together. Soil samples were filled plastic bag about 1/2 full (approximately 1 cup) with the mixed sample.

Preparation of soil suspension

1 gm of air dried soil sample was taken in l00 ml sterilized distilled water and shaken for 5 minutes stand the tube for settling the soil and upper supernatant was discarded these procedure was followed by 4 times lastly 4 th tubes supernatant was collect out for inoculation of the plate

Sterilization of Medium and Inoculation

Media, Petri dishes, were kept in an autoclave for sterilization at 121 ^{0}c for 20 min at 15 lb pressure after sterilization, plating of these media and inoculation the soil suspension by point inoculation and incubate at 37^{0}c or 2 days. At the end of incubation, PSB colonies were identified by the formation of clear zone of phosphate Solubilization around the bacterial colony (De, freitas *et al,* 1997).

Identification

The organisms were identified on the basis of standard procedure including gram staining and IMViC test (Indole, MR, VP, Citrate test) for identification of Phosphate Solubilization bacteria selective medium were used.

Analysis of Phosphate Solubilizing Activity

The phosphate Solubilizing activities of isolated strains were observed by zone of phosphate solubilization on Pikovskaya's agar medium.

RESULTS AND DISCUSSION

In present study a total of 60 isolates were isolated from general crop and citrus crop field. The results showed that out of 30 isolates from various crop fields, 2 isolates (PSBV 14 and 22) showed highest phosphate solubilizing (28 mm). Whereas out of 30 strains from citrus field, 1 isolates (PSBC 16) showed highest zone of solubilization up to 25 mm. besides this strains PSBC22, 24 and 25 given 24 mm zone of phosphate solubilization. It was found that 17 isolated strains were given zone of Phosphate Solubilization more than 20mm, as compare to citrus field, in citrus field only 11 isolates given zone of Phosphate Solubilization in this range.

In 2005 Chen *et al* studied on phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities are they reported that, the ability of a few soil microorganism to convert insoluble forms of phosphorus to an accessible form is an important trait in a plant growth Promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants. In this study, isolation, screening and characterization of 36 strains of phosphate Solubilizing bacteria from central Taiwan were carried out.

In various crop fields, 18 isolates (PSBV) were showed highest phosphate Solubilization activity whereas 12 isolaets solubilized the phosphate moderately (Fig. 1).

In 2006 Ponmurugan and Gopi studied the distribution pattern and screening of phosphate solubilizing

Bacteria isolated from different food and forage crops are they found that the distribution pattern and population density of phosphate solubilizing bacteria (PSB)was assessed in cultivated soils. PSB isolates were assessed for phosphate Solubilizing capacity, production of growth regulators, phosphatase activity, PH changes and titrable acidity. The population levels of PSB were highest in the rhizosphere soil of groundnut and lowest in the rhizosphere of Ragi, Sorghum and Maize.

It could be observed from the data that the distribution pattern of PSB in the Rhizosphere soils showed that the Population levels decreased with the distance of soil sampling from the plants. A wide variation the capacity of solubilize phosphorous by the PSB isolates was observed. Further, all the isolates were able to secrete phytohormones like gibberelic acid (GA3) and Indole acetic acid (IAA) and acid phosphatase under in vitro condition.

In present study from both field the common phosphate solubilizers *Bacillus spp*. *Pseudomonas, Enterobacter, Erwinia* spp. Were isolated. It was found that the *Bacillus* spp. given highest zone of solubilization whereas *Pseudomonas, Enterobacter, Erwinia* show low phosphate solubilization activity in both the field.

Vazquez *et al.* (1999) Studied on phosphate-Solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon and they found that the Phosphate-Solubilizing potential of the rhizosphere microbial community in mangroves was demonstrated when culture media supplemented with insoluble, tri-basic calcium phosphate and incubated with roots of black (*Avicennia germinans* L.) and White mangrove became transparent after a few days of incubation. He was isolated phosphate-Solubilizing bacterial strains from the Rhizosphere of both species of mangroves. *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus atrophaeus, Paenibacillus maceran,, Pseudomonas stutzeri.*

In present study total of 30 isolate were isolated from citrus field. The results analysis were showed that isolates PSBC 16 isolate showed highest phosphate solubilization zone i.e. 25mm. where isolate no. PSBC3, PSBC5, PSBC9 and PSBC 26, showed low phosphate solubilization zone (Fig. 2).

When the Phosphate solubilization activity of Normal crop fields isolates were analyze and compared with citrus field isolates. It was found that 17 isolates gives zone of Phosphate Solubilization more than 20mm, 28 isolates give zone of Phosphate Solubilization more than 10mm and 3 isolates gives zone of Phosphate Solubilization more than 25mm.

As compare to citrus field, 11 isolates give zone of Phosphate Solubilization more than 20mm, and 25 isolates give zone of Phosphate Solubilization more than 10mm and only one isolate give zone of Phosphate Solubilization more than 25mm (Fig. 1 & 2).

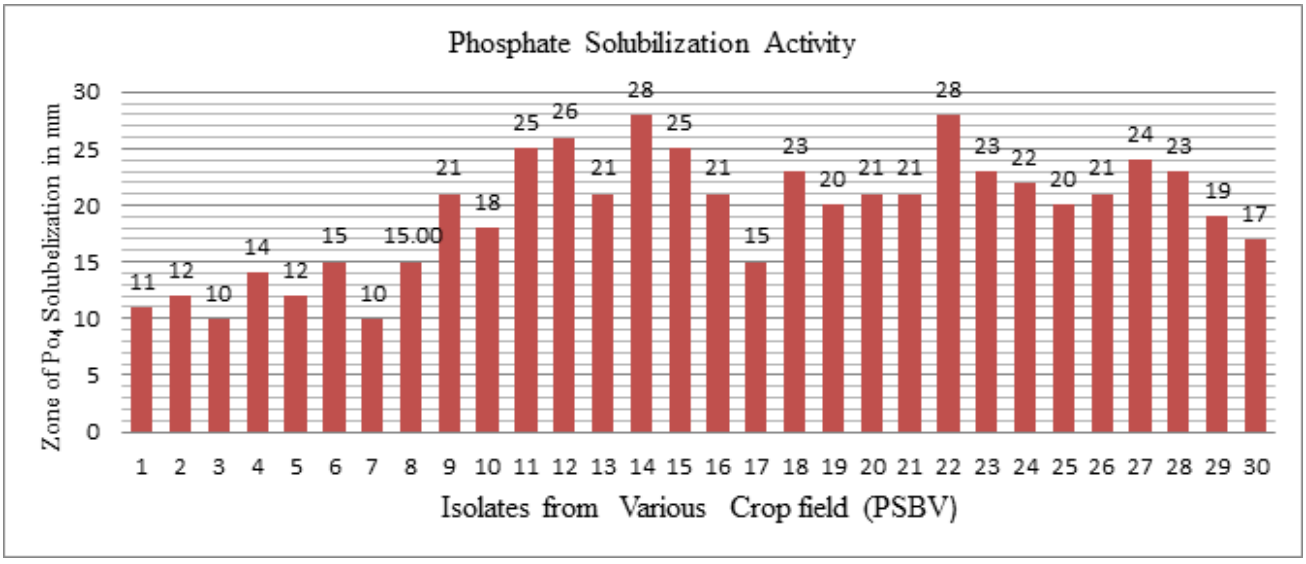

Fig . 1: Phosphate Solubilization Activity of normal crops fields

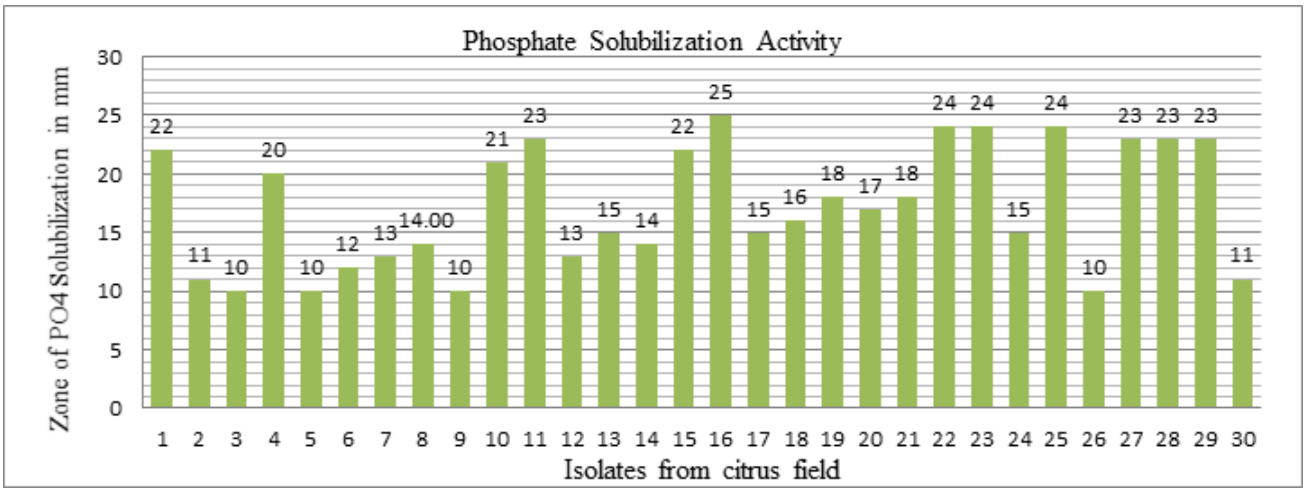

Fig . 2 Phosphate Solubilization Activity of citrus fields

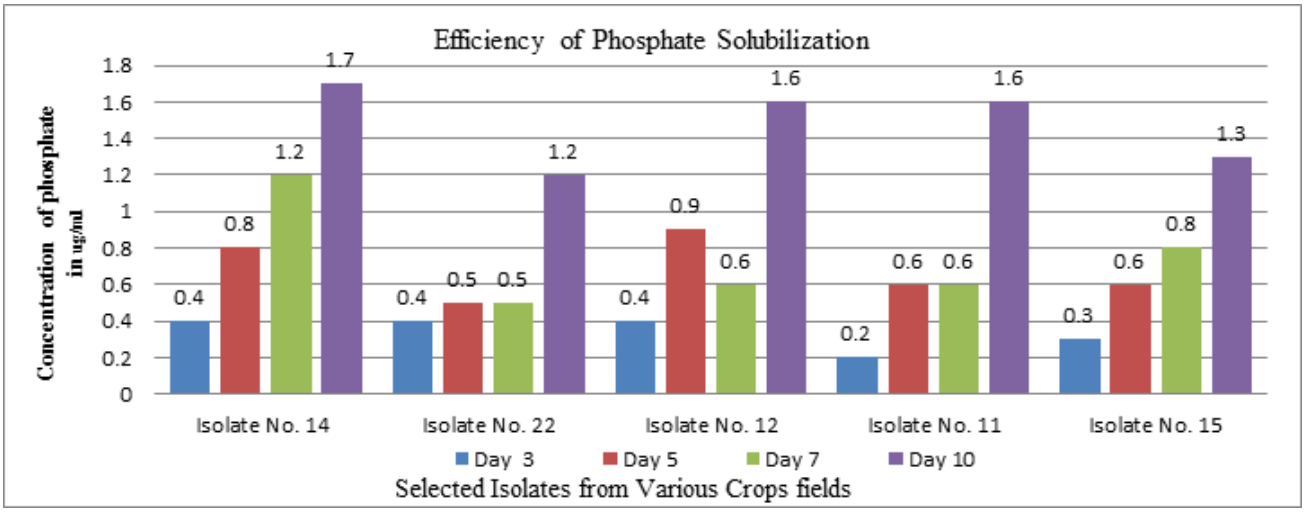

Fig No:-3:- Efficiency of Phosphate solubilization by isolated strains. PSBC 11 showed the solubilization of phosphate day 3, day 5, day 7, and day 10 are 0.4 µg/ml ,0.5 µg/ml , 0.7 µg/ml and 1.5 µg/ml. PSBC 23 showed the solubilization of phosphate in day 3, day5, day 7, and day 10 are 0.2µg/ml, 0.4µg/ml, 0.8µg/ml 1.5 µg/ml. respectively (Fig 3).

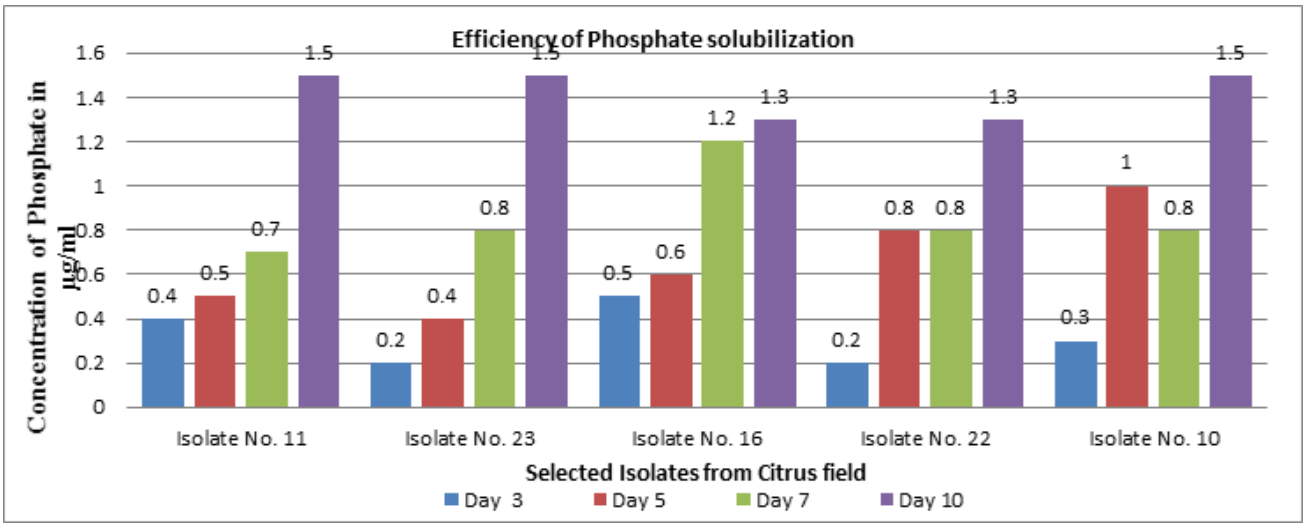

Fig No. 4 Phosphate Solubilizing Efficiency of Isolates from Citrus Field.

The efficiency of Phosphate solubilization was checked by using colorimetric method. The results and data obtained from various crop field (PSBV) and Citrus Crop filed (PSBC) were compared.

The Results showed that out of 30 isolate, only five isolates (PSBV) were subjected to estimate solubilization of phosphate and found the efficiency of phosphate solubilization at within 3,5,7,and10 days (Fig. 3). PSBV 14, PSBV 22, PSBV 12, PSBV 11, PSBV 15 showed highest solubilization at the end of 10 dayPSBV14 showed amount of phosphate in day 3, day5 day 7and day 10 are 0.4µg/ml, 0.8µg/ml, 1.2µg/ml,1.7ug/ml respectively. PSBV 22 showed the solubilization of phosphate within day 3, day 5, day7, and day 10 are 0.4µg/ml, 0.5µg/ml 0.5µg/ml, and 1.2µg/ml respectively. PSBV 12 showed solubilization of Phosphate in 3, day 5, day7, day10, was 0.4ug/ml, 0.9µg/ml, 0.6µg/ml, 1.6µg/ml respectively.

When isolates from citrus field (PSBC) were subjected for analyzing the colorimetric efficiency it was found that out of 30, PSBC 11, PSBC 23, PSBC16, PSBC 2 and PSBC10were showed highest zone of phosphate solubilization.

PSBC16 showed the solubilization of phosphate in day 3, day 5, day 7 and day 10 are 0.5 µg/ml 0.6µg/ml 1.2 µg/ml 1.3 µg/ml respectively.

PSBC 22 showed the solubilization of Phosphate in day 3, day 5, day7. Day10, are 0.2µg/ml,0.8 µg/ml, 0.8µg/ml, 1.3 µg/ml (Fig. 4).

Rudresh *et al* 2004 was found that, Improvement of Phosphate Solubilizers an alternative approach for the use of phosphate solubilizing bacteria as microbial inoculation is the use of mixed inoculation of PGPR strains comprising phosphate solubilizing Bacteria. The effect of a combined inoculation of Rhizobium, a phosphate solubilizing *Bacillus megaterium sub sps. phosphaticum strains-PB* and a biocontrol fungus, *Trichoderma sps*. On growth, nutrient uptake and yield of chickpea were studied under glass house and field condition combined inoculation of these three organism showed increased germination, nutrient uptake plant height, number of branches, nodulation, pea yield and total biomans of chickpea compared to either individual inoculation or an uninoculated control .

Balamurugan *et al* in 2010, carried out the study on isolation characterization are they found that phosphate solubilizing bacteria of tea garden soil were isolated, screened in vitro and studied its bio ecology. Among the 25 isolates, the five strains were shown higher phosphates enzymes activity compared to other strains. (PSB 12,PSB 25 and PSB 37) and among these three strains PSB 37 was found to be superior in forming halo zone of phosphate solubilization followed by PSB 25. All strains brought down the pH of culture medium. The biochemical characterization of the isolates PSB7, PSB12, PSB20, PSB22, PSB25, PSB26, PSB37 were closer to Pseudomonas species. The selected PSB strains preferred temperature at 28^0c to 35^0c for its better growth.

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RESEARCH ARTICLE

Screening of Antifungal Activity of Endophytic Fungi From *Dioscorea bulbifera*

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ABSTRACT

Antifungal potential of endophytic fungi was studied in the present investigation. Secondary metabolites were produce by endophytic fungi isolated from *Dioscorea bulbifera* to investigate their antifungal activity against two pathogenic fungi, one as a human pathogenic (*Candida albicans* MTCC 7315) and one as a plant pathogenic (*Colletotrichum acutatum* MTCC 2213) taken from IMTECH, Chandigarh, India.

Key words; Secondary metabolites, Antifungal Potential.

INTRODUCTION

Metabolites produced by endophytes are being recognized as versatile arsenal of antimicrobial agents. Some endophytic fungi have been known to have capabilities to produce a bioactive compounds, owing to their presumable gene recombination with their host while living and reproducing within the host tissues (Li *et al*., 2005). 80% endophytic fungi produce biologically active compounds with antibacterial, antifungal, antioxidant, anticancerous and herbicidal properties (Schulz *et al*., 2002). The development of new antimicrobial metabolites is important to prevail the problems related to the treatment of diseases caused by resistant pathogens (Petersen *et al*., 2004). Thus, endophytic fungi have emerged as an alternative source to synthesize new antimicrobial compounds.

Due to the ability of production of important secondary metabolites by endophytic fungi, the study of these fungi from selected medicinal plants provide greater understanding of its diversity and potentials to synthesize bioactive metabolites. In India from long period medicinal plants have been used for the treatment of different diseases and provides specific atmosphere to endophytic fungi. Many endophytic fungi with novel and natural bioactive secondary metabolites are reported previously from medicinal plants (Strobel *et al*., 2004). In view of these earlier observations, the present study was carried out to investigate the bioactive potential of endophytic fungi.

MATERIALS AND METHODS

Dioscorea bulbifera was collected from Pohara Ferest of Amravati district and brought to laboratory in sterile bags and stored at 4°C till further use. Collected samples were rinsed gently in running water to remove adhered dust and debris. Surface sterilization were done according to the method described by (Suryanarayanan *et al,* 2011). The sterilized samples were inoculated on potato dextrose agar (PDA) to isolate the endophytes.

Solvent Extraction for Isolation of Secondary Metabolites

Liquid-liquid extraction procedure was adopted to extract the spent broth of endophytic fungal isolates. The aqueous layer was extracted using solvent ethyl acetate. This art was repeated thrice. Solvent layer and residue was separated with the help of separating funnel. Then organic layer containing compounds of interest was dehydrated with anhydrous sodium sulphate. The organic layer was then collected in a pre-weighed crucible. After incubating, the solvent was removed and stock solutions of extracts were preserved in DMSO and stored at 4ºC till use. Antifungal activity was tested by disc diffusion method.

Disc Diffusion Assay

The assay was conducted as per the procedure defined by Jorgensen and Turnidge (2007). The crude extracts were dissolved dimethyl sulfoxide (DMSO). The test organisms with the inoculum size of 10^5 colony-forming units (CFU)/ mL were streaked on the surface of the media Muller-Hinton agar (Hi-media) using sterile cotton swab.Sterile Whatman paper disc impregnated with 20 µl of each extracts. DMSO was applied as a negative control to detect the solvent effects. The plates were incubated at 28°C for 48 hrs. The diameter of the clear zones surrounding the disc were measured.

RESULTS AND DISCUSSION

Several endophytic fungi have been isolated from a variety of plant which have proved as a rich source of biologically active metabolites. (Devi *et al.*, 2013).

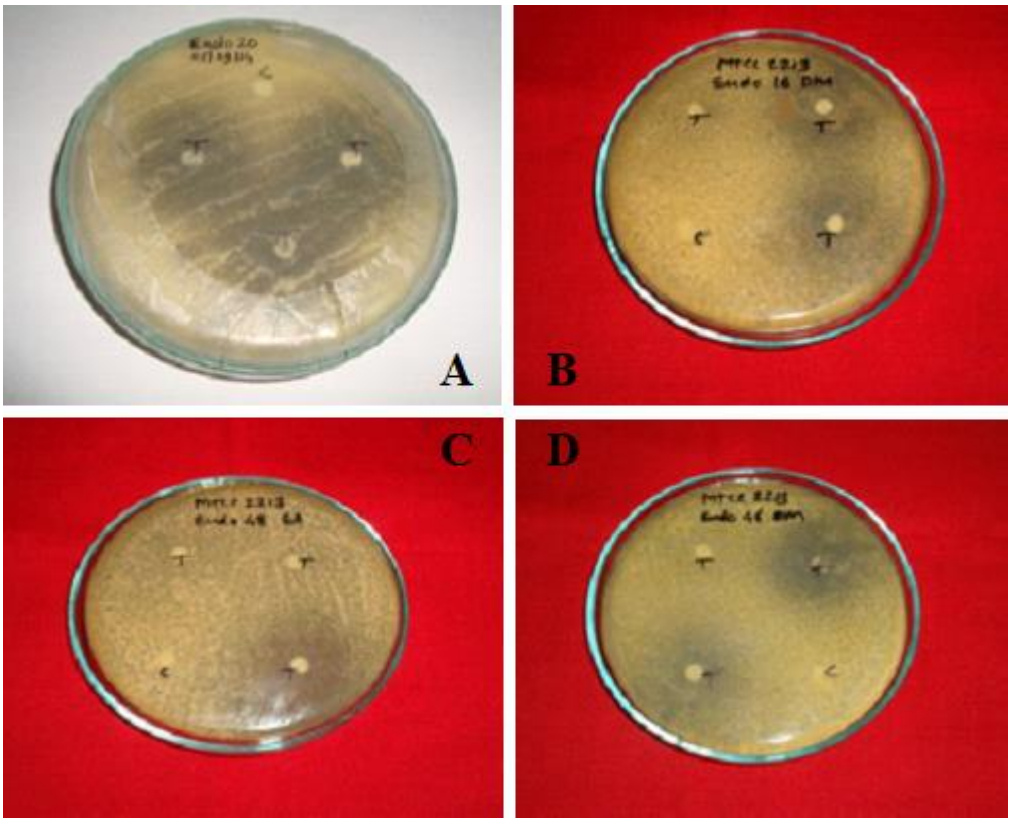

Fig. 1Antifungal activity of Endophytes against *Colletotrichum acutatum.*
A. *Aspergillus stellatus* **B.** *Epicoccum nigrum* **C.** *Penicillium chrysogenum* **D**. *Stachybotrys nilgirica*

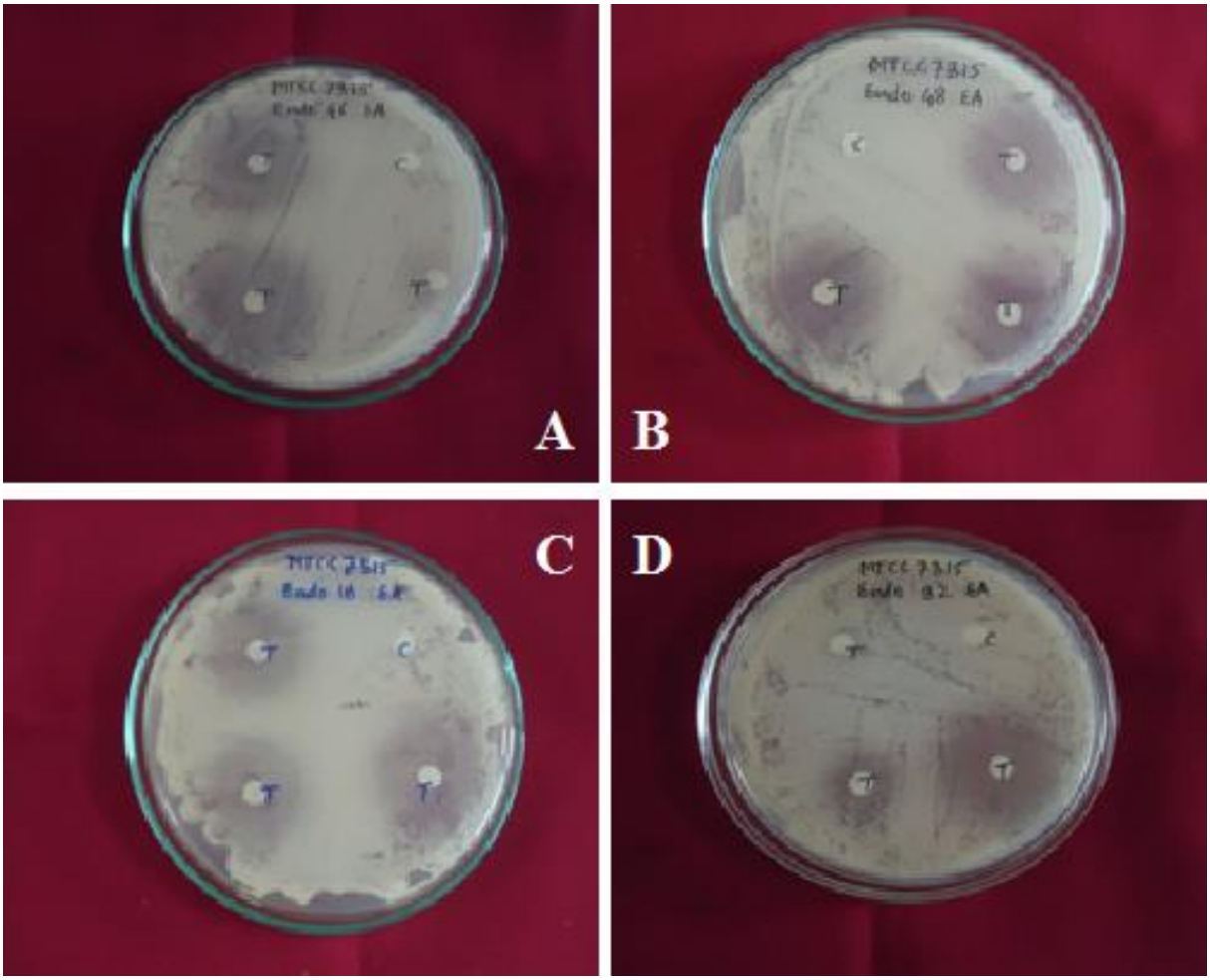

Fig. 2: Antifungal activity of Endophytes against *Candida albicans*.
A. *Stachybotrys nilgirica* **B.** *Penicillium chrysogenum* **C.** *Epicoccum nigrum* **D.** *Aspergillus stellatus*

Medicinal plants have been considered potential source of endophytes synthesizing associated plant natural products (Strobel and Daisy, 2003).

In this study total nine endophytic fungi were isolated from the *Dioscorea bulbifera* namely *Arthrinium phaeospermum, Aspergillus stellatus, Curvularia lunata, Epicoccum nigrum, Nigrospora oryzae, Penicillium chrysogenum, Pithomyces chartarum, Phoma crysanthemicola* and *Stachybotrys nilgirica.* Crude extracts of all the isolated endophytes were extracted. Ethyl acetate crude extracts produced by all the endophytic isolates were screened for their antifungal action and most of the isolates revealed great inhibitory activity against tested pathogen. Among all tested extracts ethyl acetate crude extracts produced by *Aspergillus stellatus*, *Epicoccum nigrum, Penicillium chrysogenum, Stachybotrys nilgirica* exhibited promising results for growth inhibition of pathogenic fungi) (*Colletotrichum acutatum* MTCC 2213) (Fig.1) and (*Candida albicans* MTCC 7315) (Fig.5 to 8).

CONCLUSION

The results of this study demonstrate the great antifungal potential of endophytic fungi isolated from *Dioscorea bulbifera* agaist pathogenic fungi. Therefore, it suggest that these endophytes can be important sources of bioactive substances which may useful for new drug discovery.

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

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RESEARCH ARTICLE

Phytochemical screening of selected medicinal plants of the family Lamiaceae

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ABSTRACT

The members of Lamiaceae family include aromatic plants that are being used in traditional medicine for various disorders. To study the secondary metabolites present in the leaves of the family Lamiaceae (*Ocimum sanctum*, *Leonotis nepetifolia* (L.)*, Mentha arvensis* L.). The samples were extracted using solvents like acetone, chloroform, ethanol, petroleum ether and water. These mixtures were shaken at room temperature for 24 h. After incubation, the extracts were filtered using Whatman No.1 filter paper, collected and stored at 4°C. Preliminary phytochemical screening was performed by standard methods. The phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, phytosterols, proteins, steroids, terpenoids, phenols, saponins, quinones, coumarins and glycosides. The result reveals the presence of bioactive constituents comprising alkaloids, flavonoids, phenolics, tannins, glycosides, steroids and saponins in different solvents. The presence of these phytochemicals can be correlated with the medicinal potential of this plant

Keywords: Plant material, Acetone extract, methanol extract, water extract phytochemicals

INTRODUCTION

Medicinal plants play a major role in meeting the medical and health needs of about 70% of populations in developed and developing countries, which serve as an important resource for the treatment of various maladies and illnesses (Ngari et al., 2010). Globally, about 85% of the traditional medicines used by different ethnic groups inhabiting various terrains for primary healthcare are derived from plants, especially in India; medicinal plants are widely used by all sections of the population with an estimated 7500 species of plants used by several ethnic communities (Farnsworth, 1988). The plant is being used by the local peoples and tribal of Maharashtra as ethno medicine on various ailments. This plant is also being used for its anti-inflammatory, anti-diarrheal properties by various communities in Indian subcontinent and also across the world. The present study was designed to evaluate the fundamental phytochemical constituents of this wild medicinal plant.

They are known to have various biological activities such as antimicrobial, antifungal, antioxidant, etc. The important bioactive components in plants are usually the secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds (Edeoga et al., 2005). The Medicinal plants have potent phytochemical components which are important source of antibiotic compounds and are responsible for the therapeutic properties (Jeeva et al., 2011; Jeeva and Johnson, 2012; Florence et al., 2012 & 2014; Joselin et al., 2012 & 2013; Sainkhediya and Ray, 2012; Sumathi and Uthayakumari, 2014). Therefore, the present work aims at evaluating the phytochemical composition, by qualitative and quantitative methods, of methanol, ethanol and chloroform extracts of three other members of the Lamiaceae family, namely, *Ocimum sanctum*, *Leonotis nepetifolia, Mentha arvensis* L. are known to be of medicinal use. The use of *Ocimum sanctum*, *Leonotis nepetifolia, Mentha arvensis* L., \in traditional medicine is represented in table 1.

MATERIALS AND METHODS

The plant material was collected from agriculture waste-land of Dr. PDKV agriculture campus, Akola. The plants were identified and authenticated by a taxonomist.

Preparation of crude extracts

Fresh leaves were collected, washed with distilled water, shade dried till it is crisp (approximately 15 days) and cut into small pieces. These dried samples were powdered and stored at 4^{o} C until further use. Crude extracts (10% w/v) were made using 3 solvents i.e., methanol, ethanol and chloroform. The extracts were filtered through fine muslin cloth and the clear filtrate was evaporated to dryness to form the crude extract and stored at 4^{o} C for further use.

Phytochemical Screening: The chemical tests were carried out with the crude extracts of each plant i.e., methanol extract (ME), Ethanol extract EE and Chloroform extract CE.

Tests for Tannins: About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl^3$ Solution were added. Formation of green precipitate was indication of presence of tannins.

Tests for Saponins: 5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for phlobatannins: About 2 ml of aqueous extract was added to 2 ml of 1% HCL and the mixture was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.

Tests for Flavonoids: To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for terpenoids: 2ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

Test for glycosides: Liebermann's test:
2ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully, a colour change from violet to blue green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

Table 1: Medicinal uses of the plants in the study

Plant species	Common name in English	Traditional uses
Ocimum sanctum	Basil	Cough cold, chronic fever, sore throat, bronchial asthma, malaria, bronchitis, skin diseases, arthritis, diarrhea, dysentery.
Leonotis nepetifolia (L.)	Lion's ear	Bronchial asthma, diarrhoea, fever, influenza and malaria and is also an analgesic
Mentha arvensis L	Mint	Digestive Ailments Acne, Bronchitis, Burns, Colds, Liver Problems, Headaches, Toothache

Table 2: Phytochemical constitute of the leaf extract:

Phyto-constituents	*Ocimum sanctum*			*Leonotis nepetifolia*			*Mentha arvensis* L		
	EE	ME	CE	EE	ME	CE	EE	ME	CE
Flavonoids	+	+	+	_	+	+	+	+	+
Tannin	_	_	+	_	_	_	+	+	+
Steroids	+	+	+	+	_	_	+	+	+
Terpenoids	+	+	+	_	+	+	+	+	_
Saponins	+	+	_	_	_	+	+	_	+
Glycosides	+	+	+	_	+	+	+	_	+
Phlobatannins	+	+	+	+	+	_	+	_	+

EE: Ethanol extract; **ME:** methanol extract; **CE:** Chloroform extract;
'**+**': presence of phytochemical; '**-**': absence of phytochemical

Test for steroids: 1. A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids. 2. Development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

RESULTS AND DISCUSSION

Fresh plant leaves of were collected, the leaves were washed thoroughly with normal tap water followed by sterile distil water. Then leaves were dried under shaded condition at room temperature. Leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder was stored at 4°c in light air container bottle for further analysis. The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities (Mukeshwar et al., 2011). The phytochemical characteristics of the leaf extract were investigated are summarized in table-2. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds has also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

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

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RESEARCH ARTICLE

Preliminary phytochemical screening and antibacterial activity of *Parthenocissus quinquefolia* (*L.*) Planch

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ABSTRACT

The screening and study of selected Indian medicinal plant *Parthenocissus quiquefolia (*L) Planch, were selected for phytochemical screening and antibacterial studies. The solvents used for the extraction of plant roots were ethanol, benzene, chloroform, acetone, petroleum ether and distilled water. The Gram-Postive and Gram-negative bacteria *Yeast candida, Aspergillus niger, Staphylococcus aureus, Eschericha coli, Salmonella typhi, Bacillus subtilis, Pseudomonas fluorescence, Klebsiella pneumonia* and *Streptococus pyogenes* were tested. The results obtained in the present study suggest that preliminary phytochemical analysis detected the presence of Alkaloids, Flavonoids, Terpenoids, Steroids, Cumarins, Carbohydrates and Tanins. The *Parthenocissus quiquefolia* (*L.*) Planch. Could be used in treating diseases caused by the test organisms.

Keywords: Phytochemicals, *Parthenocissus quiquefolia (L)* Planch. , Antibacterial activity and Pathogens.

INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases.A large number of the country's rural population depends on medicinal plants for treating various illnesses. These plants played a significant role in various ancient traditional system of medication in India. Phytochemical, Antibacterial Screening and Spectroscopic Analysis of the Crude Samples of Stem Bark Extract of *Lonchocarpus cyanescens* (*Nwokonkwo et al*., 2017).

Preliminary phytochemical and Fourier Transform Infrared Spectral analysis and Antimicrobial Studies of solvents extracts of *Urginea indica* (*Roxb*.) *Kunth*(Liliaceae) and *Cyclea peltata Arn. ex Wight* (Menispermaceae), results were clearly revealed that the plant contained different bioactive compounds such as of Alkaloids, Anthoquinones, Coumarins, Steriods and Flavonoids compounds were rich in the extracts of *Urginea indica* (Liliaceae) and *Cyclea peltata*(Menispermaceae) are connected with defense mechanism against many microorganisms (*Patil*

et al., 2015). Plants are a source of large amount of drugs comprising to different groups such asantispasmodics, emetics,anti-cancer, antimicrobials etc (*Tiwari et al*., 2011). Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of Methanolic Extract of *Barleria cristata Linn*. Roots in Experimental Animals (*Banu et al*., 2017).

Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Kirby-Bauer method was followed for disc diffusion assay (*Shihabudeen et al*., 2010). Preliminary studies on phytochemicals and antimicrobial activity of solvent Extracts of *Eichhornia crassipes (Mart*.) Solms. They had study the fresh plant contain alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein (*Thamaraiselvi et al*., 2012) . Studies on the phytochemistry, spectroscopic characterization and antibacterial efficacy of *Salicornia brachiata* (*Krishnan et al*., 2014). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of *Dennetia tripetala* G. Baker (*Solomon et al*., 2013).

Seed ethanolic extract showed high content of phytochemicals, highest antimicrobial and antioxidant activity and results supported the usage of Vernonia anthelmintica in folk and traditional medicine (*Santosh et al*., 2013) Phytochemical screening and anti-microbial activity of medicinal plant Pergularia daemia FromChandrapur Forest Region (*Jogi et al*., 2012). Phytochemical screening, functional groups and element analysis of Tylophora Pauciflorawight and Arn. They had concluded that traditional use of *Tylophora pauciflora* for human ailments and partly explained its use in herbal medicine as rich sourch of phytochemicals with the precence of tanins , phenol , saponins , steroids , flavoinoids , and terpenoid (*Sarlin et al*., 2012). Preliminary phytochemical screening of different solvent extracts of stem Bark and roots of *Dennetia tripetala* G. Baker (*Ugochukwu et al*., 2013). The most essential of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food (*Amin et al*., 2013). Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approach (*Saha et al*., 2010).

MATERIALS AND METHODS

Plant collection

The following medicinal plants were selected and collected for the study from the local area of Etawa forest of Betul district. The Medicinal Plants *Partheno-cissus quiquefolia (L)* Planch. was collected from follow land in and around Etawa forest brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study.

Sterilization of Plant Materials

The disease free roots were selected for this investi-gation. About 2gm dried roots were taken. Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the materials were washed thoroughly with distilled water.

Preparation of Plant Extracts

The organic solvent extract was prepared by adding 5 gm powder of ethno veterinary medicinal plants in 250 ml of organic solvent for 6 hrs. BySoxlhet method and filtrate was evaporated in controlled conditions of temperature of active constituents of preparations. Dried extracts were stored in labelled sterile wide mouthed screw capped bottle at 40c and used for further study.

Preliminary Phytochemical screening

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions, the methods of *Harbone* (1973), *Trease et al.* (1983) were used to identify the major secondary metabolites like Alkaloids, Flavonoids, Saponins, Carbohydrate, Protein, Phenols, Steroids, Tannins, Glycosides, Terpenoids, Phlobatannins, Coumarins, Emodins, Anthoquinones, Anthocyanins, Leucoanthocyanins in the extracts.

Antimicrobial screening

All solvent extracts were screened *in vitro* growh inhibitory activity against different microbes *E. coli, Pseudomonas fluroscene, Salmonella typhi, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Streptococus* Yeast candida, *Aspergillus niger*. using disc-diffusion method. The bacteria rejuvenated in Nutrient broth (Hi-media – laboratories, Mumbai, India) at 37^0c for 18 hrs. and then stored at 40^0c on Nutrient agar subcultures were prepared from the stock for bioassay.

RESULTS AND DISCUSSION

Phytochemical screening: From the above table no. 1 it is clear that,

Alkaloids: It was found that concentration of alkaloids have been extracted in Ethanol and Acetone extracts. This is evident from positive test with Hager's reagent . Benzene, Chloroform, Petroleum ether and Distil water have shown negative test for Alkaloids.

Glycosides : All extracts have shown negative test for Glycosides with Libermann's reagent.

Phenols: All extracts have shown negative test for Phenols.

Saponins: All extracts have shown positive test for Saponins.

Tannins : All extracts have shown negative test for Tannins with Braymer's reagent.

Flavonoids: It is found that concentration of Flavonoids have been extracted in Ethanol and Acetone extract. This is evident from the positive test. Benzene, Chloroform, Petroleum ether and Distil water have shown negative test for Flavonoids.

Terpenoids: All extracts have shown negative test for Terpenoids.

Steroids: All extracts have shown positive test for Steroids with Salkowski reagent.

Phlobatannins : All extracts have shown negative test for Phlobatannins.

Coumarins: It was found that concentration of Coumarins have been extracted in Ethanol and Acetone extract. This is evident from the positive test. Benzene, Chloroform, Petroleum ether and Distil water extract have shown negative test for Coumarins.

Proteins : All extracts have shown negative test for Proteins with Xanthoproteic reagent.

Table 1:-Phytochemical activity of root extracts of *Parthenocissus quiquefolia*(*L*)Planch.

Plant parts	Test / Reagents Used	Ethanol extract	Benzene extract	Chloroform Extract	Acetone extract	Petroleum Ether	Distil Water extract
		E	B	C	A	P	W
Root	Alkaloids (Hager's Test)	+	-	-	+	-	-
	Glycosides (Libermann's Test)	-	-	-	-	-	-
	Phenols	-	-	-	-	-	-
	Saponins (Foam Test)	+	+	+	+	+	+
	Tannis (Braymer's Test)	-	-	-	-	-	-
	Flavonoids	+	-	-	+	-	-
	Terpenoids	-	-	-	-	-	-
	Steroids (Salkowski Test)	+	+	+	+	+	+
	Phobatannins (Precipitate Test)	-	-	-	-	-	-
	Coumarins	+	-	-	+	-	-
	Proteins (Xanthoproteic Test)	-	-	-	-	-	-
	Emodins	-	-	-	-	-	-
	Carbohydrates (Molisch Test)	-	-	-	-	-	-

Present : **+ve ;** Absent: **- ve**

Table 2 :- Antimicrobial activity of root extracts of *Parthenocissus quiquefolia* by Disc Diffusion Method (Zone of Inhibition in mm at 100 µg/disc)

S. No	Microorganism	Ethanol	Benzene	Chloroform	Acetone	Petroleum ether	Distil water
1	**YC**	0	0	0	0	0	0
2	**AN**	0	0	0	0	0	0
3	**SA**	17	0	0	0	0	0
4	**EC**	18	0	0	16	0	0
5	**ST**	7	0	0	0	0	8
6	**BS**	0	0	0	0	0	0
7	**PF**	0	0	0	0	0	0
8	**KP**	0	0	0	0	0	0
9	**SP**	0	0	0	0	0	0

*Data represented in mean of three replicates.

YC = *Yeast candida*, **AN** = *Aspergillusniger*, **SA** = *Staphylococcus aureus*, **EC** = *Escherichia coli*, **ST** = *Salmonella typhi*, **BS** = *Bacillus subtilis*, **PF** = *Pseudomonas fluorescence*, **KP** = *Klebsiellapneumoniae*, **SP** = *Streptococuspyogenes*

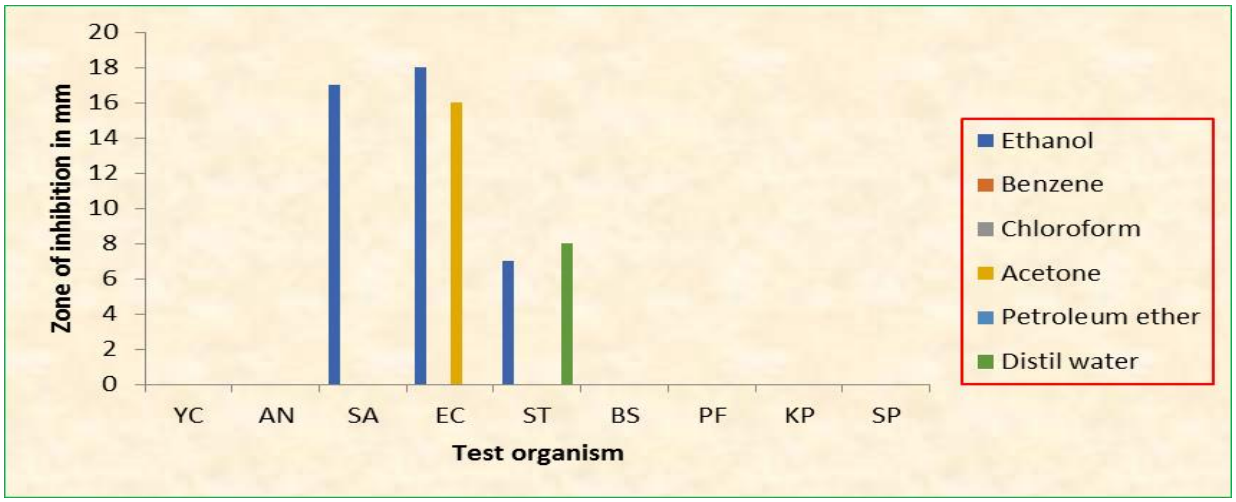
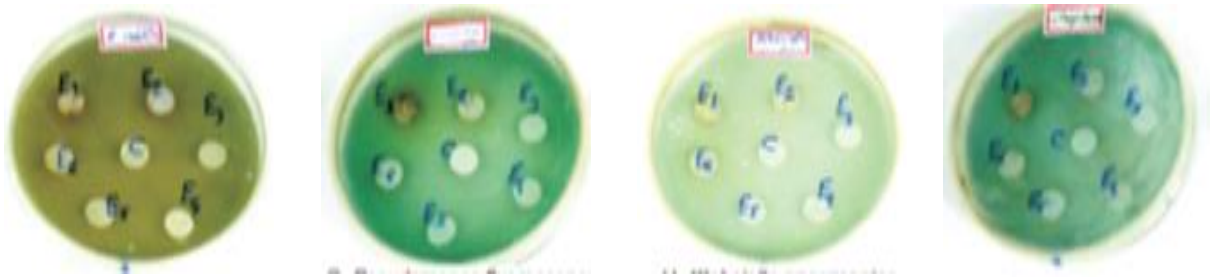


Fig. 1: Analysis of antimicrobial sensitivity of root extracts of *Parthenocissus quiquefolia*(*L*)

Yeast candida, *Aspergillusniger* *Staphylococcus aureus* *Escherichia coli,* *Salmonella typhi*



Bacillus subtilis *Pseudomonas fluorescence* *Klebsiellapneumoniae* *Streptococuspyogenes*

Fig.2 :- Antimicrobial activity of root extracts of *Parthenocissus quiquefolia(L)*Planch.

Emodins : All extracts have shown negative test for Emodins.

Carbohydrates : All extracts have shown negative test for Carbohydrates with Molisch reagent.

Antimicrobial activity

Ethanol extracts showed very promising results against three pathogens like *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.* The maximum zone of inhibition of 18 mm was observed in ethanol extract against pathogen *Escherichia coli*. Ethanol extracts was found nonreactive to other test organisms. Acetone extracts also showed positive results against *Escherichia coli*. The maximum zone of inhibition of 16 mm was found in acetone extracts against pathogen *Escherichia coli.* The acetone extracts were found no reactive to other test organisms. The aqueous extractsalso showedmicrobial zone of inhibition against Salmonella typhi. The maximum zone of inhibition of 8 mm was observed in aqueous extracts. The aqueous extracts was found non reactive to other test organisms.

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

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RESEARCH ARTICLE

Toxicity of Fluoride on Essential Trace Elements of the Rat, *Rattus rattus* (Wister)

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ABSTRACT

Fluorine being most electronegative element holds its ubiquitous presence. Fluorides are naturally occurring harmful contaminant in the environment. The aim of the present study was to analyse fluoride – induced toxicity on trace element such as Zn, Cu, Mn and Fe on kidney and thigh muscles of rat, Rattus rattus (Wister). For the present study experimental sets were arranged, one was of 7 days and another was of 14 days. Both set was arranged as short duration exposure period. Each set were intoxicated with fluoride water for different concentration i.e for 7 and 14 days periods. Present study data reveals that rats exposed with fluoride disturbs the concentration of essential trace elements and imbalances the quantity of all studied trace elements as compared to control rat.

Key words: Fluoride, Rat, Days.

INTRODUCTION

Fluoride is an essential trace element for human beings and animals. In small amounts fluoride is beneficial as it is believed to impart stability to bone and enamel, thereby preventing dental carries and osteoporosis to some extent but its higher concentration is highly toxic to humans and animals alike. The permissible limits of fluoride in drinking water as suggested by Bureau of Indian Standards varies between 0.6 to 1.2 ppm BIS (1984), and World Health organization WHO (WHO 1984). permits a maximum of 1.5 ppm of it. Chronic exposure to fluoride above the permissible limits, causes a disease called “Fluorosis”. Fluorosis is an important clinical and public health problem in several parts of the world. As fluoride is found in small quantities in almost all foods, it enters the human body mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through stomach, small intestine, mouth, lungs and skin.10 (Khandare *et al.,* 2001). High exposure to fluoride may occur from natural or industrial sources and from misuse of fluoride-containing dental care products.(Borke and Whitford ,1999) The most obvious early toxic effects of fluoride on humans are dental and skeletal fluorosis that are endemic in areas with elevated exposure to fluoride. Detrimental effects of high fluoride intake

also affect soft tissues, (Zhavoronkov, 1997: Monsour and Kruger, 1985) including the liver, (Kolodziejczyk *et al.,* 2000) lungs, (Chen *et al.,* 1999) brain, (Shivarajashankara *et al.,* 2002) and kidneys (Borke and Whitford ,1999).

MATERIALS AND METHODS

Chemicals

Sodium fluoride (NaF) were obtained from Chaiga traders.

Experimental Animals

20 Adult albino rats, 60-day-old (weighing 250-300g) were obtained from wadhwani pharmacy Collage Yavatmal. The animals were kept under standard laboratory conditions at 21± 2 °C, fed with balanced diet and water ad-libitum and exposed to 12h light / 12 h dark cycle for one week prior to the start of the experiments. The rats were housed in cleaned and husk filled sterilized polypropylene cages and fed with pellet feed and purified water *ad libitum*. The temperature and humidity were maintained at 23±2ºC and 50 to 70%, respectively. The present study was approved by the Institutional Animal Ethics Committee and conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). 20 albino wister rats were divided into four groups, Control groups given deflouridated, deionized water, while experimental groups 2, group 3, and group 4 administered sodium fluoride (Naf) of different concentration for 72 days. At the end of the experiment, animals were sacrificed and their kidney and thigh muscles, will quickly excised. Metal concentrations in the tissue digest will be determined by Atomic absorption spectrophotometer.

Mineral concentrations:

Tissue samples were blotted to remove extra water, weighed, and wet digested with a 3:1 mixture of 70% nitric acid and 70% perchloric acid by heating below 80ºC.12 The digested samples were cooled, and diluted with triple glass-distilled water to a final volume 5.0 ml. The concentrations of Zn, Cu, Fe, and Mn were measured with an atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Manganese is a cofactor in many enzymatic systems and has roles in bone formation and metabolism of carbohydrates and cholesterol (Santos *et al*., 2013). This enzyme is involved in fatty acid and protein synthesis as well as melanin and dopamine production. (Carbonell *et al.*,2008) After oxidation in its trivalent form, manganese is bound to transmanganin and is successfully deposited in the liver, skin, and skeletal muscle.

Table 1: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Kidney of rats given varied concentration of sodium fluoride in drinking water 7 days.

Parameters	Control	Expt- 1	Expt- 2	Expt- 3
Zinc	1.66±1.29	1.69±1.3	1.70±1.3	1.72±1.31*
Copper	0.184±0.42	0.182±0.42	0.179±0.42*	0.177±0.42
Maganese	1.95±1.39	1.92±1.38	1.91±1.38	1.89±1.37**
Iron	13.22±3.63	13.19±3.63	13.15±3.62*	13.02±3.61**

Table 2: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Thigh muscles of rats given varied concentration of sodium fluoride in drinking water 7 days.

Parameters	Control	Expt- 1	Expt- 2	Expt- 3
Zinc	2.49±1.56	2.46±1.57	2.44±1.56	2.40±1.54**
Copper	0.092±0.30	0.096±0.30	0.097±0.31*	0.102±0.31**
Maganese	1.36±1.16	1.38±1.17	1.39±1.18*	1.40±1.81*
Iron	12.63±3.55	12.56±3.55	12.69±3.56*	12.69±3.56*

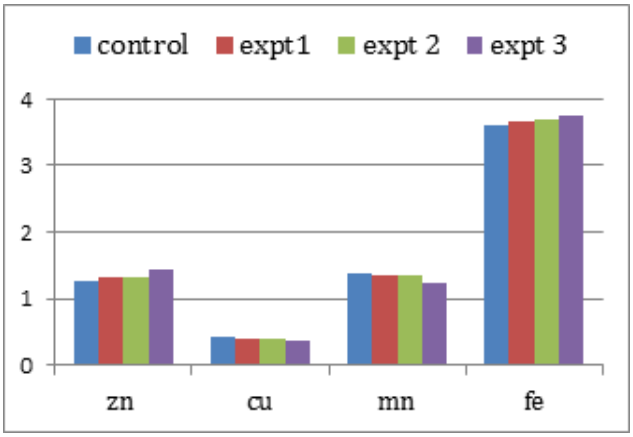

Fig. 1: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Kidney of rats given varied concentration of sodium fluoride in drinking water 7 days.

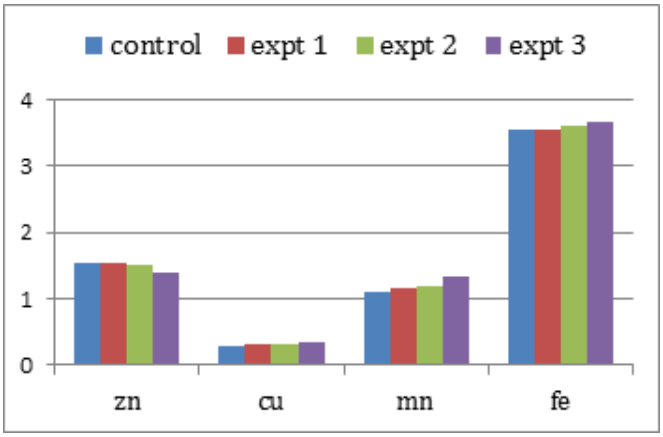

Fig. 2: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Thigh muscles of rats given varied concentration of sodium fluoride in drinking water 7 days.

Table 3: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Kidney of rats given varied concentration of sodium fluoride in drinking water for 14 days.

Parameters	Control	Expt- 1	Expt- 2	Expt- 3
Zinc	1.64±1.28	1.71±1.31	1.71±1.31	1.73±1.31*
Copper	0.183±0.42	0.178±0.42	0.177±0.42*	0.173±0.41**
Maganese	1.92±1.38	1.88±1.37	1.85±1.36	1.80±1.34**
Iron	13.09±3.61	13.04±3.61	13.01±3.60	12.94±3.59*

Table 4: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Thigh muscles of rats given varied concentration of sodium fluoride in drinking water for 14 days.

Parameters	Control	Expt- 1	Expt- 2	Expt- 3
Zinc	2.51±1.58	2.49±1.57	2.46±1.56*	2.38±1.54*
Copper	0.093±0.30	0.099±0.31	0.104±0.32	0.111±0.33*
Maganese	1.37±1.17	1.41±1.18	1.43±1.19	1.52±1.23*
Iron	12.62±3.55	12.67±3.55	12.74±3.56	12.79±3.57*

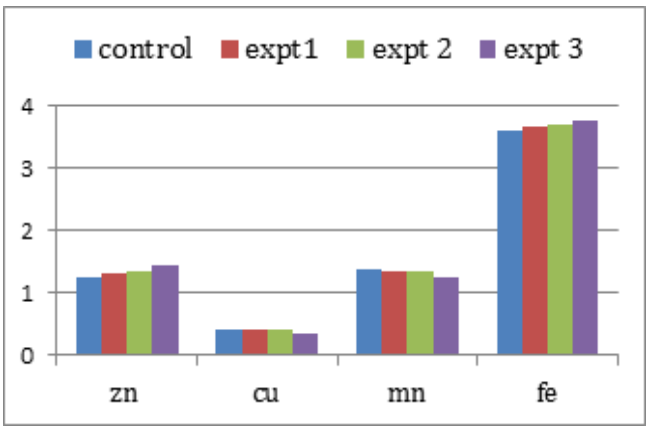

Fig. 3: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Kidney of rats given varied concentration of sodium fluoride in drinking water for 14 days.

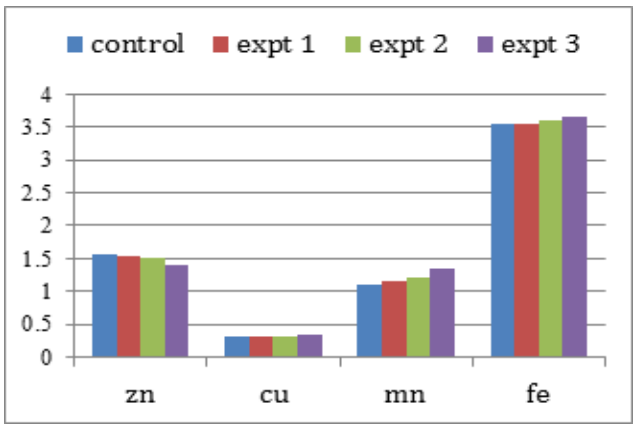

Fig. 4: Changes in level of trace elements (Zn, Cu, Mn and Fe) in Thigh muscles of rats given varied concentration of sodium fluoride in drinking water for 14 days.

Zinc is an essential nutritional and biochemical component , its deficiency has adverse health consequences. Conversely, excessive exposure to Zn is relatively uncommon, and occurs only under heavy exposure to this metal. Zn does not accumulate in proportion to it consumption, as the body content of Zn is modulated by homeostatic mechanisms that act principally on its absorption and on regulation of its liver levels (Berthof, 1988; Goyer,1991; Underwood, 1977). Level of Zn fell significantly in muscles but increases in kidney. Zinc is an essential component of numerous enzymes and is part of the structure of many proteins. Zinc containing enzymes are found in all parts of the major metabolic pathways involved in carbohydrate, lipid, protein, and nucleic acid metabolism, epithelial tissue integrity, cell repair and division, and vitamin A transport and utilization (Kaneko, 1989).

Copper (Cu) is plentiful in the environment and essential for the normal growth and metabolism of all living organisms(Schroeder *et al*., 1966; Carbonell and Tarazona ,1994).In the present study Cu fell significantly in kidney while increased in muscles. Abnormal levels of copper intake may range from levels as low as to induce a nutritional deficiency to levels as high as to be acutely toxic. Copper is an essential component of the animal system and plays an important physiological role in haematopoiesis, myelin formation, phospholipids formation, connective tissue metabolism and enzyme systems.

Fe level increases significantly in kidney and muscles similar reports observed by Bhatnagar *et al.,* 2003. Iron is one of the important mineral elements necessary for the effective metabolism of the mammalian body. Although it is present in very small amounts in the body, iron plays an important role in many metabolic processes Fe is important in formation of haemoglobin molecule. It is essential consitituent of myoglobin and repiratory enzymes. Deficiency of Fe is related to restlessness, tiredness and imbalances in brain iron homeostasis during development which result into symptoms of neurodegenerative (Vaderveer, 1990) In present study level of Fe increases significantly in kidney and muscles.

Statistical analysis of the mean and standard deviation of treated and control groups was done by one –way ANOVA without replication. Data related to trace metal concentration in control and experimental tissue are summarized in Table 1 and 2 changes observed in Cu, Mn, Fe and Zn level in kidney and muscles of the rat intoxicated by fluoride

CONCLUSION

Body has an elaborate system for managing and regulating the amount of key trace metals circulating in blood and stored in cells. When this system fails to function properly, abnormal levels and ratios of trace metals can develop. One of the most common trace-metal imbalances is elevated copper and depressed zinc. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals. Zn is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes, while copper is present in every tissue of the body, but is stored primarily in the liver, with fewer amounts found in the brain, heart, kidney, and muscles. In the above experiment slight changes in the concentration is observed, comparing both experiment i.e 7 days and 14 days short day exposure changes are more in 14 days sodium fluoride intoxication.

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RESEARCH ARTICLE

Senga (Ptychobothridae) Bothriolata a parasite infestation of *Mastacembelus armatus*

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ABSTRACT

The present investigation deals with the systematic observation of Pseudophyllidean tapeworm *Senga bothriolata Sp.nov.* from freshwater *Mastacembelus armatus* (Lacepede, 2000).The worm come closer to all species of genus Senga in general topography of organs but differs due to the remarkable bothria as a part of scolex, the scolex with bothria and the position of bothria in a particular pattern, upper most portion of scolex terminate into a rostellum which in having a rostellar hook in circular manner. Rostellum with rostellar hooks. The mature proglottids are five times broader than long. The testes are small, circular in structure, 50-60 in number, spread all over in the proglottids. The cirrus pouch is rounded in shape, present in the middle position of the proglottid. The cirrus located in the cirrus pouch. The vas deferens is long. The vagina and cirrus pouch both open common in opening known as genital pore, which is small, oval in shape. The vagina is a thin tube, starting from the genital pore and forms receptaculum seminis, the receptaculum seminis is open into ootype, the ootype is rounded, small in size. The ovary is large, bilobed, dumbbell shaped. The vitellaria are follicular, present on each lateral side of proglottids.

Keywords: *Mastacembelus armatus, Senga.*

INTRODUCTION

The genus *Senga* was established by Dollfus (1934), with its type species *S. besnardi* from *Betta splendens* at Vinecunes, France. *S. ophiocephalina* Tseng (1933), as *Anchistrocephalus ophiocephalina* from *Ophiocephalus argus* at Taimen, China and identified with a form previously recorded by Southwell (1913) as *Anchitrocephalus polyptera* (Anchistrocephalus) *Monticelli* (1890), Syn. *Anchistrocephalus*, Luhe (1999), from *Ophiocephalus striatus* in Bengal, India. *S. pycnomerus* Woodland (1924) as *Bothriocephalus pycnomerus* from *Ophiocephalus marulins* at Allahabad, India. *S. lucknowensis*. Johri (1956) from *Mastacembellus armatus* in India. Fernando and Furtado (1963) recorded *S. malayana* from *Channa striata*, *S. parva* and *S. filiformis* from *Channa micropeltes* at Malacea. Ramadevi and Hanumantrao (1966) reported the *plerocercoid*

of *Senga* sp. from *Panchax panchax*. Tadros (1968) synomised the genus *Senga* with the genus *Polyonchobothrium* and proposed new combinations for the species, Furtado and Chauhan (1971) reported *S. pahangensis* from *Channa micropeltes* at Tesak Bera. Shinde (1972) redescribed *S. besnardi* from *Ophiocephalus gachua* in India and recently Ramadevi and Rao (1973) reported another species of *S. vishakapatanamensis* India.

Ramadevi (1976) described the life cycle of *S. vishakhapatnamensis* from *Ophiocephalus punctatus* in a lake at Kondakaria, Andhra Pradesh, India. But they do not agree with Tadors Statements. Wardle, McLeod and Radinovsky (1974) put *Senga* as a distinct genus in the family Ptychobothridae. Deshmukh, 1980 reported *S.khami* from *Ophicephalus marulius*, a fresh water fish from Kham river at Aurangabad. Jadhav and Shinde, 1980 reported *S. godavari* from *M. armatus* at Nanded, M.S. India. One more species *S. aurangabadensis* was added by Jadhav and Shinde,1980 from *M. armatus* at Aurangabad M.S. India. A new addition made by Kadam et al., (1981) as *S. paithaniensis* from host *M. armatus*. Majid et al., (1984) added *S. raoi* and *S. jagannathae* from *Channa punctatus*. Two more new species erected by Jadhav et al., (1991) as *S. maharashtrii* and *S.gachuae* from the intestine of *M. armatus.*

Monzer Hasnain (1992) added *S. chauhani* from *Channa punctatus*. Tat and Jadhav (1997) added new species to the genus as *S. mohekarae* from the Intestine of the *M. armatus*, at Parli, Dist. Beed, M.S. India. Patil and Jadhav added new species to this genus as *Senga tappi* from *M. armatus* in 2003. Pande et al., (2006) added two new species i.e. *S. ayodhensis* from *Amphinuous cuchia* and *S. baghui* from *Rita rita.* (Ham.) Basti, U.P. India. Bhure *et al.,* (2007) described *Senga jadhavae* from *Mastacembelus armatus* at Aurangabad. *Senga chandkapurensis* (Khadap *et al.,* 2007) was reported from freshwater teleost *Mastacembelus armatus* at Chandikapur. Dist. Bidar, Karnataka, India. *Senga kaigaonensis,* (Wankhede and Reddy, 2009) was recorded from freshwater fish *Mastacembelus armatus* (L.) Kaigaon toka, Dist. Aurangabad (M.S.) India. *Senga madhavae* (Bhure *et al*., 2010); *Senga satarensis* and *Senga mangalbaiae* (Bhure and Nanware, 2011) were reported from freshwater fish *Mastacembelus armatus* from Maharashtra state. Pardeshi and Hiware (2011) described *Senga rupchandensis* from *Channa straitus* at Jalana, M.S. India.

Dhole *et al.,* (2011) *Senga rostellarae* and *Senga chandrashekhari* from *Mastacembelus armatus*, Maharashtra state India. Puinyabati et al. (2013) reported *Senga silcharensis* from intestine of *Channa puntatus* (Bloch) from Chatla Haor, Silchar, Assam. Bhure et al. (2014) described *Senga microrostellata* from *Mastacembelus armatus* at Parabhani (M.S.) India. *Senga nandedensis* described by Fartade and Fartade (2014) from freshwater eel *Mastacembelus armatus* in Godavari river basin (M.S.) India. Deshmukh, V.S. (2015) reported *Senga rostellata* and *Senga tringulata* from freshwater fish *Mastacembelus armatus* in from Nanded (M.S.) India unpulished Ph.D Thesis S.R.T.M. University Nanded Maharashtra state in India. *Senga madhukari* reported by Fartade *et al*. (2015) from *Mastacembelus armatus* in Godavari basin (M.S.) India. More Recently Fartade and Fartade (2015) described *Senga mastacembelus* from *Mastacembelus armatus* from Godavari Basin (M.S.) India.

MATERIALS AND METHODS

Fourty five species collected from the intestine of fresh water fish *Mastacembelus armatus* from Warkhed, Tehsil Telhara, Dist. Akola (M.S.) India during the period of January 2013 to December 2014.

These Cestodes are preserved in hot 4% formalin and Six specimens are stained with Harris haematoxylin and Borax carmine, dehydrated in ascending grades of alcohol, cleared in xylene, mounted in D.P.X. and drawings are made with the aid of camera lucida attachments. Photomicrographs were taken by Trinocular computerized Research microscope. All measurements are recorded in millimeters.

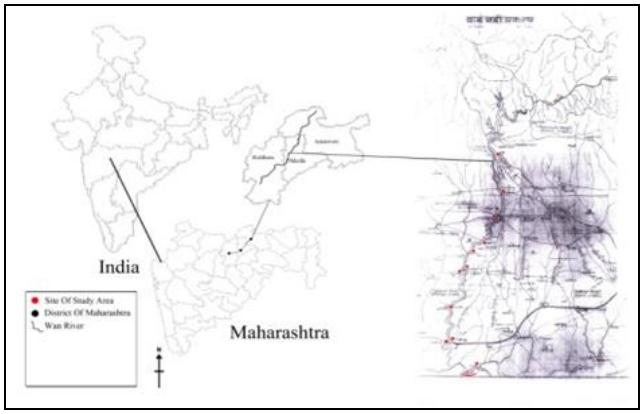


Fig. 1: Map location of survey spots on River Wan and Tributaries

Fig. 2: *Mastacembelus armatus* (Lacepede)

RESULTS AND DISCUSSION:

Description

(Based on Six Specimen)

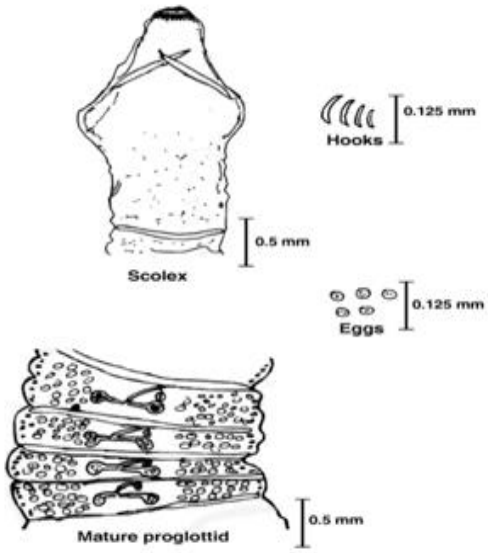
45 Piscean Cestode parasites were collected from the 28 infected intestines out of 59 examined freshwater fish host *Mastacembelus armatus* (Lacepede, 1800) Warkhed, Tehsil Telhara, Dist. Akola (M.S.) India during the period of January 2013 to December 2014.

These cestodes are preserved in hot 4% formalin and Six specimens are stained with Harris haematoxylin and Borax carmine, dehydrated in ascending grades of alcohol, cleared in xylene, mounted in D.P.X. and drawings are made with the aid of camera lucida attachments. Photomicrographs were taken by Trinocular computerized Research microscope. All measurements are recorded in millimeters.

All the cestodes are long having scolex, immature, mature and gravid proglottids. The scolex having bothria, rostellum with rostellar hooks, narrow anteriorly and broad posteriorly and measures 1.030 (0.970-1.090) in length and 0.360(0.213-0.517) in breadth.

scolex with bothria and the position of bothria in a particular pattern, the upper most portion of scolex terminate into a rostellum which having a rostellar hook in circular manner. Rostellum measure 0.455(0.290-0.621) in length and 0.120(0.090-0.150) in width. Rostellum is armed with 23-25 hooks which measures 0.084 (0.073-0.095) in length and 0.007 (0.006-0.008) in breadth.

The mature proglottids are five times broader than long and measures 0.394 (0.279-0.510) in length and 2.775 (2.450-3.100) in width. The testes are small, circular in structure, 50-60in number, spread all over in the proglottids and measures 0.036 (0.026-0.046) in length and 0.057 (0.051-0.064) in width. The cirrus pouch is rounded in shape, present in the middle position of the proglottid and measures 0.167 (0.155-0.179) in length and 0.076 (0.066-0.086) in width. The cirrus is small, located in the cirrus pouch and measures 0.179 (0.175-0.183) in length and 0.013 (0.009-0.018) in width. The vas deferens is long and measures 0.214 (0.195-0.233) in length and 0.012 (0.008-0.016) in width. The vagina and cirrus pouch both open in common opening known as genital pore,



Photoplate : *Senga bothriolata* sp.nov. Camera lucida drawing of *Senga bothriolata* sp.nov.

which is small, oval and measures 0.049 (0.042-0.057) in length and 0.033 (0.029-0.038) in breadth. The vagina is a thin tube, starting from the genital pore and forms receptaculum seminis and measures 0.388 (0.324-0.453) in length and 0.015 (0.010-0.020) in width. The receptaculum seminis is open into ootype and measures 0.179 (0.175 - 0.184) in length and 0.031 (0.027 - 0.036) in width. The ootype is rounded, small in size and measures 0.039 in diameter. The ovary is large, clearly bilobed, dumbbell shaped and measures 0.475 (0.451-0.499) in length and 0.240 (0.225-0.256) in width. The vitellaria are follicular, present on each lateral side of proglottids.

DISCUSSION

Species of the genus *Senga* are reported from labyrinthiform and cypriniform fishes of South East Asia. *S. besnardi* Dollfus, 1934 is from *Betta splendens* the Siamese fighting fish, in an aquarium at Vincennes, France. *S. ophiocephalina* Tseng, 1933, syn. *Anchistrocephalus o. T., A. polyptera* Southwell, 1913 nec *A. polypteri* (Leydig) of Monticelli, 1890, occurs in *Ophiocephalus argus;* Tsinan and Pieping, China.

S. pcynomera (Woodland, 1924), syn. *Bothriocephalus p.W.,* is reported from *Ophiocephalus marulius* at Allahabad, India. Johri, 1956 described *S. lucknowensis* from the spiny eel, *Mastacembelus armatus* Lacep. from Lucknow, India. Subsequently following species of the genus *Senga* were described and furnished information on the diagnostic features of the species by various workers.

The present worm differs from *S. besnardi* in the shape of scolex which is triangular, no. of hooks 50, neck absent, mature segment wider than long. Total number of testes 160-175.

The present cestode differs from *S. ophiocephalina* which shows pear shaped scolex, no. of hooks 47-50 and no. of testes 50-55.

The present tapeworm differs from S. pycnomerus in the shape of scolex, elongated scolex bear 68 no. of hooks, mature segments are in distinct.

The present worm differs from *S. lucknowensis* which have pear shaped scolex with 36-48 large hooks.

The present cestode differs from *S. malayana* which shows circular scolex with 60 hooks.

The tapeworm differs from *S. parva* in having pear shaped scolex with 38-40 hooks.

The present cestode differs from *S. pahangensis* in the shape of triangular scolex, no. of hooks 52 neck is short, segmentation clear, proglottids broader than long, testes laterally situated in the proglottids.

The worm differs from *S. vishakapatanamensis* which shows circular scolex, no. of hooks 46-52, two large rudimentary hooks. Neck absent, testes 50-55 in number.

The present worm differs from *S. khami* which have rectangular; pear to oval scolex, shallow bothria, no. of hooks 55-57, short neck, mature segment broader than long, testes rounded 155 in number and arranged in two fields; cirrus pouch is elongated.

The cestode differs from *S. aurangabadensis* which bear oval scolex, two bothria, 50-52 large hooks in two half rows, overlapping on each other, neck absent, mature segment longer than broader. Testes 240-260 in number cirrus pouch is medullary.

The present tapeworm differs from *S. godavarii* which have pear shaped scolex which is broader in center and tapering at anterior and posterior end, bothria present, hooks 40-42 in number arranged in two half rows, which overlap on each other. Neck absent, mature segment broader than long and testes are rounded which are arranged in fields and 220-230 in number, cirrus pouch is oval, situated in anterior half of the segment.

This cestode differs from *S. paithanensis* which shows prominent large triangular scolex with two bothria that extends up to posterior end. no. of hooks are 54 and arranged in two half rows which overlap on each other. Neck is present. Mature segment broader than long. Testes are rounded, oval, 130-135 in number arranged in two lateral groups. Cirrus pouch oval and curved anteriorly to the isthmus of the ovary in the centre of the segment.

The present form differs from *S.raoi* in having pear shaped scolex, hooks 46 in numbers, absence of neck and testes 65-170 in numbers.

The present of cestode differs from *S.jagannathae* in having pear shaped scolex, hooks 44 in numbers and testes 240 - 250 in numbers.

The present parasites differs from *S. gachuae* in having pear shaped scolex, hooks 22-25 in numbers and testes 60-70 in numbers.

The present cestode differs from *S. maharashtrii* which shows muscular scolex; broader anteriorly and narrow posteriorly. Bothria oval and two in number that extends up to the posterior end of the scolex. Hooks 45-46 in number; large and arranged in two half crowns. Neck absent mature segment broader than longer, testes oval 80-90 in no. cirrus pouch small oval in the posterior half of the segment in the medullary region and anterior to the ovary.

The present worm differs from *S.chauhani* in having scolex oval, hooks 40-44 in numbers and testes 200-210 in numbers.

The present cestode differs from *S. mohekarae*, which shows pear shaped scolex, two bothria, extend up to the posterior end, hooks 151 in number. Neck is short and broad, mature segment broader than long, testes 300-310 in number. Cirrus pouch oval and situated in the anterior half of the segment and ovary bilobed, posterior to middle of the segment.

The present Cestodes differs from *S. tappi* which have triangular scolex, bothria two in number and extended from anterior to posterior end of the scolex. Hooks 42-44 in number, neck is very short and squarish. Mature segment three times broader than long, testes small rounded in shape distributed in 2 fields on either side of cirrus pouch oval; pre-ovarian in anterior half of the segment and ovary bilobed elongated post equatorial, medially situated.

The present parasites differs from *S.ayodhensis* in having conical scolex, absence of neck, hooks 29 in numbers.

The present cestode differs from *S.baughi* in having pear shaped scolex, hooks 28 in numbers.

The present worm differs from *S jadhavae* Bhure et al., 2007 in having scolex triangular, rostellum rounded, rostellar hooks 50-54 in numbers, neck short, testes small, rounded, ovary bilobed, vagina coiled tube, vitellaria follicular, arranged in 4-5 rows, uterus Saccular and recovered from *Mastacembelus armatus* Aurangabad (M.S.), India .

It differs from *S. chandkapurensis* Kahadap et al.,2007 in having scolex barrel shaped, rostellum armed, rostellar hooks 28-30 in numbers, circularly arranged, neck short, mature proglottid broader than long, testes small, rounded, 170-180 in numbers, anterior to cirrus pouch, Vitellaria granular and reported from intestine of freshwater fish *Mastacembelus armatus* at Chandikapur Dist Bidar, Karnataka, India.

The present form differ from *S. kaigaonensis,* Wankhede and Reddy, 2009 in having scolex triangular, anterior end pointed, rounded and posterior end broad, testes 285-295 in number, cirrus pouch pre-ovarian, obliquely placed and reported form fresh water fish *Mastacembelus armatus* (L.) Kaigaon toka, Dist. Aurangabad (M.S) India.

The present cestode species differs from 25. *Senga madhavae* Bhure *et al*., 2010 in having Scolex triangular, Rosetellum armed with 40-44 hooks, neck absent, mature proglottids 5-6 times broader than long, Vagina thin tube, Vitellaria granular, uterus Saccular and collected from *Mastacembelus armatus* (Lacepede,1800); Pune (M.S.), India.

The present form differ from *Senga mangalbaiae* Bhure and Nanware, 2011 in possessing Scolex conical, tapering at the apex and broad at the base, distinctly marked off from the stroblia, uterus Saccular, vitellaria granular, arranged in 2-3 rows and collected from *Mastacembelus armatus* (Lacepede,1800); Osmanabad (M.S.), India.

The reported cestode species differs from *Senga rupchandensis* Pardeshi and Hiware, 2011 in having body long, scolex flat, tubular, cylindrical, scolex bears two bothria, rostellum flat, having two rows of semicircular hooks, 42-55 in number, neck absent, vitellaria follicular, eggs oval, non-operculate and recovered from *Channa striatus* (Bloch, 1793) at Jalna District (M.S.), India.

It differs from *Senga rostellarae* Dhole *et al.,* 2011 in having body long, Scolex pear shaped, medium, elongated, bothria two, large, cirrus pouch elongated,oval, vitellaria follicular, arranged in one

row and collected from intestine of *Mastacembelus armatus* L.; M.S. India.

The present cestode parasite *Senga chandrashekhari* Dhole *et al.,* 2011 in having scolex large, broad at the posterior end, narrow at the anterior end, neck short, , testes medium, rounded, 98-117 in numbers, evenly distributed, in two lateral fields, vagina long, broad tube, eggs oval, operculate, and reported from intestine of *Mastacembelus armatus* L.; M.S. India.

It differ from *Senga silcharensis* Puinyabati, Shomorendra and Kar Devashish, 2013 in having Scolex pear shaped, bluntly rounded apically, Anterior region of scolex having rostellum with 44 hooks in two semi-circles, ovary post-equatorial, bilobed, collected from the intestine of *Channa punctatus* (Bloch) from Chatla Haor, Silchar, Assam.

The *S.microtrigularis Sp.nov.* is differ from *Senga microrostellata* Bhure *et al.,* 2014 in having Scolex triangular, tapering at apex and broad at base, distinctly marked off from stroblia, bothria two, sessile, rostellum oval, armed with 18-20 hooks, arranged in a circle, neck absent, mature proglottids 8-9 times broader than long, testes small, oval to rounded, 250-300 in numbers, scattered lateral side of segment on either side of ovary and Cirrus pouch small, elongated, transversely placed, cirrus thin, short, straight, vas deferens short, thin tube, genital pore small, oval, vagina arises from gonopore, thin tube, runs towards posterior side, receptaculum seminis thin, short tube ootype oval to rounded, Ovary large, distinctly bilobed, dumbell shaped, Uterus Saccular, eggs oval, non-operculated, vitellaria follicular, arranged in a line and recovered from *Mastacembelus armatus* (Lacepede,1800); Parbhani (M.S.), India.

It differs from *Senga nandedensis* Fartade Asawari and Fartade Madhukar,2014 in having scolex large, well developed, triangular, bothria two, spatulate, neck absent, testes oval, small, 150-200 in numbers cirrus pouch oval, medium ovary small, bilobed, dumbell shaped vitellaria follicular, arranged in two lateral margin of the segment and reported from *Mastacembelus armatus* in Godavari basin (M.S.) India.

The present form differs from *Senga rostellata* Deshmukh V.S, 2015. The scolex having pair of bothria, which is sessile, extends from the anterior end to posterior end of the scolex. The anterior end of the scolex terminates in a rostellum, which is oval to rounded in shape. The rosetellum is armed with 20-22 hooks, neck is long, mature proglottids are about three times broader than long, testes are small, oval in shape, pre-ovarian 25-30 in number, scattered in two groups. The cirrus pouch is cylindrical in shape, pre – ovarian in position, situated in the centre of the segment, cirrus is thin, present within the cirrus pouch, vas deferens is short, thin, straight tube, The vagina and cirrus pouch open a common pore known as genital pore, which is small in size, oval in shape, vagina is a thin tube, slightly curved. The receptaculum seminis is straight tube open into ootype. Ootype is oval, medium in size, ovary is distinctly bilobed, The vitellaria are follicular, on each lateral side from anterior to posterior margin of the proglottids and reported from freshwater fish *Mastacembelus armatus* (Lacepede, 1800); Nanded (M.S.) India.

The *S.microtrigularis Sp.nov.* differs from *Senga tringulata* Deshmukh V.S, 2015. scolex having pair of bothria, which are sessile, extends from the anterior end to posterior end of the scolex. Scolex bears rostellum with hooks which are arranged in circle unequal length, neck is absent. The mature proglottids are about 4-5 times broader than long, The testes are medium, oval in shape, 55-60 in number, scattered throughout the proglottids, The cirrus is thin, curved tube, present within the cirrus pouch, vas deferens is short, vagina is a thin tube, ootype is oval, medium in size ,ovary is large, distinctly bilobed, dumbbell shaped , vitellaria are follicular. Eggs are elongated, tapering at both ends and collected freshwater fish host *Mastacembelus armatus* (Lacepede, 1800) at Hadgaon Dist. Nanded (M.S.).

The present form differs from *Senga mastacembelusae* having scolex triangular, hooks 20-22. Mature segment rectangular, genital pore rounded, which are reported from *Mastacembelus armatus* in India

It differs from *Senga madhukarii* Fartade, A. (2015) in scolex is large and well developed, cylindrical in shape, scolex bear rostellum armed with hook 45 in numbers. The bears two bothria spatulated overlapping each other, long extend upto posterior end of scolex, neck is absent, the testes are oval in shape, medium in size 130 in numbers spread in the segment at each side of the ovary. The cirrus pouch is oval medium in size, anterior to ovary, situated in the middle of the segment. Ovary is bilobed each lobe is different with

long isthmus. Vitellaria are follicular arranged in two to three rows at each lateral margin of the segment and reported from *Mastacembelus armatus* (Lacepede, 1800); Godavari basin Maharashtra India.

Senga bothriolata Sp.nov.differs from earlier described *Senga microtrigularis* Sp.nov. (2016) In having The scolex is small and triangular, acute at anterior side and broad at posterior side, scolex having rostellum and bears pair of bothria, starting from the anterior end and terminate into posterior end of the scolex, The neck is long and broad, The testes are minute, rounded in shape, 50-55 in number, scattered in two groups . The cirrus pouch is cylindrical in shape, present in the middle position of the proglottid. Ovary is bilobed , Genital pore is minute in size, vagina is a thin tube, vitellaria are granular, on each lateral side margin of the proglottids and reported from *Mastacembelus armatus* (Lacepede, 1800); Wan river at Takali, Tehsil Sangrampur, Dist Buldhana (M.S.) India.

The above noted characters are valid enough to erect a new species hence the name *Senga bothriolata* Sp.nov. is proposed after the remarkable bothria as a part of scolex

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

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RESEARCH ARTICLE

Impact assessment of avifauna from the selected lakes around Adani thermal power station in Gondia district, MS, India

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ABSTRACT

The study was conducted during February 2014 to January 2016 aims to examine the monthly diversity and impact assessment of the avifauna from the selected lakes in Gondia district, Maharashtra. A total of 103 species including 51 water bird species and 52 land bird species belonging to 13 orders and 40 families were recorded during two years study period. Out of recorded 103 bird species, 65 species were residents (63%), 25 species were winter visitors (24%), 03 species were summer visitors (03%) and 10 species were passage visitors (10%). According to relative abundance as per their sightings frequency, 35 species were very common (34%), 12 species were common (12%), 20 species were uncommon (19%), 26 species were occasional (25%) and 10 species were rare (10%) for the study area. The population diversity of the birds in the study area is significantly fluctuating between months and diversity index values ranged from 1.804 to 4.028 at study area. The present study does not take into more consideration as the climate change and global warming is still a nascent in the present study area. The less impact might be because of acclimatization phenomenon. No more impacts on the avifauna noticed due to Adani thermal power station in Gondia district. From conservation point of view, the situation is still within control, and we still have chance to save and preserve most bird species. But in future the present study area may be affected due to the warming by Adani thermal power station in Gondia district, Maharashtra.

Keywords: Avifauna, impact assessment, diversity, lakes, Gondia.

INTRODUCTION

Biodiversity is the variety of life on the Earth within and between all species of plants, animals and microorganisms and the ecosystems within which they live and interact. The birds found in the World and India shows great biodiversity. The eastern Vidarbha region of Maharashtra State, especially Gondia district is important to the water birds and land birds as there are many lakes in the district. A bird has been described as a feathered biped. Birds are vertebrate warm-blooded animals that is whose temperature remains more or less constant and independent of

the surrounding temperature (Ali, 2002). Wetlands and water birds are inseparable elements and thus form a rich array of water bird communities (Grimmett and Inskipp, 2007). Water birds are an important component of most of the wetland ecosystems as they occupy several trophic levels in the food web of wetland nutrient cycles (Rajashekara and Venkatesha, 2010). The bird habitats of the Indian subcontinent can be roughly divided into forest, scrub, wetlands, marine, grassland, desert and agricultural land habitat. Many bird species require mixed habitat types (Grimmett *et al*., 2011). The species density, diversity, richness and relative abundance of water birds depend upon wetland characteristics such as size, water level, quality of water, availability and distribution of food resources (Manikannan, 2011). The value of each wetland is intimately tied up with the culture and the needs of the people who exploit it and is dependent to a great degree upon its location. In a developing country like India, large number of people living around wetlands depend heavily on their resources for subsistence and traditional activities like fishing, grazing, farming, reed-gathering, etc. (Vachanth, 2013).

Bock *et al.* (1993) studied that the livestock grazing showed positive, negative and mixed responses on neotropical migratory birds in four major ecosystems in western North America: grasslands of the Great Plains and Southwest, riparian woodlands, intermountain shrub steppe, and open coniferous forests. The North American breeding bird survey has shown that grassland birds areunder going declines that are more widespread than any other group of birds. Due to the dynamic nature of the ecosystems and the anthropogenic impacts on them, the status of wetland biodiversity in India had changed drastically in the recent past (Mishra, 1999). Tasker *et al.* (2000) studied the impacts of fishing on marine birds in view the most direct effects of fishing on the birds involve killing by fishing gears, although on lesser scale some fishing activities also disturbs the birds. The loss of shade fromcattle trampling andgrazing on riparian vegetation raises the water temperature and reduces water oxygen levels (Carter, 2002).

The study at Kurukshetra has revealed that the anthropogenic activities like mass bathing in holy pondsm, cutting of emergent and fringed vegetation, draining of water, release of sewage, throwing of domestic garbage, developmental activities like construction of roads and retaining walls are some major threats to the bird diversities of the aquatic habitats (Kumar and Gupta, 2009). Different anthropogenic activities in the wetlands of Kolhapur city of Maharashtra State have increased the pollution and human encroachment area which adversely affected the bird population (Kachare *et al.*, 2011).

Gondia is known as the district of lakes as numbers of waterbodies are present in the district. Even though it represents only a small fraction of the geographical area of the Vidarbha region of Maharashtra State, India, Gondia district has a unique diversity of flora and fauna. The rich faunal wealth of Gondia district, especially near and around the Navegaon National Park has been recorded notably by several researchers; especially near and around the Srinagar lake (Chinchkhede and Kedar, 2012), Navegaon lake (Chitampalli, 1976; Chinchkhede and Kedar, 2013; Paliwal and Bhandarkar, 2014), Shrungarbandh lake (Bhandarkar and Paliwal, 2014). But the other lakes had not been given proper attention in terms of avifaunal research and the unavailability of detail report on the avifaunal wealth was the inspiring force behind the selection of the presented study. As the different anthropogenic activities and environmental changes are the biggest threats to the avifauna and our current knowledge about behaviour, natural history and status of avifaunal species is far from complete. Thus, the scientific study was launched to examine the "*Impact assessment of avifauna of the selected lakes around Adani thermal power station in Gondia district, Maharashtra.*" Hence, this research makes the contribution to the theoretical knowledge and conservation of the avifauna in and around the Gondia district, Maharashtra State, India.

MATERIALS AND METHODS

Study Area

The study area comprises three reservoirs around Adani thermal power station from Gondia district. A reservoir is impounding of water of a flowing river and is called a lake. These three reservoirs are Bodalkasa, Chorkhamara and Khairbandha. As these are the artificial lakes, hence these are locally called as Bodalkasa lake, Chorkhamara lake and Khairbandha lake. The location of three study sites are shown in the figure 1.1 and 1.2 by the help of google map. Bodalkasa lake lies at the geographic coordinates of 21°21'15"N

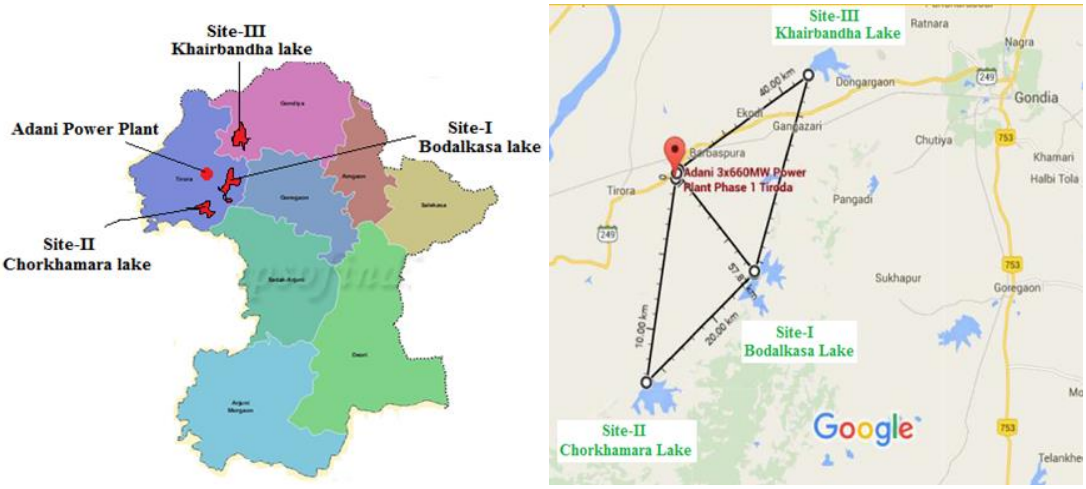

Fig. 1.1 & 1.2: Position View & Satellite View of three study sites around Adani Thermal Power Station in Gondia district (M. S.) India

latitude and 80°01'00"E longitudes, and situated near about 9 km south of Adani thermal power station. Chorkhamara lake lies at the geographic coordinates of 21°18'00"N latitude and 79°57'00"E longitudes, and situated near about 14 km south-west side of Adani thermal power station. Khairbandha lake lies at the geographic coordinates of 21°28'30"N latitude and 80°03'45"E longitudes, and situated 13 km away from Adani thermal power station on north-east side in Gondia district, Maharashtra.

The flora and fauna of the study area is typical of the aquatic, semi aquatic and surrounding habitats. There are floating weeds, grasses, herbs, bushes and large trees in and around the lakes. The observed floral species were Typha species, Water hyacinth, Hydrilla species, Azolla species, *Durva* grass, Crown grass, Paspalum species, Ipomoea species, etc. The shrubs and larger trees include *Bamboo*, Butea species, Ziziphus, *Jambul*, Mango tree, *Tendupatta*, *Mahua* tree, Banyan tree, Tamarind tree, *Bael* tree, Indian Fig tree (*Umbar*), *Neem* tree, Jackfruit tree, etc. The lakes also harbours the different species of fauna as insects (Moths, Butterflies and aquatic insects), crustaceans (Prawns), crabs, molluscs (Snail), fishes (Catla, Rohu, Mrigal, *Magur, Shingur, Botri, Pabda, Kanoshi, Poshti, Chital, Bhadar*, etc.), amphibians (Frogs), lizards and other animals (Deer, Dogs, Cows, Buffaloes, Goats, Monkeys, Squirrels, etc.

Survey and Methodology

The study was conducted during February 2014 to January 2016 aims to examine the monthly and seasonal diversity, and population dynamics of the avifauna from the present study area. Data were collected weekly during three seasons viz. summer (February to May), monsoon (June to September) and winter (October to January) of two successive years. The study was started with the permission of irrigation and forest departments of Gondia district, Maharashtra State, India. The counting of the birds was made at morning and evening timings of the day when the birds are most active (Rajashekara and Venkatesha, 2010, 2014) and depending on the light conditions (Namgail *et al.,* 2009). Weekly visits to the site were made for two years and an average of 4 weeks was accounted for a month (Wanjari, 2012).

No single survey can provide all data to answer every research question (Turner, 2003). There is no single technique that can be used for counting all types of birds. This is mainly because birds differ in terms of their size, behavior traits, habitat preferences etc. (Urfi*et al.,*2005). Based on the experience in the field considering the habitat status and area of each study site, the visual encounter surveys were conducted to the entire lake for 'direct counts' of the birds by randomized walking along the bank of lakes (Crump and Scott, 1994; Whitakar, 2002; Manley *et al.,* 2005; Manikannan, 2011; Vachanth, 2013; Joshi, 2014). Because most species of birds tend to be visible, methods to survey them generally rely on observers seeing them. Stationary and double counting methods were also employed for the counting the birds in the flocks (Gregory *et al.,* 2004).

A field binocular was used to observe the birds and the photographs were taken from the study area by using

Nikon camera with different zoom lenses. After detection, specimens were photographed by camera and identified with the help of visible structural features (Ali, 2002). The threats to the present study were assessed during the study period by direct observations in and around the study lakes and also by enquiring local villagers. For assessing the impact of these factors on the birds the Impact matrix method was used (Nalavade, 2013).

A systematic checklist of all recorded bird species from three sites was prepared on the basis of observations. The scientific names, common names, family sequence and IUCN status were ascertained as per BirdLife international (2013 version 6) and Grimmett *et al.* (2011). The order sequence was followed from a field guide by Manakadan *et al.* (2011). The residential local status of the bird species was assigned strictly with reference to the study area on the basis of presence or absence method as followed the techniques developed by Thakur *et al.* (2010); Grimmett *et al.* (2011); Koli (2014); Shekhawat and Bhatnagar (2014) as resident (R), winter visitor (WV), summer visitor (SV) and passage visitor (PV). The abundance status of the birds was recorded on the basis of the percent frequency (encounter rates) of sightings as followed the techniques developed by Kasambe and Wadatkar (2007), Kasambe and Sani (2009), Tak *et al.* (2010), Priyanka (2012); as Vc-Very Common (75-100%), C-Common (50-74%), Uc-Uncommon (25-49%),O-Occasional (5-24%) and Rr-Rare (less than 5%). The IUCN status was ascertained as per Bird Life International (2013 version 6) as *LC-Least concern and **NT-Near threatened.

RESULTS AND DISCUSSION

The results of the observed species of avifauna are inventory at three study lakes in Gondia district, Maharashtra State, India. A total of 103 species including 51 water bird species and 52 land bird species belonging to 13 orders and 40 families were recorded during two years study period from February 2014 to January 2016 (Table 1.1).

Table 1.1: A systematic list of Bird Species at three lakes in Gondia district, Maharashtra, India (Feb. 2014 to Jan. 2016)

Order / Family	Sp. Sr. No	Scientific Names	Common Names	Residential Status #	Abundance Status¤
Order - I: Anseriformes 1) Anatidae	1	**a) Water Birds:** *Dendrocygnajavanica**	Lesser Whistling-duck	SV	Uc
	2	*Anseranser**	Greylag Goose	PV	Rr
	3	*Anserindicus**	Bar-headed Goose	WV	O
	4	*Sarkidiornismelanotos**	Comb Duck	PV	Rr
	5	*Tadornaferruginea**	Ruddy Shelduck	WV	Uc
	6	*Nettapuscoromandelianus**	Cotton Pygmy-goose	R	Vc
	7	*Anasstrepera**	Gadwall	WV	O
	8	*Anaspenelope**	Eurasian Wigeon	PV	Rr
	9	*Anasplatyrhynchos**	Mallard	PV	Rr
	10	*Anaspoecilorhyncha**	Western Spot-billed Duck	R	C
	11	*Anasacuta**	Northern Pintail	WV	Uc
	12	*Anascrecca**	Common Teal	WV	O
	13	*Nettarufina**	Red-crested Pochard	WV	O
	14	*Aythyaferina**	Common Pochard	WV	O
	15	*Aythyanyroca***	Ferruginous Duck	PV	Rr
	16	*Aythyafuligula**	Tufted Duck	WV	O
Order II: Podicipediformes2) Podicipedidae	17	*Tachybaptusruficollis**	Little Grebe	R	Uc
Order III: Ciconiiformes 3) Ciconiidae	18	*Mycterialeucocephala***	Painted Stork	WV	O
	19	*Anastomusoscitans**	Asian Openbill	R	Vc
	20	*Ciconianigra**	Black Stork	WV	O
	21	*Ciconiaepiscopus**	Woolly-necked Stork	WV	O
4) Threskiornithidae	22	*Threskiornismelanocephalus***	Black-headed Ibis	SV	Uc
	23	*Pseudibispapillosa**	Red-naped Ibis	R	C
5) Ardeidae	24	*Ardeolagrayii**	Indian Pond Heron	R	Vc

	25	*Ardeacinerea**	Grey Heron	WV	O
	26	*Ardeapurpurea**	Purple Heron	R	C
	27	*Bubulcus ibis**	Cattle Egret	R	Vc
	28	*Casmerodiusalbus**	Great Egret	R	Vc
	29	*Mesophoyxintermedia**	Intermediate Egret	WV	Uc
	30	*Egrettagarzetta**	Little Egret	R	Vc
Order IV: Pelecaniformes 6)Phalacrocoracidae	31	*Phalacrocoraxniger**	Little Cormorant	R	Vc
	32	*Phalacrocoraxfuscicollis**	Indian Cormorant	WV	O
	33	*Phalacrocoraxcarbo**	Great Cormorant	WV	O
7) Anhingidae	34	*Anhinga melanogaster***	Oriental Darter	PV	Rr
Order V: Gruiformes8) Rallidae	35	*Porphyrioporphyrio**	Purple Swamphen	R	Uc
	36	*Gallinulachloropus**	Common Moorhen	R	Uc
	37	*Fulicaatra**	Common Coot	WV	O
Order VI: Charadriiformes 9)Recurvirostridae	38	*Himantopushimantopus**	Black-winged Stilt	R	C
10) Charadriidae	39	*Vanellusduvaucelii***	River Lapwing	PV	Rr
	40	*Vanellusindicus**	Red-wattled Lapwing	R	Vc
	41	*Pluvialisfulva**	Pacific Golden Plover	PV	Rr
	42	*Charadriusdubius**	Little Ringed Plover	R	Vc
11) Jacanidae	43	*Hydrophasianuschirurgus**	Pheasant-tailed Jacana	R	Uc
	44	*Metopidiusindicus**	Bronze-winged Jacana	R	Uc
12) Scolopacidae	45	*Gallinagogallinago**	Common Snipe	WV	O
	46	*Tringastagnatilis**	Marsh Sandpiper	WV	Uc
	47	*Tringanebularia**	Common Greenshank	WV	O
	48	*Tringaglareola**	Wood Sandpiper	WV	Uc
	49	*Actitishypoleucos**	Common Sandpiper	WV	O
	50	*Calidristemminckii**	Temminck's Stint	WV	O
	51	*Calidrisalpina**	Dunlin	PV	Rr
Order VII: Columbiformes13) Columbidae	52	**b) Land Birds:** *Columba livia**	Rock Pigeon	R	Uc
	53	*Streptopeliadecaocta**	Eurasian Collared Dove	R	Vc
	54	*Stigmatopeliachinensis**	Spotted Dove	R	Vc
	55	*Stigmatopeliasenegalensis**	Laughing Dove	R	Vc
	56	*Treronphoenicopterus**	Yellow-footedGreen Pigeon	R	C
Order VIII: Psittaciformes 14) Psittacidae	57	*Psittaculakrameri**	Rose-ringed Parakeet	R	Vc
	58	*Psittaculacyanocephala**	Plum-headed Parakeet	R	Uc
Order IX: Cuculiformes15) Cuculidae	59	*Cuculusvarius**	Common Hawk Cuckoo	R	O
	60	*Eudynamysscolopaceus**	Asian Koel	R	Uc
	61	*Centropussinensis**	Greater Coucal	R	Vc
Order X: Caprimulgiformes16) Caprimulgidae	62	*Caprimulgusasiaticus**	Indian Nightjar	PV	Rr
Order XI: Coraciiformes 17) Coraciidae	63	*Coraciasbenghalensis**	Indian Roller	R	Vc
18) Alcedinidae	64	*Halcyon smyrnensis**	White-throated Kingfisher	R	Vc
	65	*Alcedoatthis**	Common Kingfisher	R	Vc
	66	*Cerylerudis**	Pied Kingfisher	R	Vc

19) Meropidae	67	*Meropsorientalis**	Little Green Bee-eater	R	Vc
20) Upupidae	68	*Upupaepops**	Common Hoopoe	R	Vc
21) Bucerotidae	69	*Ocycerosbirostris**	Indian grey Hornbill	R	Uc
Order XII: Piciformes 22) Ramphastidae	70	*Megalaimahaemacephala**	Coppersmith Barbet	R	O
23) Picidae	71	*Dinopiumbenghalense**	Black-rumpedFlameback	R	Vc
	72	*Chrysocolaptesfestivus**	White-naped Woodpecker	R	Vc
Order XIII: Passeriformes 24) Pittidae	73	*Pitta brachyura**	Indian Pitta	SV	O
25) Aegithinidae	74	*Aegithinatiphia**	Common Iora	R	O
26) Oriolidae	75	*Oriolusoriolus**	Eurasian Golden Oriole	R	C
	76	*Oriolusxanthornus**	Black-hooded Oriole	R	Uc
27) Dicruridae	77	*Dicrurusmacrocercus**	Black Drongo	R	Vc
28) Corvidae	78	*Dendrocittavagabunda**	RufousTreepie	R	Vc
	79	*Corvusculminatus**	Indian Jungle Crow	R	Vc
	80	*Corvussplendens**	House Crow	R	Vc
39) Alaudidae	81	*Ammomanesphoenicura**	Rufous tailed lark	R	C
	82	*Eremopterixgriseus**	Ashy-crowned Sparrow Lark	R	Vc
30) Pycnonotidae	83	*Pycnonotuscafer**	Red-vented Bulbul	R	Vc
31) Sylviidae	84	*Orthotomussutorius**	Common Tailorbird	R	C
32) Timaliidae	85	*Turdoidesmalcolmi**	Large Grey Babbler	R	Uc
	86	*Turdoidesstriata**	Jungle Babbler	R	Vc
33) Zosteropidae	87	*Zosteropspalpebrosus**	Oriental White-eye	R	C
34) Sturnidae	88	*Acridotherestristis**	Common Myna	R	Vc
	89	*Sturnus contra**	Asian Pied Starling	R	Vc
	90	*Sturnuspagodarum**	Brahminy Starling	R	Vc
35) Muscicapidae	91	*Copsychussaularis**	Oriental Magpie Robin	R	C
	92	*Saxicoloidesfulicatus**	Indian Robin	R	Vc
36) Nectariniidae	93	*Nectariniazeylonica**	Purple-rumped Sunbird	R	O
	94	*Nectariniaasiatica**	Purple Sunbird	R	C
37) Passeridae	95	*Passer domesticus**	House Sparrow	R	C
38) Ploceidae	96	*Ploceusphilippinus**	Baya Weaver	R	Uc
39) Estrildidae	97	*Amandavaamandava**	Red avadavat	WV	O
	98	*Lonchurapunctulata**	Scaly-breasted Munia	R	Uc
	99	*Lonchuramalacca**	Black-headed Munia	R	O
40) Motacillidae	100	*Motacillaflava**	Yellow Wagtail	WV	O
	101	*Motacilla alba**	White Wagtail	WV	O
	102	*Motacillamaderaspatensis**	White-browed Wagtail	R	Vc
	103	*Anthusrufulus**	Paddyfield Pipit	R	Vc

 # Grimmett*et al.* (2011); Koli (2014); Shekhawat and Bhatnagar (2014): R - Resident, WV - Winter visitor, SV - Summer visitor, PV - Passage visitor.

¤ Kasambe and Sani (2009), Tak*et al.* (2010), Priyanka (2012): Rr - Rare (<5%), **O -** Occasional (5-24%), **Uc -**Uncommon (25-49%), **C -** Common (50-74%), **Vc -** Very common (75-100%).

*** BirdLife International (2013): LC* -** Least concern, **NT** -** Near threatened.

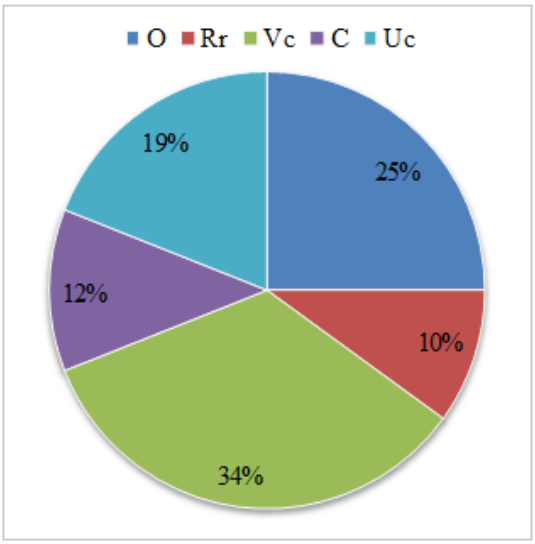

Fig. 1.3: Overall Residential status of Avifauna at study area

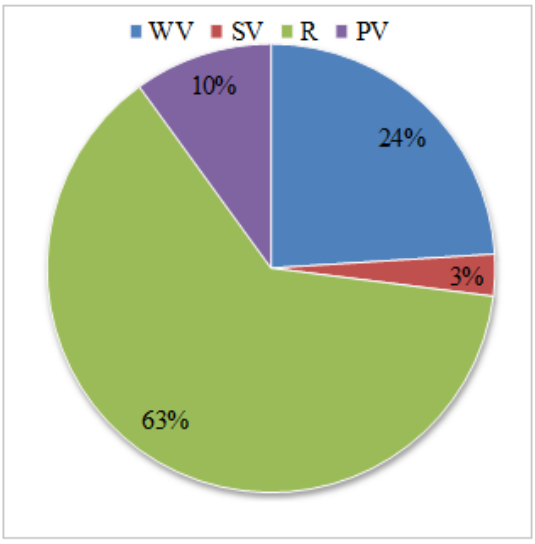

Fig. 1.4: Overall Abundance status of Avifauna at study area

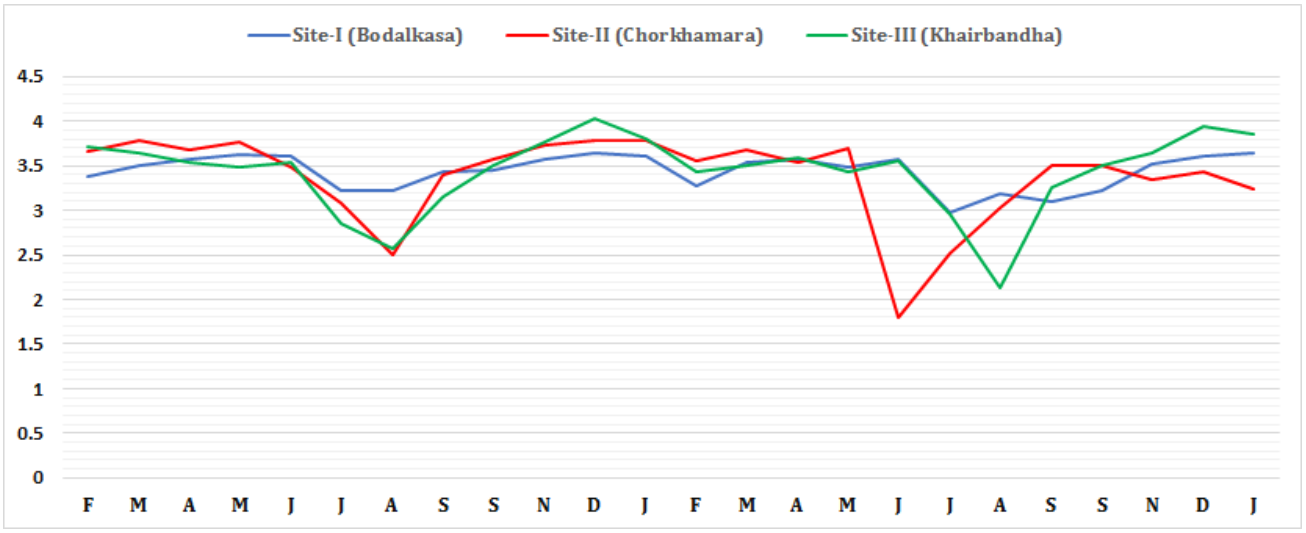

Figure 1.5: Monthly Shannon Diversity

Out of recorded 103 bird species, 65 species were residents (63%), 25 species were winter visitors (24%), 03 species were summer visitors (03%) and 10 species were passage visitors (10%) (Figure 1.3). According to relative abundance as per their sightings frequency, 35 species were very common (34%), 12 species were common (12%), 20 species were uncommon (19%), 26 species were occasional (25%) and 10 species were rare (10%) for the study area (Figure 1.4). As per IUCN status (2013), 98 bird species (95%) were least concern (LC) and 05 were near threatened (NT) species (05%).

The population diversity of the birds in the study area is significantly fluctuating between months. The monthly diversity index values ranged from 1.804 to 4.028 at study area (Figure 1.5). Every year from October onwards a considerable number of birds reach to the present study area. The peak population of the birds has been during November to January, this is due to the arrival of migratory birds and availability of food at study area. The low population was during the month of July and August due to the heavy rain fall the birds leave the study area and food sources disturbed due full water level in the lakes. The basic requirement of migratory water birds at their wintering sites are adequate food supply (Lakshmi, 2006; Mohan and Gaur, 2008), which are fulfilled by the lakes. Most of the water bird species leave the lakes by March-end or early April. The land birds were commonly seen throughout the year simultaneously.

The anthropogenic activities and other impacting factors influence the bird diversity (Walls, 1999; Datta, 2011; Manikannan, 2011; Sharma and Saini, 2012; Bhadja and Vaghela, 2013; Vachanth, 2013; Nalavade, 2013; Ramamurthy and Rajakumar, 2014; Sulaiman*et al.* 2014). Inallthereare13drivers (impacting factors) and two bird groups. During present study the main threats were noticed from the study area namely, population growth (PG), washing activities (WA), recreational activities and tourism (RA), MFP collection (FC), agricultural practices (AP), cattle grazing (CG), fishing practices (FP), forest fires (FF), loss and degradation of habitats (LH), poaching of birds (PB), dogs menace (DM), pollution (P), industrialization (I), etc. The impact of each driver on the concerned bird group is judged on the basis of actual field observations, field experience of the author supported by the data collected during the study period. The intensity of impact was arrived at by assigning a certain score to each bird group. The range of score is expressed numerically on a scale ranging from 0 to5.

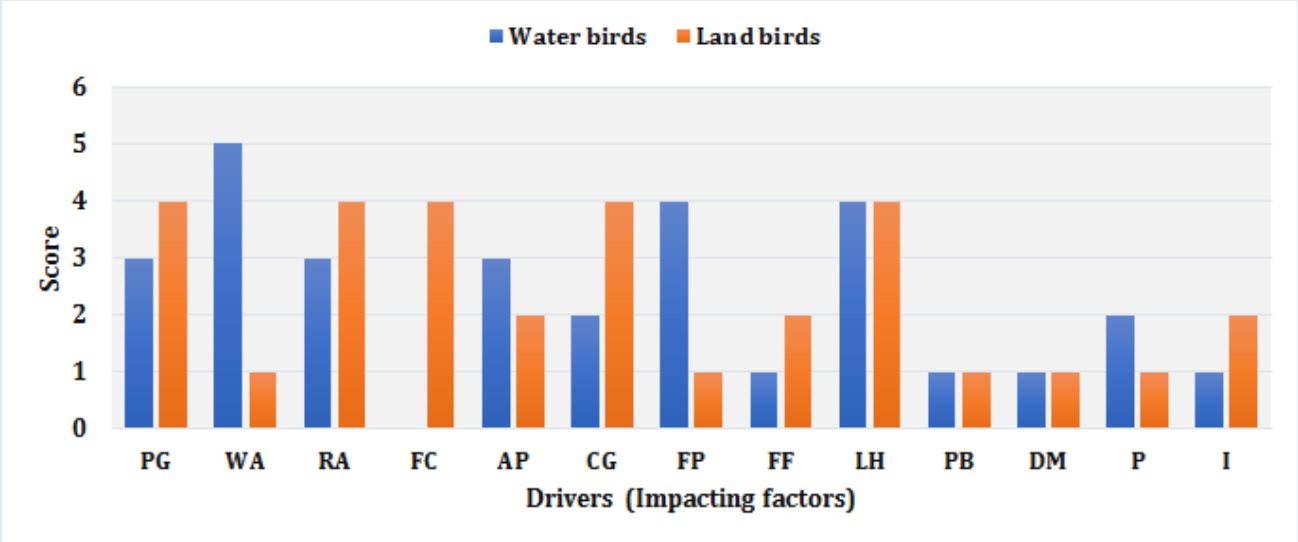

Figure 1.6: Impacts of drivers on Bird groups

Table 1.2: Grouping of Drivers on the basis of Impact level

Impact Category	Impacting drivers	Total Score	% Score
High	Loss and degradation of habitats	22	36.07
	Population growth		
	Recreational activities and tourism		
Moderate	Washing activities	26	42.62
	Cattle grazing		
	Agricultural practices		
	Fishing practices		
	MFP collection		
Low	Forest fires	13	21.31
	Pollution		
	Industrialization		
	Poaching of birds		
	Dogs menace		
Total:		**61**	**100.00**

Where,
0 = No impact.

1 = In significant impact: There is very weak relation between driver and bird group.

2 = Low impact: The Impact may not be clearly recognizable to a layman unless watched carefully.

3 = Moderate impact: The impact is visible but not threatening to the bird group.

4 = High Impact: The impact level is such that the diversity and population level of the group of species is likely to suffer.

5 = Very high Impact: The impact level is so intense, the group of species concerned is already threatened or is likely to become critical in the near future.

Total score for every driver and every bird group was also calculated (Figure 1.6).

On the basis of impact category, three drivers fall under the high impact category (36.07%), five in moderate impact category (42.62%) and five in low impact category (21.31%) (Table 1.2).

As Adani thermal power plant was started in 2012 in Tirora tehsil of Gondia district, the population of birds is decreasing. Even though the super-critical technology used by Adani power plant, but there is a lot of steam evolving from the boilers of the power plant. The maximum temperature 47.5^{o}C was recorded in the year 2011 in Gondia district. After two years, the maximum temperature 48^{o}C was recorded in the month of May 2013 at Tirora in Gondia district (India Weather Report) and this may be because of the increasing industrialization like Adani thermal power station in the district. The change is not abrupt, it is gradual so may not have direct impact. That may be responsible for the day by day increasing temperature which affects the nutrient cycle in the lakes, hence adversely affects the birds directly or indirectly.

Only 103 birds species have been recorded from three lakes viz. Bodalkasa, Chorkhamara and Khairbandha in Gondia district, Maharashtra during February 2014 to January 2016. Earlier, Chitampalli (1976) has recorded 209 birds species, Chinchkhede and Kedar (2013) recorded 126 bird species from the Navegaon National Park respectively in Gondia district. Misra (undated) recorded 166 bird species from Nagzira Wildlife Sanctuary including Chorkhamara lake in Gondia district. While Pimplapure and Sawji (2009) were recorded 412 bird species from Vidarbha region of Maharashtra State. Compared to previous studies in the district, the present study indicates a decline in the number of birds species. The main reasons for decline of birds in the study area may be due to anthropogenic pressure, irrational practices of fish catching in the lakes, cattle grazing, agricultural practices in encroachment area, changing climate and many other factors.

CONCLUSION

Various different studies have already been done, or are still going on, world over, on the possible impact of global warming on biodiversity and wild life in general and birds in particular. Global warming has set in motion and is affecting the timing of migration of birds (Jain, 2015). The present study does not take into more consideration as the climate change and global warming is still a nascent in the present study area. The less impact might be because of acclimatization phenomenon. No more impacts on the avifauna noticed due to Adani thermal power plant in Gondia district. This power plant near about 10-15 km away from each site of present study area. Also the power plant runs on the basis of super critical technology which helpful to reduce the pollution. Hence no more pollution caused, only steam and ash is produced in large quantity. The steam may be responsible to increase in temperature of surrounding present study area in future. Also the ash is responsible for degradation and loss of the bird habitats. Hence, in present study the avifauna was not impacted due to Adani thermal power plant in Gondia district except slight variations.

From conservation point of view, the situation is still within control and we still have chance to save and preserve most bird species. But in future the present study area may be affected due to the warming by Adani thermal power plant in Gondia district, hence particular conservative management is needed. The laws and legislations are not just sufficient for protection of the avifauna but it is necessary to raise the awareness levels by providing important information about birds among different sections of people for conservation of avian biodiversity in Gondia district, Maharashtra State. To overcome the problems related bird diversity, this research work has been done in the hope that it will help in raising the conservative awareness about the bird biodiversity among the people in Gondia district as well as

neighbor hood. Some following recommendations are suggested here to conserve the bird biodiversity and natural resources.

Recommendations

- Integrated grazing policy like alternative grazing, and based on the recommendations of advisory and expert committee must follow to help in the preservation of forest resources and biodiversity. Direct cattle grazing near the edges of the lake should be totally prohibited. The local people must be educated in this aspect.
- Stocking lakes through fishermen cooperative societies with a particular guideline manual. Fishermen only allowed at noon time and after a particular gap of days and not continuously in the lakes to catch fishes. The fisheries department should initiate immediate steps for the sustainable use of these lakes in this regarded.
- Ground fires for the roasting of the prawns and smaller fishes by fishermen must be prohibited near the lakes. Instead of it, they may use any particular large utensil for the roasting purposes.
- Dumping and flying of ash from Adani power plant in Gondia district must be properly managed by following the rules of environmental pollution and industrial acts. Also that ash should be used for making the bricks.
- Regular checking of the poaching and illegal hunting by appointing more staff from concerning departments, Lake protection committees (LPCs) and NGOs to prevent further population loss of the birds.
- Pollution and siltation may be avoided by maintaining special water tanks beside to the lakes separately for the immersion of the idols. After one month of the immersion of idols, the lakes may be desilted by the department if necessary.
- Setting up of Bird-clubs in schools to impart environment education, to encourage and mobilise participation of school children in various bird conservation activities in their localities. The curriculum should include chapters on the importance of birds in nature and to humans, and the need to conserve them.
- As there are Sarus Conservation Committees and organisation of Sarus-Vulture Conservation Rally in Gondia district (Dhurve*et al.,* 2010); that should be follow for all other birds by increasing the scope of these committees modifying as Bird Conservation Committee and Bird Conservation Rally in the present research study area.

Every people at local level must participate with own interest for the prevention of the forests, water bodies, bird diversity and all other natural resources at present study area as well as throughout the World.

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

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RESEARCH ARTICLE

Ichthyofaunal diversity of upper Morna Reservoir, Medshi (MS) India

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ABSTRACT

Reservoir is main resources exploited for inland fisheries and understanding fish faunal diversity is a major aspect for its development and the sustainability management. In the 2012-2013 investigation total 14 species of fishes belonging 4 orders and 6 families were reported in Upper Morna Reservoir, situated at Medshi village in Washim district. In all 4 orders cypriniformes were found most dominant followed by perciformes and others. In all fishes three species was found dominant i.e. *Catla catla, Labeo rohita, Cirrhina mrigla* is due to stocking of seed of this fishes intensely so far as the records of fish fauna of other water bodies. Total 14 fishes are recorded as *Rasbora daniconius , Puntius sarana, Schiatura denisoni, Catla catla, Cirrhinus mrigala, Labeo rohita Cirrhinus reba, Cyprinus carpio, Garra mullya, Glossogobius, Channa gachua, Mystus cavasius, Ompak bimaculatus, Mastacembelus armatus.*

Keywords: Fish diversity, Washim, Upper Morna Reservoir, Medshi

INTRODUCTION

Fresh water ecosystem is exploited in every possible way and one of it fish production, which is directly depends on the productivity of water ecosystem. The productivity of the fresh water community that determines the fish growth is regulated by the dynamics of its physico-chemical and biotic environment (Wetzel, 1983).

Fisheries sector in India has come a long way since independence; contributing immensely to the country's protein requirements as also export earnings. With annual growth rate of 4-6%, the fisheries sector is contributing about 5.4 million metric tons of fish, enabling per capita availability to the tune of 9kg for the Indian population. With the increasing population pressure and corresponding demanded for fish, various strategies are being formulated for enhancing production for marine inland resources. It is significant that inland fish production has been increasing at a high rate. Aquaculture, highly compatible with other farming systems with the flexibility of operation an investment contribute to more than 1\3rd of the fish production of the country.

It has also been identified as a farming activity that would ensure domestic food security and rural development by organization all over the world particularly in the Asian continent.

Fishes from one of the most important groups of vertebrates, influencing his life in various way. Millions of human beings suffer from hunger and malnutrition and fishes from rich sources of food and provide a meal to tide over the nutritional difficulties of man. In addition to serving as an important item of food, fishes provide several by product to us.

Freshwater fishery comprising species of carps, catfish, freshwater prawns and provides for a highly profitable economic enterprise and the traditional practices in the eastern India have taken shape of an industry in the states like Andhra Pradesh and Punjab. A survey indicates that only about a third of the freshwater aquaculture resources in the country have been put to use and hence great potential for enhancing fish production exists. At a time when the Indian Council of Agricultural Research is making long term plan for the next 25 years, in the form of "Vision -2020", it is highly appreciable that a systematic effort is made to project the potentials of fresh water aquaculture in the country by duly considering the resources and demand in different part of the country. The National Freshwater Aquaculture Development Plan provides the base for planning development strategies in the coming years. The fisheries sector is a very important part of the economy of rural region and provides a valuable source of employment.

MATERIALS AND METHODS

The Upper Morna reservoir is located ($18^0$36"44'N and $76^0$56"33.61'E) at Medshi, Malegaon Taluka in Washim district of Maharashtra. It is constructed on the Upper stretch of the Morna River, one of the minor river of Vidarbha region of Maharashtra and one of the tributary of the Purna River. The Morna River originates from the village Nagzari located in Washim district & meet the river Purna in Akola district at Andura. The main aim of construction of this reservoir was to save Akola city from the flood conditions, which was generally being occurring in the rainy seasons. Beside this the reservoir is used for irrigations, fishing activities & drinking purposes by the people residing around reservoir.

Fishes are main economic source for the people in Medshi villege, therefore Society is established called as the Shree Ganesh Machchhimar Co-operative Society Limited Medshi. (Reg. No.369) and the data on fish capture for two consecutive years was collected from it during study period.

Collection of fishes:

The Fishes were collected monthly with the help of fishermen by using different nets like gill net, cast net, traps, hooks & hand picking and brought to laboratory and morphometric study done immediately. Coloration, general pigmentation & fin formula, length and weight were recorded and preserved in 10% formalin.

The identification of fishes was carried out by using standard literature of (Talwar and Jhingran, volume 1 and 2, 1991) and some of them were sent to Zoological survey of India.

RESULTS AND DISCUSSION

Reservoir is main resources exploited for inland fisheries and understanding fish faunal diversity is a major aspect for its development and the sustainability management. In the present investigation total 14 species of fishes belonging to 4 orders and 6 families were reported in Upper Morna reservoir. The collected specimens and their systematic are depicted in given in Table 1 and photoplate I & II.

In all 4 orders cypriniformes were found most dominant followed by perciformes and others. In all fishes three species was found dominant i.e. *Catla catla, Labeo rohita, Cirrhina mrigla* is due to stocking of seed of this fishes intensely so far as the records of fish fauna of other water bodies, similar findings are recorded by (Kulkarni *et al*., 2008) they reported cultural fishes Catla, Rohu and Mrigal and 6 species of local fish fauna in Derala tank, Dist. Nanded, Maharashstra. Potentially the vast and varied Inland fishery resources of India are one of the richest countries in the world. Sreekantha and Ramachandra (2005) recorded 43 species of fishes in Linganamakki reservoir, Sharavathi river. Devi Prasad *et al*., (2009)

reported 45 species of fishes belonging to 15 families, 31 genera in which 6 fishes were threatened, 7 species were vulnerable and 40 species of fish endemic in this region i.e. major wetland of Mysore.

Shinde *et al*., (2009) investigated 15 fish species belonging to 3 orders, 4 families and 12 genera. The order cypriniformes found dominant with 11 species, 3species of perciformes and one species of siluriformesin Harsool Savangi dam, Aurangabad (M.S.) India. Kar *et al.*, (2006) revealed 69 species of fishes in biggest water tectonic lake Sone in Assam, India; belonging to 49 genera, 24 families and 11orders out of which 84.2% belonging to fresh water group, while rest to the peripheral class in aquatic ecosystem of Northeastern india.

Table 1: Fish diversity

Sr. No.	Order	Family	Species
1.	Cypriniformes	Cyprinidae	*Rasbora daniconius* (Hamilton)
2.	Cypriniformes	Cyprinidae	*Puntius sarana* (Hamilton)
3.	Cypriniformes	Cyprinidae	*Schiatura denisoni(Day)*
4.	Cypriniformes	Cyprinidae	*Catla catla* (Hamilton)
5.	Cypriniformes	Cyprinidae	*Cirrhinus mrigala*(Hamilton)
6.	Cypriniformes	Cyprinidae	*Labeo rohita*(Hamilton)
7.	Cypriniformes	Cyprinidae	*Cirrhinus reba* (Hamilton)
8	Cypriniformes	Cyprinidae	*Cyprinus carpio* (Linnaeus)
9.	Cypriniformes	Cyprinidae	*Garra mullya* (Sykes)
10.	Perciformes	Gobiidae	*Glossogobius* (Hamilton)
11.	Perciformes	Channidae	*Channa gachua* (Hamilton)
12.	Siluriformes	Bagridae	*Mystus cavasius*(Hamilton)
13.	Siluriformes	Silridae	*Ompak bimaculatus* (Bloch)
14.	Synbranchiformes	Mastacembelidae	*Mastacembelus armatus* (Lacepede)

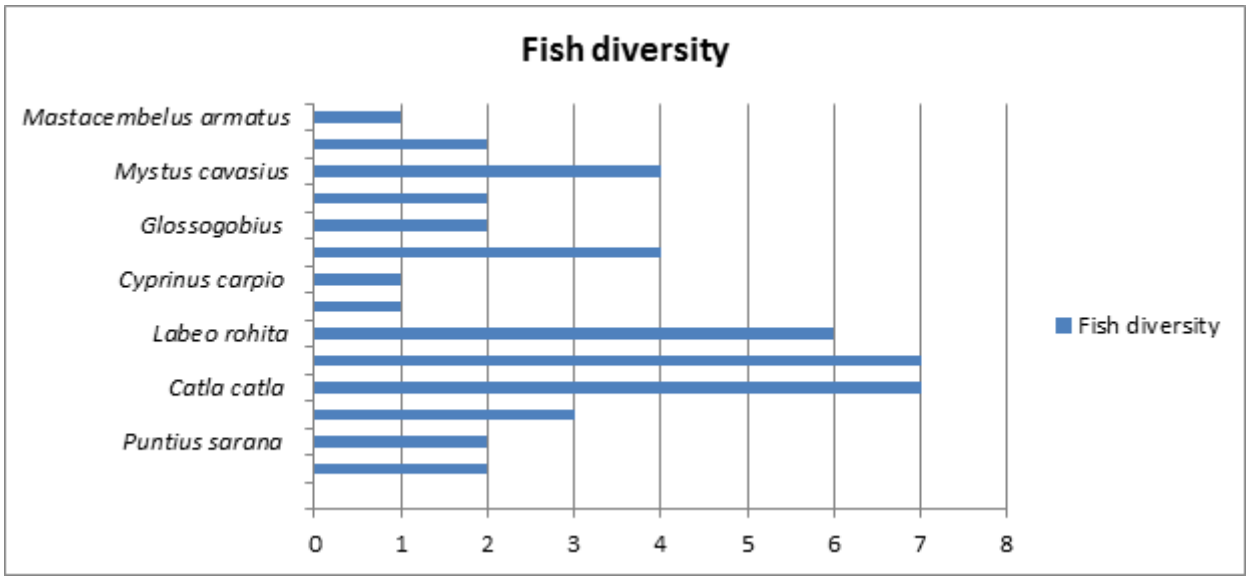
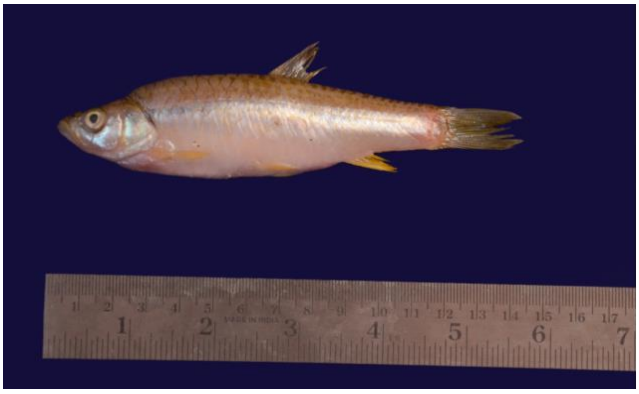


Fig 1: seasonal variation in fishes during 2012-2013.



Rosbora daniconiu

Puntius sarana

Ompok cavasius

Mastacembelus armatus

Catla catla

Cirrhinus mrigala

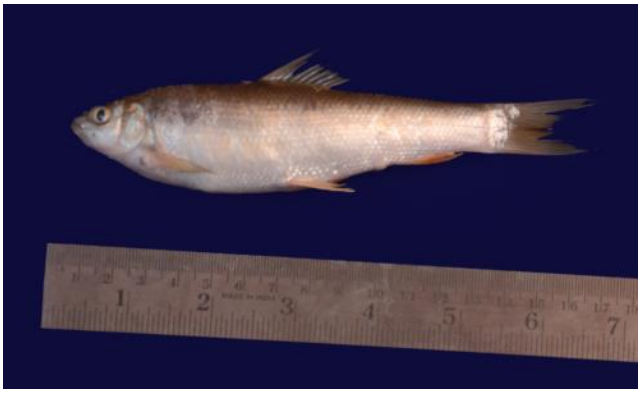
Channa gachu

Garra mullya

PHOTOPLATE I :(FISHES)

Mystus cavasius

Labeo rohita



Cirrhinus reba

Schistura denisoni

Glossogobius giuris

Cyprinus carpio

PHOTOPLATE – II

CONCLUSION:

To know the economic value of reservoir fish fauna play important role, because fisheries are the main economic source in Morna reservoir, therefore it is essential to introduce systematic management strategies both for conservation and sustained fish production. To attract increasing investment from private sector. Substitute traditional method by introduction of advanced technology in operation of reservoir fishery. It is a great need of conservation of depleted and endangered species of fish and fishery resources. It is also important to know how from the reservoir sources we up lift the fisherman's co-operative society and how to increases the socio-economic status of traditional fisherman.

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RESEARCH ARTICLE

Spider Density & Diversity in Agroecosystem of Akola district (Vidharbh) India

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ABSTRACT

Spiders are one of the predatory fauna found in agriculture fields which feeds on a wide range of insect pests and hence acts as buffer to limit pest populations. In our present study spiders were collected from cotton,banana,citrus field.Area of Akot region,disAkola,Vidharbha.Akot is located at 20.7000° N 77.0142° E. The investigation was carried out for a period of six months from September 2016 to February 2017.spider were collected from using hand picking,visual search method.During this study 14 species belonging to 12 genera and 7 families. Araneidae ,Salticidae ,Oxyopidae , Philodromidae ,Scytodidae ,Uloboridae ,Lycosidae .Araneidae represented maximum number of species. The richness of the spider species based on the fluctuation in different months by the seasonal variation.

Keywords: Spider density &diversity agroecosytem in Akola region Cotton, banana and Citrus.

INTRODUCTION

Cumulative studies made on spider from three major agricultural fields of Akola district Vidharbha. The agricultural ecosystem in Akola district is entirely dependent on rainy season as there is hardly any irrigation facility available in this area. Also, the agriculture fields are continuously been disturbed by farmers for getting fodder (weeds grown in between the main crops) to feed their cattle's. Spiders belonging to the order Araneae are generalist predators and one very potential biological agent in controlling insect pests in agricultural ecosystems (Marc1999). Spiders are ubiquitous in terrestrial ecosystems and abundant in both natural and agricultural habitats (Turnbull 1973). They play an important role in regulating insect pests in agriculture ecosystems. Spider feed on insect and other Arthropods. They play important roles in pest's control. 46'617 species of spiders have been identified in the world (World Spider Catalog Version 18.0) Family of spiders that are often found in agro-ecosystems and play an important role in the natural control of insect pest species are members of the Araneidae, Linyphiidae, Lycosidae, Oxyopidae, Salticidae, Tetragnatidae, and Thomisidae (Susilo F. 2007). Spiders are considered to be of economic value to farmers as they play valuable role in pest

management by consuming large number of prey in the agriculture fields without any damage to crops (Rajeshwaram 2005), (Sundaeland 1999).

MATERIALS AND METHODS

The study area was located in district Akola region,. Maharashtra, India Akola is located at 20.7000° N 77.0142° E

MAP: AKOLA DISTRICT, VIDHARBHA

The investigation was carried out for a period of six months from September 2016 to February 2017. Sampling was conducted in 6 month at the randomly from selected cotton, banana and orange field. Sampling was done every month from quadrates. Spider were collected from 1 quadrates (1sq.m x 1sq.m) placed at four corners and centre of 10 sq.m x 10 sq.m area by vivvsual search and hand picking method. Spiders were preserved after proper stretching into 70% alcohol. Morphological characters were noted down. Identification was done on basic of 0morphometric characters of various body parts and genitalia. The help was mainly taken from the keys and catalogues provided by Biswas&Biswas (2003,2004) Nentwig (2004) and Plantik (2004), world spider catlogoue version 15 (2015)and various literature and information and photographs available on internet and other relevant literature

RESULTS AND DISCUSSION

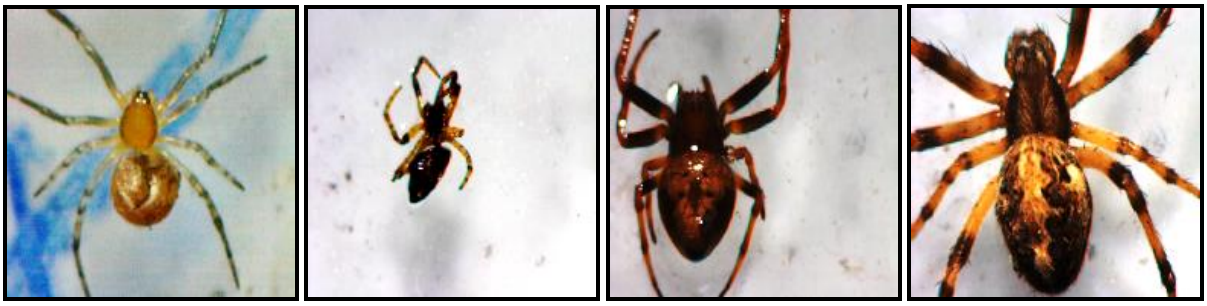
Present study made on spider density in agroecosystem of Akola region . At random sampling were made from cotton, banana, and citrus cultivated area during this study we collected 14 spider species belonging to seven families (Table 1).

The population dynamic of spider collection yielded 14 species belonging to 12 genera and 7 families. Among the seven families, Araneidae 42%, Salticidae 14.28%,Oxyopidae 14.28%,, Philodromidae 7.14%,Scytodidae 7.14%,Uloboridae 7.14%,Lycosidae 4.14%.Araneidae represented maximum number of species followed by Salticidae, Oxyopidae, Philodromidae, Scytodidae, Uloboridae, Lycosidae. (Table 1).

Generic density & diversity study show 4 genera belonging to family Araneidae, one genera to lycosidae, two genera represent by oxypidae, one genera to philodromidae two genera belonging to salticidae and one genera to uloboridae (table 1).

Table 1: Taxonomical density & diversityof spider from different habitat of Akola district during September 2016-February 2017.

Family	No. of Genera	No.of Species	% of Species
Araneidae	04	06	42%
Lycosidae	01	01	4.14%
Oxyopidae	02	02	14.28%
Philodromidae	01	01	7.14%
Salticidae	02	02	14.28%
Scytodidae	01	01	7.14%
Uloboridae	01	01	7.14%
Total	12	14	



1. *Araaneus sp.* 2. *Cyclosa sp* 3. *Eriovixia sp.* 4. *Neoascona theisi sp*

5. *Neoscona oaxacensis female* 6.*Neoscona female* 7. *Neoscona male* 8. *Neoscona oaxacensis female*

9. *Neoscona oaxacensis male* 10. *Neoscona theisi female* 11. *Neoscona mukharji* 12. *Paradosa sp.*

13. *Oxypuss sp.* 14. *Oxyopes macilentus* *15. Haarvest man* 16. *Phintela vitata*

17. Stenaelurillus albus 18. *Spitting spider* 19. *Uloborous sp.*

PHOTO PLATE 1 : Dorsal view 1- 11: ARANEIDEA; Fig. 12 LYCOSIDAE; Fig. 13-14 OXYOPIDAE; Fig. 15- *PHILODROMIDAE*; Fig. 16-17 SALTICIDAE; Fig. 18- SCYTODIDAE; Fig. 19 - ULOBORIDAE :

Table 2: Taxonomical density of spider from different habitat of Akola district, during September 2016-February 2017.

Habitat	Family	Genus/species
Banana	Araneidae	1) *Araneus sp*
		2) *Cyclosa sp*
		3) *Eriovixia sp*
	Philodromidae	1) *Haarvest man*
Cotton	Araneidae	1) *Neoscona theise* female
		2) *Neoscona oaxacensis* female
		3) *Neoscona sp* female
		4) *Neoscona sp* male
		5) *Neoscona theise*
		6) *Neoscona mukharjis*
	Lycosidae	1) *Paradosa sp*
	Oxyopidae	1) *Oxypus sp*
	Salticidae	1) *Phintella vitata*
		2) *Stenaelurillus albus*
	Scytodidae	• *Spitting spider*
	Uloboridae	1) *Uloborous sp*
Orange	Araneidae	1) *Araneus sp*
	Oxyopidae	1) *Oxyopesmacilentus*

Table 3: Species richness of spider from different habitat of Akola district September2016-February 2017.

Family	Species/Genera	No.of Ind.	% of species
Araneidae	*Araneus sp*	01	0.78%
	Cyclosa sp	02	1.56%
	Eriovixia sp	01	0.78%
	Neoscona theisi female	25	19.53%
	Neoscona oaxacensis female	29	22.65%
	Neoscona sp female	18	14.06%
	Neoscona sp male	06	4.68%
	Neoscona mukharji	1	0.78%
Lycosidae	*Paradosa sp*	2	1.55%
Oxyopidae	*Oxypus sp*	5	3.90%
	Oxyopes maciletus	1	0.78%
Salticidae	*Phintela vitata*	10	7.81%
	Stenaelurillus albus	3	2.34%
Scytodidae	*Spitting spider*	3	2.34%
Uloboridae	*Uloborous sp*	20	15.62%
	Total	128	

Species density & diversity study shows that maximum species belong to family Araneidae i.e. six species they are *Neoscona theisi* followed by *Neoscona oaxacensis*, *Neoscona mukharji*, *Araneus sp, Eriovixia sp* and *Cyclosa sp*. In the studied area Table 2. Whereas Oxyopidae and Salticidae represent two species each they are *Oxypussp, Oxyopes macilentus, Phintella vitata* and *Stenaelurillus albus* respectively while remaining family represent one species each such as *Paradosa sp*, spitting spider and *Uloborous sp* respectively (Table 2). Similar results were also reported by Keshwani and Vankhede (2012) from the agroecosytem of Amravati district.

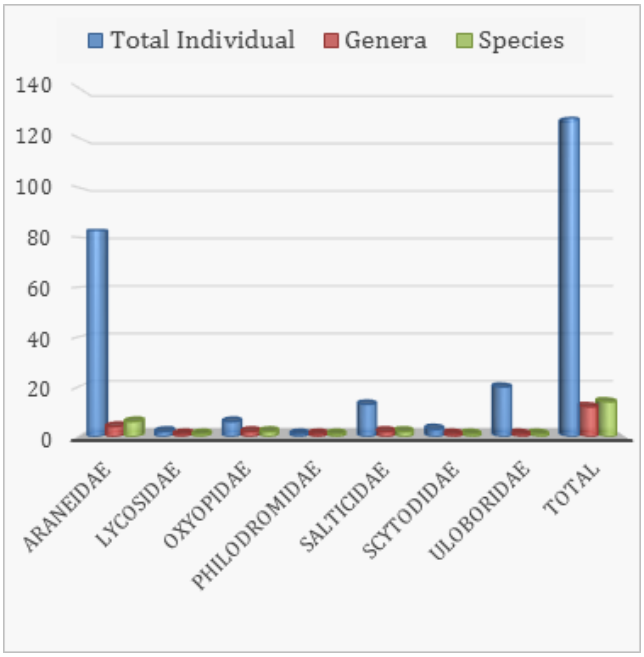


Fig. 1: Spider density & diversity of Agro-ecosytem in Akola District during September 2016 to February 2017.

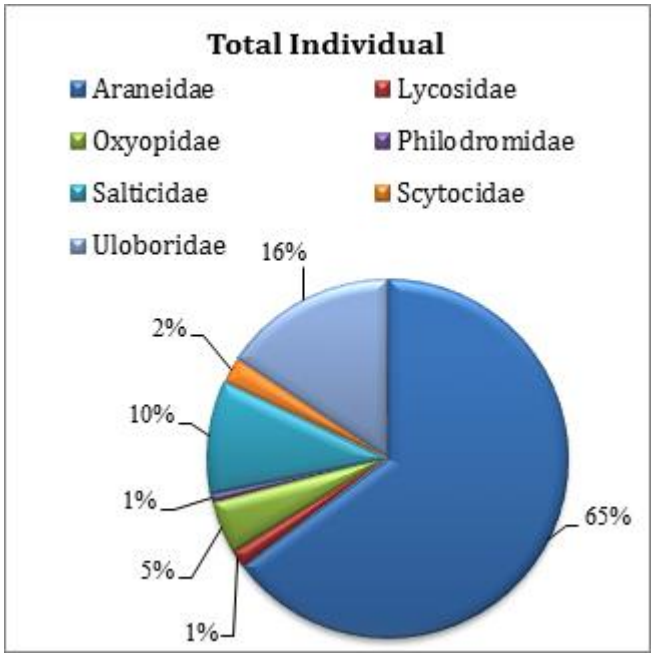


Fig. 2: Species richness of agro-ecosytem Akola District during September 2016 to February 2017

Comparative microhabitat study reveals that banana agro-ecosystem shows more spider diversity followed by Cotton, Banana and Orange agroecosystem.

In the present study we collected 128 individuals of the different species belonging to the six family (Table 3) .

Species richness study reveals that *Neoscona oaxacensis* (22.65%); *Neoscona theisi* (19.53%) *Neoscona sp* (14.06%); *Cyclosa sp* (1.56%); *Neoscona sp* male (4.68%) and *Araneus sp, Eriovixia sp, Neoscona mukharji* (0.78%) in the collected sample (Table 3 and photoplate 1-6).

Graphical study shows that member of family Araneidae predominant throught the period of investigation (Fig. 1)

DISCUSSION

In the present study, Fourteen species of spiders belonging to seven families in Akola district collected and identified. These spiders were belonging to the family Araneidae, Lycosidae, Oxyopidae, Philodromidae, Salticidae, Scytodidae, Uloboridae. In this study two species of spiders were observed, one is web weaver and another one is non – web weaver. The web weaving spiders were belonging to the family Araneidae and Lycosidae. The non-web weaving spiders were belonging to the family Saltididae, Oxyopidae. The seasonal variation of spider population dynamics from thissites have been observed in the cotton field, maximum web – weaving individual had been found in cotton field November while less number of individual, were recorded during February. The study was resulted to identification of fourteen species belonging to twelve genera and eight families. The major families were, Araneidae, Oxyopidae and Salticidae, Scytodidae. Spiders are ubiquitous predators that are abundant and diverse in agricultural ecosystems. Spider assemblages have the ability to limit population growth of arthropod pests alone or in combination with other natural enemies (Mansour *et al.,* [1980], Oraze and Grigarick (1989), Riechert and Bishop [1990]; Carter and Rypstra (1995).

CONCLUSION AND SUGGESTION

Spiders are common and occur in high numbers in cotton fields, where they are also some of the very first predators to colonize the fields. In cotton fields they occur on the plants as well as the soil surface. Spiders

have a very wide range of prey, including all stages of a pest such as eggs, larvae, pupae and moths. They can show a reproductive response to increased numbers of a pest and prey preferentially on pests occurring in large numbers Owing to the different guilds they occupy various families are affected differently by pesticides. Their presence in cotton fields should be encouraged and steps should be taken to protect them from harmful chemicals. Although spiders may be incapable of controlling major pest outbreaks by themselves, their role in a complex predatory community could be important in regulating pest species at low densities early in the season and between peaks of pest species activity. They may play an important role in keeping pests at endemic levels and prevent outbreaks from occurring in the first place. The total collected sample of spider comprised 128 individuals consisting of 14 species, 12 genera, and 7 families. We collected from Cotton, Banana, Orange habitat.

In present study during Sept. 2016 to Feb. 2017 the population dynamic of spider collection yielded14 species belonging to 12 genera and seven family in regions. Comparative study of spider density&density in different habitat reveals that more spider were recorded from Banana, Cotton and Orange field among the seven family observed.

Highest species were found in Araneidae family six species followed by Uloboridae, Salticidae, Oxyopidae, Scytodidae, Philodromidae respectively.

Spider is important biological agent which help to control pest population in agroecosytem so there is need to use spider in agroecosytem for maintaining harmonious nature of environment

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

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RESEARCH ARTICLE

Histopathological study of *Clarias batracus* (Bloch) infected with *Lytocestus indicus* Moghe

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ABSTRACT

The present study deals with Histophathological Study of Cestode parasites from some fresh water fishes from Amravati Division. Pathogenic effects of cestode are due to attachment of the adult parasite in the gastrointestinal tract and also to the encapsulation of larval stages in the tissues. Cestode live in a very hazardous environment as on there is continuous movement of the gut lining, food gut surface and the nature of its related glands, they have a hold fast organ (scolex) for attachment, which may be acetabulate with 4 suckers, bothridiate with 4 bothria (muscles hold fast organs), or bothriate with 2 bothria (holdfast grooves), some cestode scolices may also be armed with hooks and spines or have a retractable rostellum or proboscis covered with fine hooks.

Key words: Scolex, Suckers, Bothridia etc.

INTRODUCTION

Parasitism is intimate relationship between two organisms in which one (the parasite) lives on, off or at the expense of the other (host). The parasitism is having the ecological relationship between two different organisms. The parasite is metabolically or physiologically depending upon it's host. Heavily infected host may be killed by their parasites. The reproductive potential of the parasite exceeds that of their hosts. The helminth infections are very common in man, domestic animals and wild-life. Mostly the poor tropical and sub-tropical areas with poor socioeconomic status, endemic helminth infections are very common.

Fish diseases and histopathology, with a broad range of causes, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure, it is a mechanism which can provide an indication of fish health by determining early injury to cells and can therefore be considered an important tool to determine the effect of parasites on fish tissue.

Data on the pathological mechanism of Caryophyllid cestodes were presented by Mackiewicz and Cosgrove (1972), who made a comparative pathological study on the mode of attachment and scolex morphology of 15 Caryophyllid species. These authors found that Caryophyllid species without attachment organs could cause considerable pathology at the attachment point. They reported about some mechanical displacement and ephithelial loss adjacent to the scolex proper and a narrow eosinophilic interface layer at the neck region.

The physiological conditions of a specific species depend mainly on the type of site which is available; this may be favourable or unfavourable where the parasites get sufficient nourishment. The type of diet available will have profound effect on the growth rate of the cestode parasites and also the distribution of cestode is likely to be related not only to the physiochemical conditions within the gut, but also the actual topography of the gut surface and the nature of the related glands.

In low to moderate infections, pathological effects are localized around the attachment of the adult worm. The extent damage is proportional to the depth of penetration of the scolex. It is negligible when parasites are attached to the ephithelial mucosa only and becomes extreme, with extensive qranuloma and subsequent fibrasis, when the scolex is anchored in the muscle layer or entirely perforates the intestinal wall (Paperna & Zwerner, 1976, McDonough & Gleason, 1981, Kabata 1958). The depth of penetration of some species may vary in different host fishes (Tarachewski. H 1989). Although reports of the presence of the tapeworm, *Proteocephales* sp., in liver of freshwater fishes have been discussed (Wardle and McLeod, 1952). Thanapon Yooyen *et al.* (2006) also found one species of cestode, *Senga chiangmaiensis* in the liver of *Mastacembelus armatus*. Often no injury results from intestinal cestodes unless they are present in high numbers, but an inflammatory reaction may occur in association with mature worm and plerocercoid larvae that exert mechanical pressure on internal organs.

Plercocercoids migrating in the visceral cavity can produce adhesions that are very damaging to fish and can even cause death when vital organs are severly injured. The large plerocercoids of *Lingula intestinalis* can cause great damage to small fish even rupturing the body. The pathogenicity of adjacent cestodes of various orders described by Rees G, in 1967. In fishes Mevicar (1972) described host parasites relationship of *Acanthobothrium, Phyllobothrium, Echinobothrium*. Murlidhar and Shinde (1987) observed histopathology of *Acanthobothrium, Uncinathum* of fish *Rhynchobatus djeddensis*. Caira (1994) observed a comparison of mode of attachment of histopathologenicity of tapeworm representing two orders infecting the spiral intestinal of the nursa shark, *Ginglymostoma cirratusa.*

At the same, it is known that tapeworm ingest nutrition by digesting the intestinal content and partially by damaging the intestinal wall with the help of their proteolytic enzymes while they protect themselves from the effect of host-produced proteoytic enzymes with their protease inhibitors. Mackiewicz *et al*. (1972) supposed that proteolitic enzymes or other lytic secretion played a role in pronounced tissue reaction. Adult cestodes are not uncommon parasites in the digestive tract of fishes. However compared to larval stages, adult helminth in general and cestode in particular are looked upon as of not having much adverse effect on their fish hosts. Never the less quit a few reports on the pathogenicity of cestodes on fishes are available (Vik .R, 1957). But often the pathology of infection is reported only in very general terms and the exact nature of damage to fish hosts by adult cestodes is not fully examined. This work is an attempt to bring out the different aspect of pathology of infection of fresh water fish *Clarias batrachus* (Linnaeus).

Fish is a cheap and important source of protein. It contains lipids, minerals, oils and vitamins. *Mastecembelus armatus*, *Mrigal, Labeo rohita, Clarius batracus, Channa orientalis, Catla, Rohu* and etc. are widely distributed in India and it occurs mainly in quiet waters, lakes, pools but may also occur in fast flowing rivers. The fish is generally classified as omnivores or predators feeding mainly on aquatic insects, fish and higher plants debris. In most part of the world, fish production is mainly from the wild. As the world population grows, fish resources are being depleted at an increasing rate as a result of environmental degradation, over harvesting, pollution thus fish production could no longer meet the demand of the growing population. This had led to increase in the involvement of stakeholders in aquaculture. This method has also been plagued by the problems of overcrowding, poor environmental conditions and pollution which often result in reduced immunity of fish and higher susceptibility to parasites and diseases.

MATERIALS AND METHODS

Freshwater fish *Clarias batrachus* (Linnaeus) were brought to the laboratory for examination. During the parasitological examination the intestines were cut open and examined under stereomicroscope to see the degree of infection. The tapeworms were collected, placed in saline solution, freed from the adhering mucus by gentle shaking, they were flattened, processed and stained for morphological studies and were identified as *Lytocestus indicus* with in short time 2 to 3 cm long pieces of proximal intestinal and liver segments containing tapeworms were fix in Bouin's solution for 24 hrs, as the tissue undergoes autolysis rapidly after death and rapid fixation is essential. The fixed material were transferred and processed through ascending grades of alcohol, dried in a wax miscible agent and impregnated in wax (M.P 58º to 60ºC). Sectioning were carried out on a rotary microtome at 6Em. Sections were floated on warm water at 48ºC and mounted on chemically cleaned slides coated with egg albumin. The mounted, unstained sections were dewaxed in three stages of xylene at 1 minute each and stained with most widely used standard haematoxylin and eosin stain, staining was carried out using haematoxylin and eosin staining technique (Bullock, 1963). This stained is often sufficient for identification of larger parasites such as helminthes, in this method the nuclei of cells are stained by the haematoxylin, the cytoplasm is coloured by the eosin. Stained mounted sections were examined under light microscope for good ones that were selected for photomicrography.

RESULTS AND DISCUSSION

Histopathological sections from intestine of *Clarias batrachus* (Linnaeus, 1758) infected with *Lytocestus indicus* cestode parasite. After their first detection in March 2015, specimen of the cestode *Lytocestus indicus* were found on several occasions, during the dissection of *Clarias batrachus* (Linnaeus) submitted for routine diagnostic examination from Khadak Purna Dist. Buldhana (M.S) India. In histological section made from the foregut the non segmented strobila of the worm, section in more or less longitudinal direction could be seen in the gut lumen. At the site of worm penetration into the mucous membrane the damage of the epithelium was well visible. In areas more distant

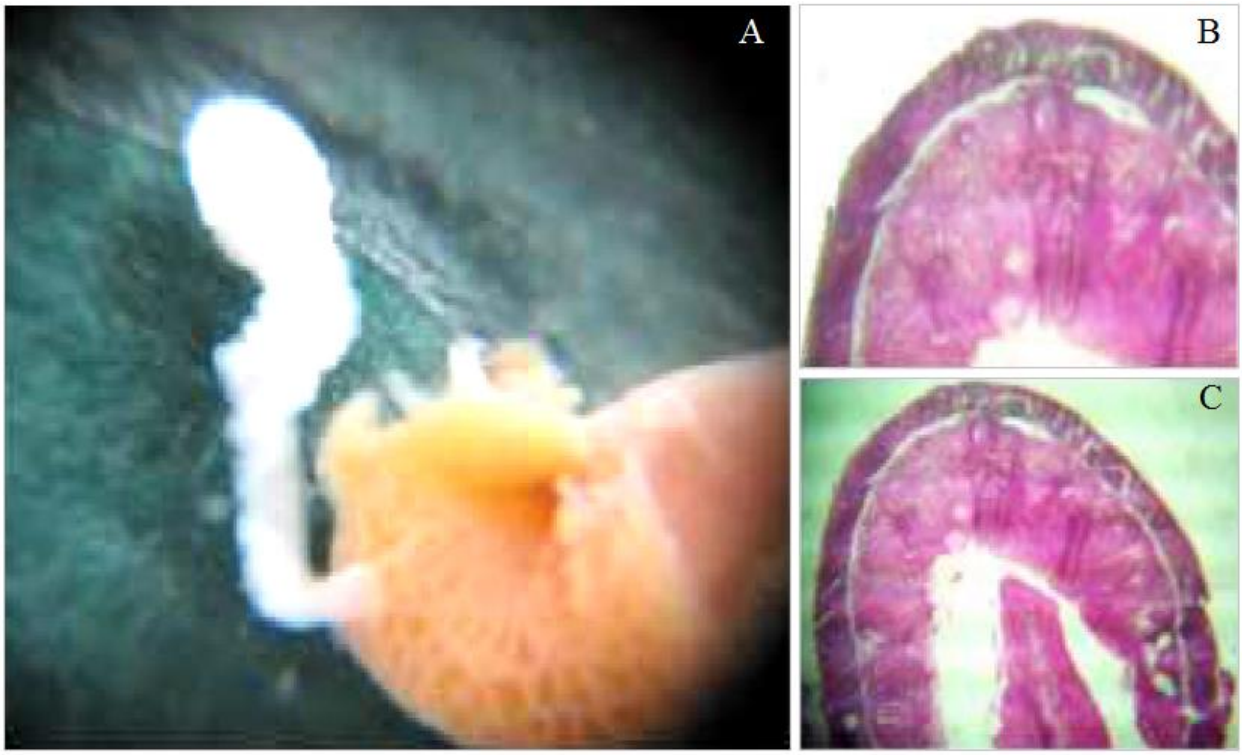

Fig. 1: Histopathological sections from intestine of *Clarias batrachus* (Linnaeus, 1758) infected with *Lytocestus indicus* (Moghe, 1925) cestode parasite
A: Attached cestode parasite **B:** Non infected Intestinal tissue **C:** Infested Intestinal tissue

from the point of entry the gut wall was still covered by an intact epithelium. Which gradually became narrower, and at the site of the penetration only epithelial debris and damage connective tissue cell could be found. Moreover in one case the complete absence of epithelium was noticed where the worm scolex was in direct contact with the connective tissue cell and damaged capillaries of the lamina propria.

In the present study case the damage of *Lytocestus indicus* is similar to the damage reported by Satpute and Agrawal (1974) also noticed shortening of villous processes and inflammatory response in the submucosa and serosa of *C. batrachus* infected with *Lytocestus indicus*. According to Karanis and Taraschewski (1993), in *Caryophyllaeus laticeps* infection of cyprinids the scolices of the worms caused local compression of the host's gut epithelium at their site of attachment, where vacuolation of the ephithelial cell and rupture of the brush border could be observed.

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

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RESEARCH ARTICLE

Dieldrin (Organochlorine) effect on reproduction of Earthworm at different toxication periods

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ABSTRACT

In the present work we establish that the lowest dose concentration of dieldrin from 4mg/kg soil to 8mg/kg soil showed non-significant impact on the reproduction of earthworm in all treated periods, but the result value produced near to significant on the replication of the crawler. It was also found that the 12mg/kg soil to 16mg/kg soil concentration of dieldrin showed non-significant difference in the breeding of the earthworm till 40, 50 and 60 days of toxication periods as compared to control. But same toxicant at highest concentrations dose at 16mg/kg and 20mg/kg soil of dieldrin produced a significant effect on the replication of the earthworm as compared to control.

Key word: - Earthworm, dieldrin, reproduction, cocoon, soil.

INTRODUCTION

The crawler is the usual epithet for the greatest number of Oligochaeta in the phylum annelid**.** Red worms are common soil organism in most environments and act as an important role in structure and fertility of soil ecosystems (Bartlett, *et al.,* 2010). Most toxicity tests for cypermethrin on earthworms had been conducted using full blown earthworms (Alshawish, 2004). However, some upshot areas showed that juveniles are practically more confidential to EDCs than adults (Booth and O'Halloran, 2001). Earthworms are universal in a wide Cordilleran belt of soils and may stand for appropriate 80% of the every one soil biomass. The species acclimate with a liberal hand to laboratory final notice and it's an arm and leg sensitivity to its circumventions makes them single of the virtually congruous bio-indicator for soil chemicals (Spurgeon, *et al.,* 2004).

Earthworms play a substantial role in decomposing litter material and in structuring soils. By burrowing they create holes and pores in the land and stabilize these structures with their slime see for an overview (Edwards, 2004). As a sound indicator of land quality, earthworms were used as testing organisms by OECD in early 1980's for the registration of industrial fertilizers and pesticides before implementing them into

the soil. More than forty of them are currently registered, although all operate the risk of acute and sub acute toxicity. Organophosphates are used in husbandry, in the home lawn, in gardens and in veterinary practice, etc. All apparently share a common mechanism of cholinesterase inhibition and can cause similar symptoms of toxicity since they share this line of action, exposure to the same organophosphate by multiple paths can run to serious additive toxicity or synergistic effect (Savage, *et al.,* 1988).

MATERIALS AND METHODS

Experimental animal

Earthworm, *Eisenia foetida* (Savigny, 1826) is a recommended earthworm test species by Organization for Economic Co-operation and Development (OECD, 1984a) and European Economic Community (EEC, 1985).

Animal collection

Earthworm, *Eisenia foetida* brought from commercial suppliers, Nursery Department of Forest, Wadali, Amravati and adopted as the test species, recommended by (OECD, 1984) guideline for testing of chemicals no. 207, earthworm, and acute toxicity tests.

Chronic test for reproduction

This test was similar to the described above, the difference in the reproductive test were cocoon production juvenile survive rate. The cocoon was assessed after 28 days by wet sieving the soil and collecting all cocoons at different time intervals and different pesticides concentrations and the rate of hatchling and the rate of survival of juvenile was also assessed. The number of cocoon and juvenile were counted and compared at the end of reproductive test.

Statistical analysis

Mortality and LC_{50} were observed and calculated. Probability analysis was used for the result of filter paper contact test method and for standardization artificial soil test mortality was assayed according to Buchatsky (*et al.,* 2007). Correlation analysis and two way ANOVA significance tests ($p<0.05$) were used for the assessment of selected pesticide on survival, biomass, growth and reproduction of earthworm. For the filter paper contact test for cythion and dieldrine, based on the resulting 48h LC_{50} values, the fertilizers were classified as super toxic (<1mg/cm^2), extremely toxic (0.8–0.9mg/cm^2), very toxic (0.6–0.7mg/cm^2), relatively nontoxic (>0.5mg/cm^2).

RESULTS AND DISCUSSION

This test was similar to the growth, but the test endpoints were a cocoon production after 30 days to 60 days and the number of hatchlings, and their development after 60 days. The worms were hand-sorted after 30 days; the numbers of cocoons produced were counted and returned for further incubation. The juveniles were weighed after 60 days and their maturity was determined from the presence of a fully developed clitellum. Chronic test was conducted to analyze the impact of selected pesticide on (cocoon) reproduction of the earthworm. It was a long duration test. The duration of time intervals were 10, 20, 30, 40, 50 and 60 days for both the pesticides and dose of different concentrations for dieldrin 4, 8, 12, 16 and 20mg/kg soil. After 30, 40, 50 days of intoxication, number of cocoons deferred than the control in all treatment groups. The earthworms are a farmer's friend and it is widely used for preserving the richness of the land. Cocoon engenderment in earthworm exposed to dieldrin for 60 days were lower than that for 30 days at concentrations.

Table 1: Impact on the reproduction (cocoon) of earthworm exposed to dieldrin at different toxication periods.

Toxicated periods (day) and concentration (mg/kg)	10	20	30	40	50	60
Control	0	0	0	8±2.8	12±3.4	19±4.3
4mg	0	0	0	0	5±2.2*	8±2.8*
8mg	0	0	0	0	3±1.8*	6±2.6*
12mg	0	0	0	0	2±1.4*	6±2.4*
16mg	0	0	0	0	2±1.4*	7±2.6*
20mg	0	0	0	0	9±3*	6±2.4*

* Significant differences ($P < 0.05$) were found between treatment and control group were found 0.01.

It is evident from (table and figure) dieldrine intoxicated earthworm showed significant decreased the rate of cocoon production in treating earthworm than the untreated earthworm. The reduction was found in rate of cocoon production due to toxic effect of dieldrine on earthworm. Estimation of the rate of production of cocoon in intoxicated earthworm determines that the duration and dose dependant effect. At the present dose of dieldrine, rate of cocoon production was found to be significantly decreased as compared to control reproductive earthworm. The significant result was observed in the rate of cocoon production during all periods of toxication. It is evident from (table and figure) that the earthworm intoxicated with dieldrine determined decrease in the production of cocoon as compared to control. From (table) the reduction in the rate of cocoon production in all treated earthworms and the result was significantly in dose and duration of days. During the chronic test it was found that the result was significantly different as compared to control and also observe that the delay in duration of cocoon production than control reproductive earthworm. (Booth and O'Halloran, 2001) reported Chlorpyrifos had adverse effect on fecundity in earthworm exposed to 5 mg/kg chlorpyrifos after 8 weeks reported. (Xiao, *et al.,* 2006) noted the field application rate (5-10 mg/kg) of acetochlor had no long term effect on the reproduction of *Eisenia fetida* but at higher concentration (20-80 mg/Kg) produced toxicity to *Eisenia fetida.* Epinoza-Navarroand and Bustos-Obreg stated treated *Eisenia fetida* with organophosphate insecticide Malathion and found that Malathion decreased the spermatic viability in spermatheca, altering the cell proliferation and modifying the DNA structure of spermatogonia (Espinoza-Navarroand and Bustos-Obreg,´2004). Sperm count also seems to be a very sensitive marker (Savage, *et al.,* 1988 and Neuhauser and Callahan, 1990). Malathion could affect the sperm count, but in addition, its metabolites could affect sperm quality (Espinoza-Navarro and E. Bustos-Obreg´on, 2004.).

CONCLUSION

Different parameters were analyzed concern to toxic effect of two selected pesticides dieldrine (organochlorine) on earthworm. Different doses of dieldrine ranging from 4, 8, 12, 16 and 20mg/kg soil for duration of 10, 20, 30, 40, 50 and 60 days as chronic test for reproduction. Reproductions (cocoon) were decreased till the end of the experiment for both duration periods and for selected toxicants. The decreased in the different parameter of earthworm may be due to the change in the biochemical and physiological function of earthworm. Experimental data showed that earthworm was more sensitive dieldrine. It also determined that decreased in the morphological parameters as period of toxication increased means that the toxicity of pesticide is directly proportional to the experimental periods.

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

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RESEARCH ARTICLE

Toxic Effect of fluoride on Neuclic acids and Lipoproteins of rat, *Rattus rattus* (Wistar)

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ABSTRACT

Fluoride is a ubiquitous substance found naturally in soil, water, plants, and animals in trace quantities and is also a common air pollutant in some industrial productions. The experimental model comprised albino rats treated with different concentration of sodium fluoride for 72 days. Parameters were neuclic acids and lipoproteins.The data indicate significant reduction in DNA, RNA and HDL concentration with significant increase in LDL and VLDL concentration.

Keywords: Albino rat, sodium fluoride, LDL, VLDL, HDL.

INTRODUCTION

Fluorine and fluoride compounds are constituents of minerals in rocks and soils, and the main sources of fluoride exposure for humans include foodstuffs, fluoride supplements, fluoride dentifrices, and water contaminated with high concentrations of fluoride compounds from geological sources (Ozsvath,2009; Ruiz-Payan *et al*.,2005). Fluoride compounds are being utilised in the life-science industry, crops, pharmaceuticals, hygiene, cosmetics, and domestic commodities. Their production has been increasing steadily over the years (muller,2007). Excessive chronic fluoride intake results in fluorosis, characterised by a vast array of symptoms and pathological changes such as dental mottling, crippling deformities, osteoporosis, and osteosclerosis (Whitford *et al*., 1979; Zahvoronkov and Strochkova, 1981). Endemic fluorosis has now become a global concern (Fawell *et al*.,2006). Fluoride crosses the cell membrane very rapidly (Sireli and Bülbül ,2004), and is distributed in the skeletal and cardiac muscle, liver, skin, and erythrocytes (Perumal,2013; Akdogan, 2002). In vivo studies (Kaushik *et al*.,2002; Reddy *et al*.,2003 and Schiffl, 2008) have proven that fluoride to be a cell toxin. The high toxicity of NaF arises from its being a very reactive ion. The chronic toxic action of fluoride also has been investigated in the liver.

Present study focuses on the sodium fluoride induced toxicological changes in nucleic acids (DNA and RNA) and lipoprotein content in rat liver.

MATERIALS AND METHODS

Animal experiment

Albino rat, *Rattus rattus* weighting 150-200 g, were used. Animals were purchased from wadhwani pharmacy Collage Yavatmal and acclimatized for two weeks in Animal House in the Department of Zoology Govt. Vidharbha Institute of Science and Humanities Amravati. The Institutional Animal Ethical Committee already approved this study for the use of Rat. The rat were housed in well-ventilated animal house and caged also well, at room temperature and exposed to 10-12 h of daylight.

Rats were divided into four groups having five animals each. 1st group was used for control and 2nd, 3rd and 4th groups were ingested with 0.02 gm, 0.04gm, and 0.06 gm of fluoride water respectively for 72 days. Animals from each dose group were deprived of food overnight and sacrificed at the end of 72 days. They were stunned by a blow on the head and operated. The liver was removed with adhering material by dipping in chilled normal saline and homogenized.

Chemical; All the reagents were purchased from Chaiga Traders, Yavatmal and were of analytical grade.

Biochemical Analysis

The estimation of DNA and RNA were done from liver tissue by using Giles and Meyer,1965. and Mejboum,1939 respectively. And lipoproteins by measuring protein concentrations.

Statistical analysis

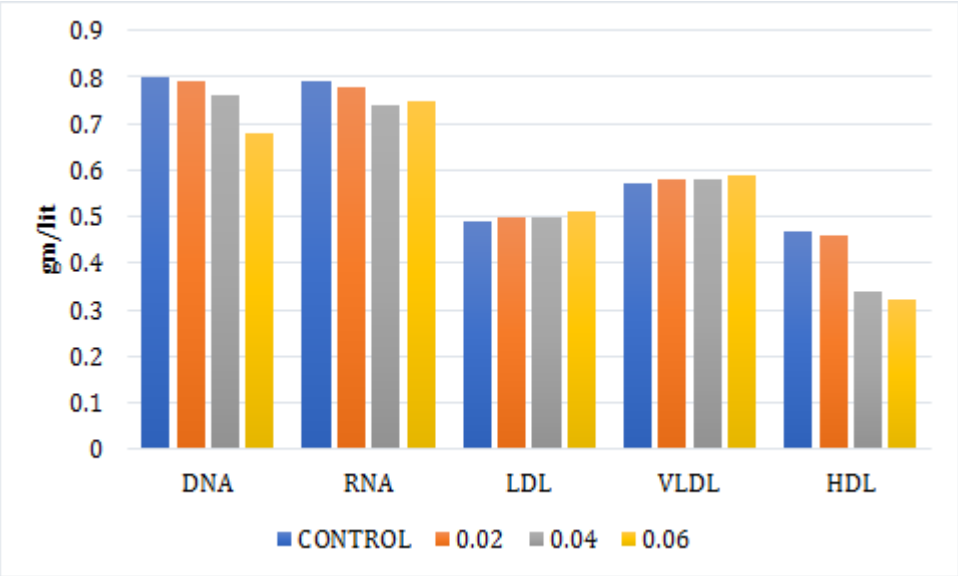
The results were expressed as the mean ± SEM. The data were statistically analyzed using one-way analysis of variance (ANOVA). The level of significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 depicts the levels of DNA, RNA in the liver of control and experimental groups of rats. There was a significant (P<0.01) decrease in DNA, RNA in the liver of rat. As shown in Table 1 LDL, `VLDL were significantly increased and decrease in HDL with higher doses of fluoride content as compared to control.

Table and fig 1 Effect of fluoride on nucleic acid and lipoprotein contents in rat liver

Parameter	Control	0.02 gm/lit	0.04 gm/lit	0.06 gm/lit
DNA	0.64±0.80	0.63±0.79***	0.57±0.76***	0.47±0.68***
RNA	0.63±0.79	0.62±0.78**	0.55±0.74**	0.54±0.75***
LDL	0.24±0.49	0.24±0.50	0.25±0.50*	0.25±0.51*
VLDL	0.33±0.57	0.34±0.58*	0.34±0.58***	0.35±0.59***
HDL	0.22±0.47	0.21±0.46*	0.12±0.34***	0.10±0.32***



Values are expressed as Mean ± SE *= p<0.05; **=p<0.01; ***=p<0.001; where nothing is shown =Non-Significant

Fluoride-induced reduction in DNA, RNA and protein content might be due to direct or indirect effect of fluorosis (Jha, *et al.,2012*). The value of DNA & RNA decreased after NaF treatment (Chinoy and Shah,2004; Trivedi *et al.*,2008 and Sarkar *et al*.,2014) this decrease might be due to the inhibitory action of fluoride on DNA synthesis or to alteration in the synthesis of RNA (Verma *et al*., 2007). Fluoride produced free radicals directly or indirectly alters the activities of DNA and RNA, which affected the transcription and translation processes, ultimately would affect the protein synthesis (Verma and Chakraborty,2008; Patel and Chinoy,1998 and Memon and Chinoy,2000).

Chronic fluoride intake has been recorded to cause hyperlipidemia and oxidative stress by many investigators (Barbier *et al*., 2010; Rupal *et al*., 2010 and Rupal *et al*., 2011b). Rupal and Narasimhacharya (2012) reported significant high level of total lipid, TC, LDL-C and VLDL-C after exposure of rats to 100ppm of NaF for four weeks.

In the present study, a significant increase level of VLDL and LDL were observed. The changes in the serum lipid profiles and other lipid compounds noted in the fluoride treated rats may be explained by the increased activity of HMG-CoA via accumulation of ROS releasing inflammatory cytokines in the liver (Afolabi *et al.* 2013). The changes in the activity of HMG-CoA reductase may depress LDL receptor gene expression. Defects in LDL receptor interfere with cholesterol uptake from the blood stream, which in turn causes excess cholesterol synthesis in liver and high levels of plasma cholesterol and LDL-C (Rupal *et al*. 2012). Lipase enzyme responsible for the controlling of triglyceride accumulation in the liver, but the buildup of ROS induced by fluoride inhibit the lipase enzyme and increase the triglycerides (TG) level in the tissue and serum. Increasing levels of TG leads to elevation of VLDL-C in the serum because VLDL particles are the main transporters of TG in plasma (Sharma *et al.,*2003). The overproduction of hepatic VLDL-C and impaired catabolism of TG-rich particles may lead to hypertriglyceridemia. Lowered level of plasma HDL- C implies the altered metabolism of the major HDL apoprotein A-I in the liver (Suttie & Phillips 1960).

High density lipoproteins (HDL) are mostly synthesized in the liver. Afolabi et.al performed a study on male rats exposed to 50 mg/L and 100 mg/L of F through drinking water for seven weeks and observed that both concentrations promoted hypercholesterolemia and decreased HDL level . In the present study decreased level of HDL may be due to interference of fluoride with lipid metabolism.

CONCLUSION

From the results, it is clearly indicated that 72 days of sodium fluoride exposure to rats caused a significant decrease in DNA, RNA and HDL but LDL and VLDL were elevated in the exposed rats.

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

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RESEARCH ARTICLE

Histopathological and Seasonal variation study of *Cotugnia aurangabadensis* in *Gallus domesticus*

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Gaikwad PR, Bhise JV and Sonune MB (2017) Histopathological and Seasonal variation study of *Cotugnia aurangabadensis* in *Gallus domesticus, Int. J.of. Life Sciences*, Special Issue, A8: 121-124.

ABSTRACT

Histological and seasonal study reveals the infection efficiency and pathogenecity among the infected animal. This study shows the infection of cestode parasite *Cotugnia auranabadensis* on *Gallus domesticus*. This infection indicates the pathogenic symptoms on the host. We basically infer the histological changes such as attachement of cestode to the intestinal villi through large rostellum. This brings the local deformities in the intestinal structure and function. Gastrointestinal parasitic infections are a worldwide problem which results in morbidity and mortality in tropical countries. It caused great economic losses through lowered fertility, reduced work capacity, involuntary culling, and reduction in food quality, and meat production, treatment coasts and mortality in heavily parasitized animals. The prevalence of gastrointestinal parasites, the genera of cestode parasites involved, species and the severity of infection also vary considerably depending on local environmental conditions such as humidity, temperature, rainfall, vegetation and management practices.

Key words: Cestode, Rostellum, Pathogenecity, Gastrointestinal, etc.,

INTRODUCTION

In low to moderate infections, pathological effects are localized around the attachment of the adult worm. The extent damage is proportional to the depth of penetration of the scolex. It is negligible when parasites are attached to the ephithelial mucosa only and becomes extreme, with extensive qranuloma and subsequent fibrasis, when the scolex is anchored in the muscle layer or entirely perforates the intestinal wall (Paperna & Zwerner, 1976, McDonough & Gleason, 1981, Kabata 1985).

At the same, it is known that tapeworm ingest nutrition by digesting the intestinal content and partially by damaging the intestinal wall with the help of their proteolytic enzymes while they protect themselves from the

effect of host-produced proteoytic enzymes with their protease inhibitors (Mackiewicz et al. 1972) supposed that proteolitic enzymes or other lytic secretion played a role in pronounced tissue reaction.

The seasonal variation of gastrointestinal helminthic infection shows the higher prevalence of parasites in monsoon (93%) followed by winter (74%) and summer (74%). The higher incidence of parasitic infection occurs in rainy season because too easy dispersal of larvae in pasture resulting in increased contact between the host and the parasites from the results of the studies on incidence of different types of helminthic infection, it is clear that the cestode parasites are predominated during all the seasons with 262 (43.67%) followed by Nematode parasites with 125 (20.83%) and Trematode parasites with 75 (12.50%). Higher incidences of cestode parasites may be due to conductive environment for growth of the parasites. The present study indicates that the occurrence of helminthic infection depends on suitable environment which require in its development. Padwal et al., (2007). Environmental variations were reflected in seasonal difference in the incidence of diseases. The existence and survival of parasite is greatly influenced by pollution of the environment the development of parasites need high temperature and sufficient moisture. The high prevalence occurs in summer followed by winter season (Jadhav and Bhure 2007).

The influence of diet on such clumped helminth distribution is ruled out. This conclusion is based on the reason because the general occurrence of a large number of intermediate arthropod hosts that breeding season of poultry enabled the availability of infective larvae to all the birds, irrespective of sex of birds. But still, the female domestic fowls exhibited greater prevalence of tape-worm infection during breeding season than male birds. The evidence provided by the earlier investigation of Von brand (1966), and Malhotra (1983) have demonstrated that the physiological resistance in female avian hosts might decline during breeding season, resulting thereby, in an increased invasion of a variety of helminthes in female birds compared to the male birds. A correlation of seasonal fluctuations in atmospheric temperature with distribution of cestodes. Chincholikar and Shinde (1976) provided evidences of greater influence of female sex on survival rate of *Heterakis gallinae* that of male sex in natural female chicken populations in Bulgaria. The consumption of increased invertebrate populations by laying female *American woodducks* during egg production and hyperphagia were identified to be the main reasons for increased invasion of female avian hosts by a variety of helminthes during breeding.

MATERIALS AND METHODS

The digestive tracts were carefully examined. Cestodes were collected and a complete record about the infected host, parasites is summarized. The parasites were flattened and kept in 4% formalin, stained by Harris-haematoxylin, mounted in DPX and identified for further observations.

The survey was carried out during the period of June 2013 to May 2014, at various places of Amravati Region *viz*. Akola, Buldhana, Amravati, Chikhali, Deolgaon Raja, the hosts examined for a year, were *Gallus domesticus* for cestode parasites. The poultry Birds were surveyed. The digestive tract of *Gallus domesticus* collected from slaughter houses

RESULTS AND DISCUSSION

Histopathological sections from intestine of *Gallus domesticus* (Linnaeus, 1758) infected with cestode parasite (*Cotugnia aurangabadensis*, Shinde, 1969.) Scolex of *Cotugnia aurangabadensis* Shinde, 1969 attached to the intestinal villi through large rostellum. The scolex of this worm is non-penetrative type, so the worm invades only the villi but not the crypts of Lieberkuhn. So, the attachment is superficial. Mature and gravid proglottids of the strobila are not attached to the wall of intestine and found freely suspended in the lumen of intestine. Cyst is encircled with connective tissue sheath and located deep in the submucosa, just above the muscularis externa. Few gravid segments were also found in posterior part of intestine. (Chincholkar,1976).

In this study, important parasites of vertebrates such as poultry birds were identified and those factors affecting the epidemiology of these parasite. High levels of prevalence, intensity and abundance of these parasites were generally observed around the rainy season. Thus, confirm that the weather conditions of the wet seasons were generally favorable for the

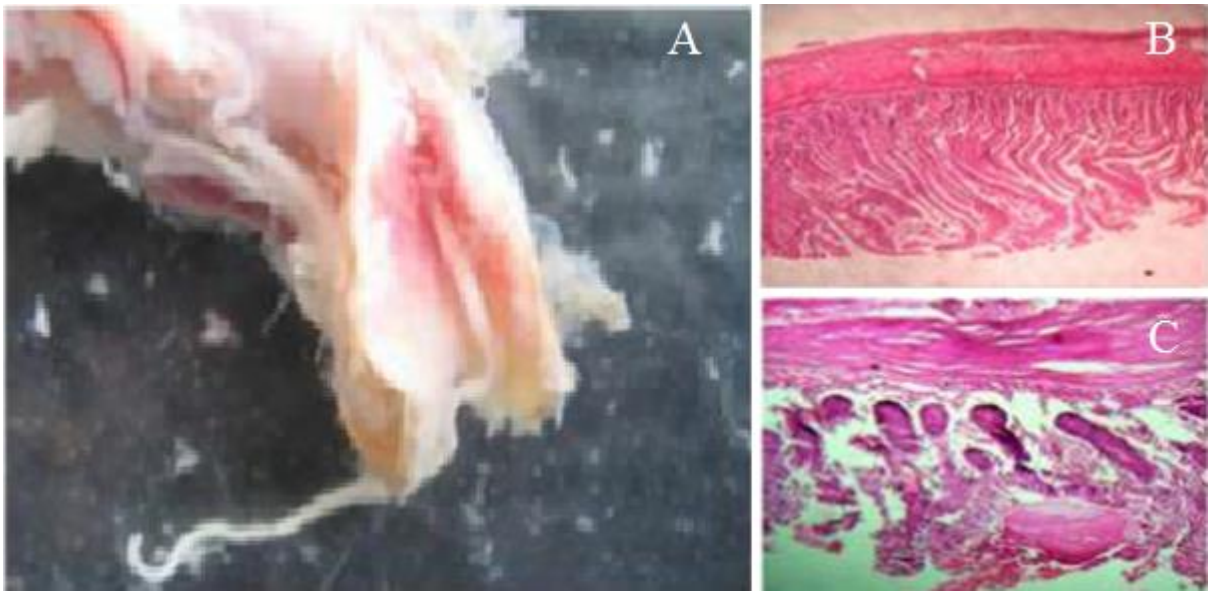

Fig. 1: Histopathology of *Cotugnia aurangabadensis* (Shinde, 1969)
A: Attached cestode parasite; **B:** Non infected intestinal tissue; **C:** Infected Intestinal tissue

Table 1: Seasonal Variation of *Cotugnia aurangabadensis* (Shinde, 1969) from *Gallus gallus domesticus* (Linnaeus, 1758) during the year 2013-14 from" Amravati Region"

Sr. No.	Month & Year	No. of dissected hosts	No. of infected hosts	No. of Cestode parasites collected	Prevalence %	Genera	Locality
1	Jul-2013	7	6	8	85.71	***Cotugnia***	Akola
2	Aug-2013	6	6	7	100	***Cotugnia***	Chikhali
3	Sep-2013	5	5	9	100	***Cotugnia***	Buldhana
4	Oct-2013	9	8	10	88.89	***Cotugnia***	Deolgaon raja
5	Nov-2013	10	6	6	60.00	***Cotugnia***	Deolgaon raja
6	Dec-2013	12	5	5	41.67	***Cotugnia***	Deolgaon raja
7	Jan-2014	8	4	5	50.00	***Cotugnia***	Akola
8	Feb-2014	6	5	5	83.33	***Cotugnia***	Chikhali
9	Mar- 2014	6	5	5	83.33	***Cotugnia***	Amravati
10	Apr – 2014	8	4	4	50.00	***Cotugnia***	Akola
11	May - 2014	8	4	4	50.00	***Cotugnia***	Buldhana

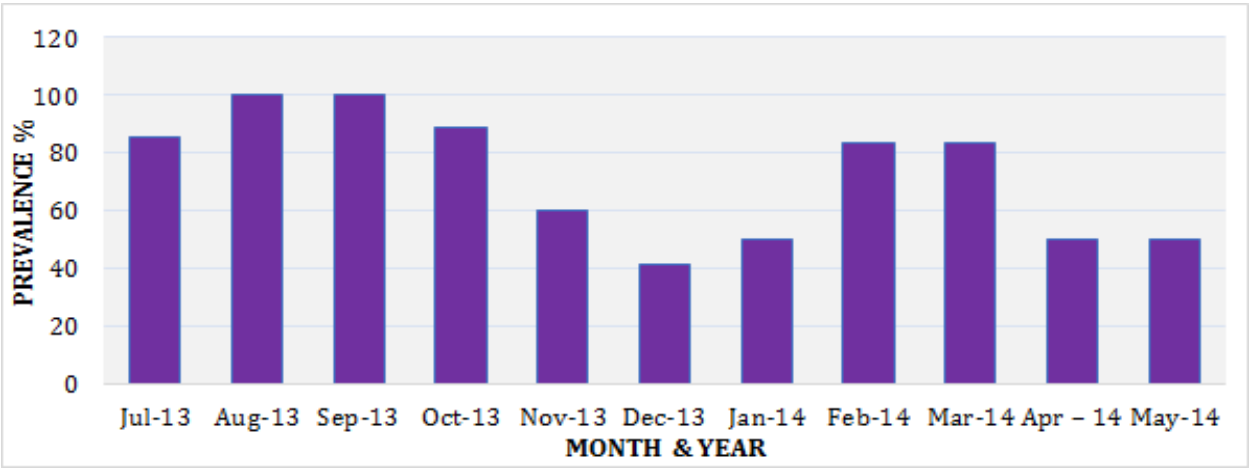

Fig. 2 : Seasonal Variation of *Cotugnia* (Shinde, 1969) from *Gallus gallus domesticus* (Linnaeus, 1758) during the year 2013-14 from"Amravati Region"

development, survival and transmission of the free-living stages of cestode. The present studies also demonstrated the roles of other factors such as age, sex and susceptibility of host.

Bird rearing is traditionally practiced in Amravati Region, M.S. (India), has to cope with many constraints, especially health related. In order to contribute to the knowledge of avian diseases in the area and to undertake improvement in traditional bird keeping, a parasitological investigation based on examining intestine and survey of cestode species was carried out for a year. Birds are characterized by relatively diverse and abundant communities of intestinal helminthes, especially cestodes, which may be related to the opportunistic habits.

The highest incidence of prevalence was observed in the period of Jul-2013 to Oct – 2013 and also Feb and Mar- 2014 of *Cotugnia* Shinde, 1969 *Sp*. in the year 2013-2014.

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

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RESEARCH ARTICLE

Effect of fluoride ingestion on trace elements on brain and liver of Rat *Rattus rattus* (Wistar)

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ABSTRACT

Fluoride (F) is highly electronegative anion with cumulative toxic effects, from prolonged ingestion that can lead to the pathogenesis known as fluorosis. Present study was aim to investigate the effect of sodium fluoride on trace elements such as Zn, Cu , Mn and Fe in brain and liver of rats. Albino rats were divided into four different group control, Group I, Group II, Group III (0.02gm/L, 0.04gm/L, 0.06gm/L) repectively treated with sodium fluoride dissolved in distilled water for 72 days. Trace elements concentration analysed by Atomic absorbtion spectrophoto-meter. In liver and brain level of Zn, Cu and Fe decreased significantly. Mn level shows significantly decreased in liver and significantly increased in brain.

Keywords: Sodium Fluoride, Brain, Liver, Trace Elements..

INTRODUCTION

Fluoride is a highly electronegative trace element which is the 13th most abundant element in the earth's crust (Jha et al., 2011). Fluoride is toxic when consumed in excess, and has lead to a condition known as fluorosis. Many vital organs and tissue in the body, such as liver (Ersan et al., 2010), kidney (Iano et al., 2014), cerebrum and cerebellum (Yaqoob 2012., Chirumari and Reddy 2007., Webb and Bradley 1966) the skeleton (Levy 2014) and teeth (DenBesten and Li 2011) may be damaged by excessive accumulation of Fluoride. Fluoride interacts with other minerals including trace elements (Nese et al., 2014). Trace minerals exist in cells and tissues of the animal body in a variety of chemical combinations, and in characteristic concentrations, which vary with the trace mineral and tissue (McDowell, 1989, 1992; Underwood and Suttle, 1999). The present study was planned to investigate the effects of fluoride on trace elements of brain and liver

MATERIALS AND METHODS

Adult albino rat, *Rattus rattus* (Wistar) were obtained from P. Wadhwani College of pharmacy, yavatmal. The rats were housed in polypropylene cages with stainless steel grill tops and were fed with standard pellet diet and given distilled water ad libitum. The animals were allowed to acclimatize to the laboratory conditions for seven days before experiments began. The rats were randomly divided into 4 groups, the first group served as controls and was given water ad libitum. The second group animals were given sodium fluoride (NaF) 0.02gm/l water ad libitium. The third group animals were given sodium fluoride 0.04gm/l water ad libitum. The fourth group animals were given 0.06 gm/l water ad libitum and maintained for 72 days. The body weight of each animal was noted before treatment and also on day 73 and rats were sacrificed and their Brain and liver were quickly excised and Metal concentrations in the tissue digest will be determined by Atomic absorption spectrophotometer at the following wavelength Zn-213.8nm; Cu-324.8nm; Fe-248.3nm; Mn-279nm.

RESULTS AND DISCUSSION

As seen in the table, depletion of zn in the rats occurred mostly in liver and brain of fluoride intoxicated rats. Similar reports were reported for zinc levels in fluorosis with decreases being reported in liver (Narayanaswamy and Piler 2010 and Krasowska and Włostowski 1981). The activity of some Zn-dependent enzymes, such as alkaline phosphatase, increases during F toxicity (Singh and Swarup 1999 ; Ranjan 2007). Oxidative stress and increased superoxide dismutase (SOD) activity with Zn involvement have also been observed in experimental F intoxication (Ranjan et al., 1999; hniak and Inkielewicz 2005). Liver is known to be as a storehouse for copper, and the kidneys and heart also maintain elevated Cu concentrations (Shenkin 2009).

Table :1

	Parameters	Zinc	Copper	Manganese	Iron
Brain	Control	24.76 ±4.97	2.75±1.92	2.75±1.65	29.32±5.41
	Experiment 1	21.97±4.68*	2.19 ±1.48*	3.25±1.80*	27.37±5.23*
	Experiment 2	21.06±4.58*	2.94±1.33**	3.98±1.99**	26.39±5.13***
	Experiment 3	19.32±4.39**	2.62±1.21**	4.27±2.22***	25.30±4.88***
Liver	Control	16.18±4.02	3.29±1.81	4.35±2.08	14.64±3.82
	Experiment 1	15.82±3.97*	3.14±1.77	4.14±2.03*	14.88±3.76*
	Experiment 2	15.70±3.88**	2.30±1.51**	3.58±1.89**	15.95±3.56***
	Experiment 3	13.28±3.64***	2.10±1.45***	3.33±1.82***	16.25±3.25***

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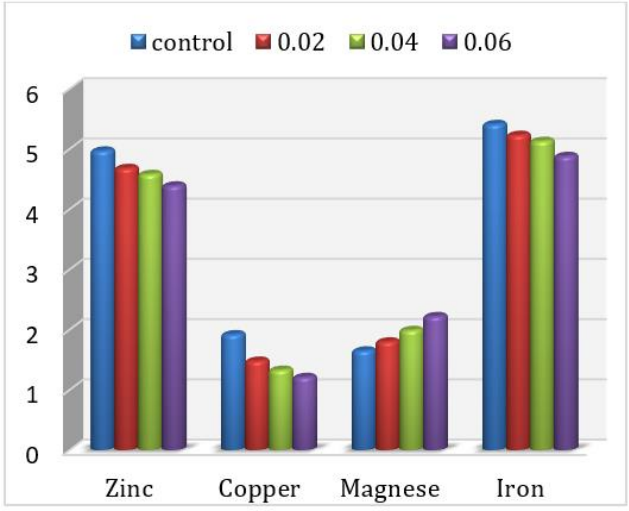

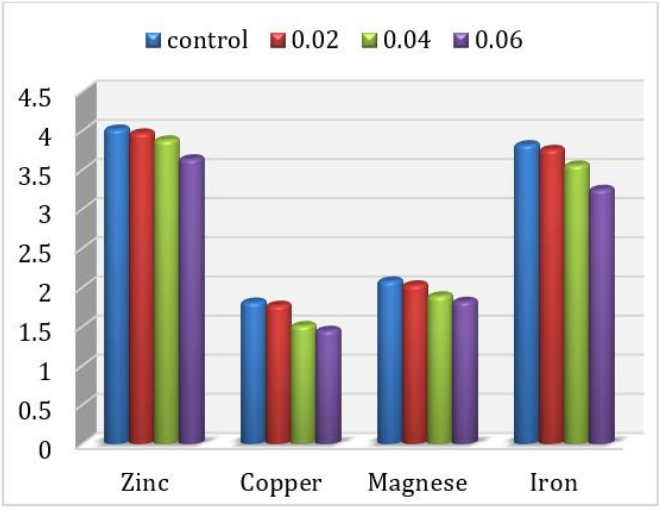

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Hepatic storage and biliary Cu secretion are predominant pathways for adjustment to fluctuations in Cu intake (Sauberlich 1999). In the present study cu level fells significantly in brain and liver of fluoride intoxicated rats. Similar results were observed by Bhatnagar *et al.,* 2003. Manganese is a cofactor in many enzymatic systems and has roles in bone formation and metabolism of carbohydrates and cholesterol (Santos et al., 2013). This enzyme is involved in fatty acid and protein synthesis as well as melanin and dopamine production (Hardy et al., 2008). After oxidation in its trivalent form, manganese is bound to transmanganin and is successfully deposited in the liver, skin, and skeletal muscle (Boullata 2013). Mn level fells significantly in liver but increase in brain Bhatnagar *et al.,* 2003.

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes c, c1, a1, etc (Malhotra, 1998). Fe is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the cytochromes (Chandra, 1990). In the present study fe also fells significantly in brain and liver. Similar reports were observed by Bhatnagar *et al.,* 2003.

CONCLUSION

Trace minerals such as zinc, copper and manganese and iron play a wide variety of biological and physiological roles in animal development and health.These minerals take part in the antioxidant defense and DNA repair, bone and tissue development, and immune function. The result of this study gives valuable information about disturbance in the metal concentration of liver and brain of fluoride intoxicated rat.

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

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RESEARCH ARTICLE

Major Cotton Pest in Akot Region District Akola, Vidharbha.

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ABSTRACT

Insects are found in all types of environment and they occupy little more than two thirds of the known species of animals in the world. Insects affect human beings in a number of ways. Many of them feed on all kinds of plants including crop plants, forest trees, medicinal plants and weeds. They also infest the food and other stored products in godowns, bins, storage structures and packages causing huge amount of loss to the stored food and also deterioration of food quality. Present study was made on major insect pest of cotton in Akot region during July -16 to Feb-17. At random collection of major pest was made from selected cotton cultivated area of Akot region. In present study we collected eight species samples from the cultivated cotton fields from Akot region area. In Akot region from the different sides like Popatkhed, Akola road, Vai area in the form of Bollworms, sucking pest, stainers,eg. *Eariasvittella* and *E. insulana*, *Pectinophora gossypiella, Amrascabiguttulabiguttula ,Aphis gossypii, Thripstabaci Bemisiatabaci, Dysdercuscingulatus*, and *Oxycarenus hyalipennis.*

Keywords: Major cotton pest , Akot region Akola .

INTRODUCTION

Insect pest are one of the major limiting factors in the cotton production. About, 1300 species of insects have been reported on cotton worldwide (Matthews and Tunstall, 1994). Out of which Caterpillars of six lepidopteron species are of great economic importance. Out of these nearly 130 species occur in India. About a dozen of these arthropods are commonly present in sufficient numbers requiring their management for better cotton yields. The key insect pests affecting cotton plant could be divided into three categories viz. boll worms, sap sucking pests and stem, leaf and foliar feeders depending on the type of damage caused. Major yield loss to the Indian cotton (even up to 60%) is due to bollworm complex consisting of three genera of bollworms viz. Helicoverpa, Earias, and Pectinophora, commonly referred to as American bollworm, Spotted bollworm and Pink bollworm respectively. Sucking pests i.e. jassids, aphids, whiteflies and thrips are deleterious during early season of the cotton plant growth and development and have the ability to build up to

serious proportions as a result of rapid breeding. The important foliage feeder includes semilooper, spodoptera, leaf roller, and ash weevil and grass hoppers. The variations are observed on geographical basis regarding occurrence of insect pests. Cotton, Gossypium hirsutum L., is subject to attack by wide variety of insect pests. The number of insect pests in cotton recorded were 1326 species (Hargreaves, 1948), 46 groups (Aston and Winfield, 1972) and 20 to 60 species (Luttrell et al., 1994). Luttrell (1994) emphasized that although the number of species recorded in the crop varied from region to region, 5-10 key pests caused significant crop damage.

MATERIALS AND METHODS

The study area was located in Akot region, in Akola district. Maharashtra, India Akot is located at 21.1° N 77.06° E it has an average elevation of 345 meters. The investigation was carried out for a period of six months from July 2016 to February 2017.Sampling was conducted in 6 month at the randomly from selected cotton filed. Sampling was done every month from quadrates. Cotton pest were collected from 1 quadrate (1 sq. m x 1 sq. m). Placed at four corners and one center of 10 sq. m x 10 sq. m area by visual search method between 8.00 -10 hours. A sufficient area was left to avoid edge effects. All 1 quadrates were searched. Pest can be collected from the plant of cotton. Fresh Specimen from each quadrate were brought in the laboratory for identification for this we take photograph with help of sterio-zoom microscope from dorsal, ventral and lateral side of the specimen then preserved in 70 % alcohol.

RESULT

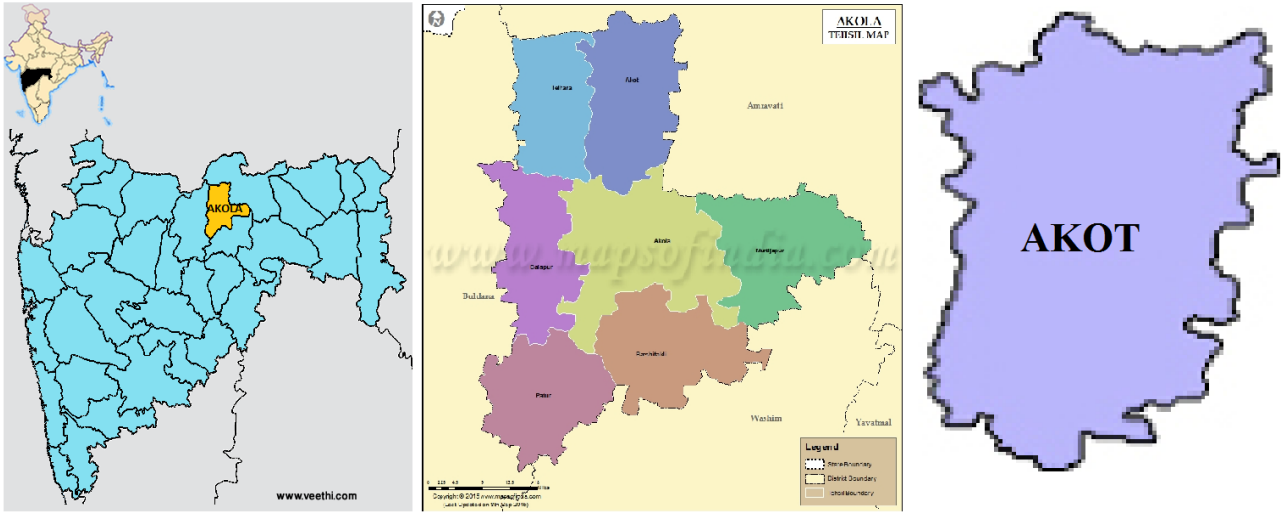
Collection identification and nature of damage, life history of important cotton pest and their management were studied from July 2016 to February 2017. Eight species of insect pest were collected and identified on the cotton crop of Akot region. Insect pests are one of the major limiting factors in cotton production. Of 1326 insect pests recorded on Cotton worldwide, nearly 130 species occur in India. About a dozen of these arthropods are commonly present in sufficient numbers requiring their management for realizing better cotton yields.

Among these insect pests we have collected different developmental stages of red cotton bugs from cotton cultivated area during month of February2017.

Nymph: There are two instars present in life cycle of red cotton bug. Both 1st and 2nd nymphal instars can shows small reared white bands on abdomen and black markings on the wings respectively.

Adult: Adult are reddish brown with white bands on the abdomen and black markings on the wing with high percentage as compared to nymphs.

In present study I have found bollworms, sucking pest and stainers. In bollworms I have found pink bollworms and spotted bollworms. In sucking pest I have found jassids, aphids, thrips, and whiteflies. And in stainers red cotton bug and dusky cotton bug present.



Map Akot Region: Distric Akola, Vidharbha, Maharashtra, India

Bollworms-


A] Spotted and spiny bollworms


B] larvae


C] Adult moth

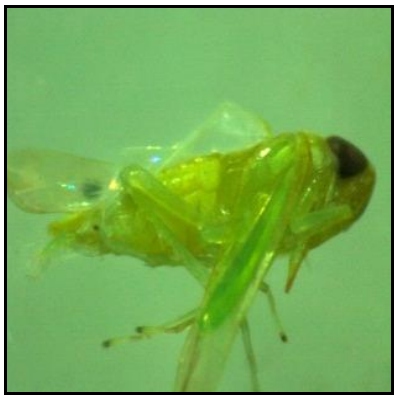

A] Pink bollworms :eggs

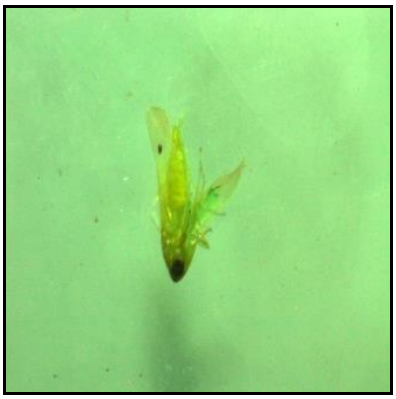

B] larvae


C] Adult moth

Sucking pests-


A] Jassids :Dorsal view


B] Lateral view


C] Ventral view


A] Aphids colony on cotton leaf


B] Aphids :nymph


C] Adult aphids


A] Thrips :Dorsal view


B] Lateral view


C] Thrips on cotton leaf


A] Whiteflies colony on cotton leaf


B] Adult whiteflies : Lateral view


C] Dorsal view

Stainers-


Red cotton bug - nymphal instar 1: Dorsal view


B] Lateral view


C] Ventral view


A] nymphal instar 2: -Dorsal view


B] Lateral view


C] Ventral view

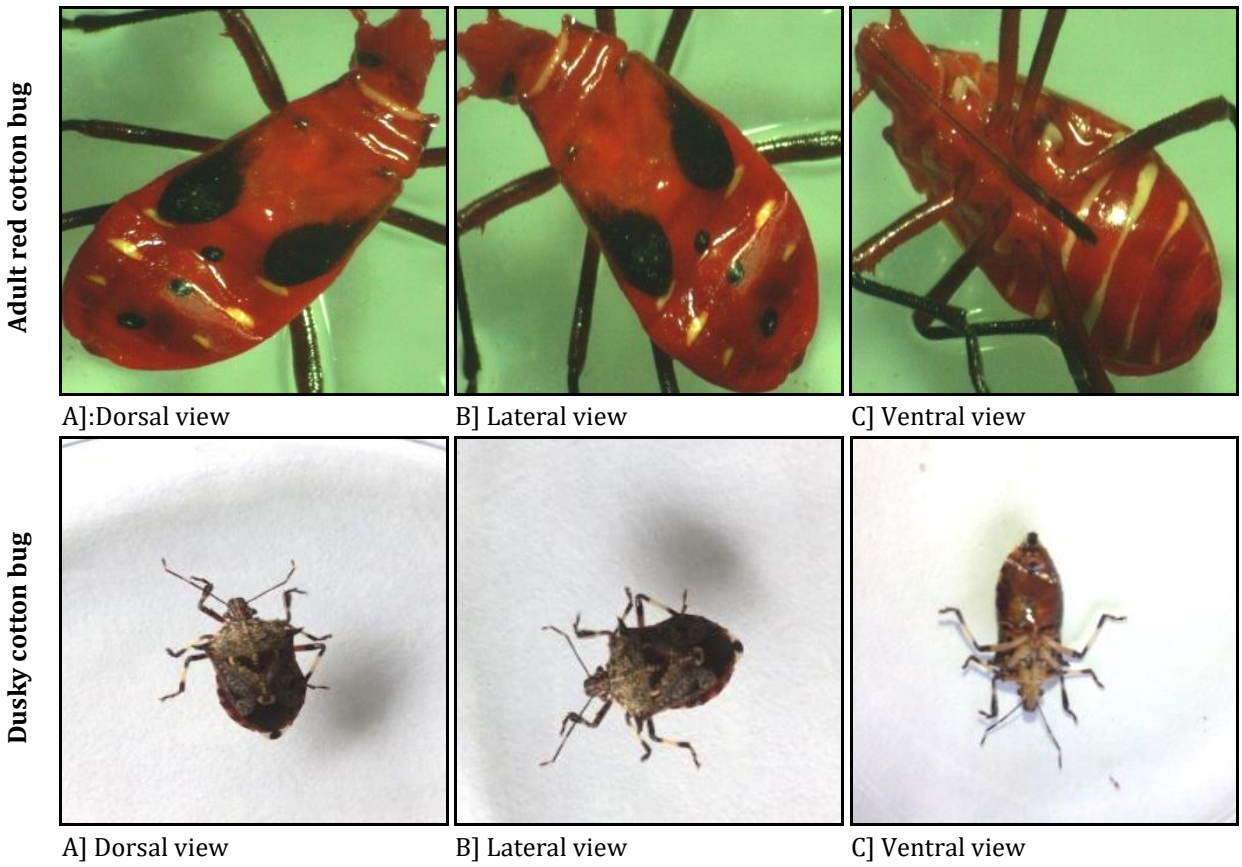


Table 1: Major Cotton pest of Akot region District Akola during July 2016- February 2017.

S. N.	Insect pest	Scientific name	Symptoms of damage
A	Bollworms		
1	Spotted and spiny bollworms	*Eariasvittella*and *E. insulana*	Bore mark in main shoot, dried and withered away shoot, twining of main stem due to axillary monopodia, feeding holes in flower buds and bolls blocked by excrement.
2	Pink bollworm	*Pectinophoragossypiella*	"Rosetted" bloom, pink larvae inside developing bolls with interloculi movement.
B	Sucking pests		
1	Jassids	*Amrascabiguttulabiguttula*	Affected leaves curl downwards, turn yellowish, then to Brownish before drying and shedding, "hopper burn" stuntsyoung plants.
2	Aphids	*Aphis gossypii*	Leaf crumpling and downward curling of leaves, sticky cottondue to deposits of honey dew on open bolls.
3	Thrips	*Thripstabaci*	Leaves of seedlings become wrinkled and distorted with whileshiny patches, older crop presents rusty appearance from adistance.
4	Whiteflies	*Bemisiatabaci*	Upward curling of leaves, reduced plant vigor, lint contamination with honey dew and associated fungi, transmission of leaf curl virus disease.
C	Strainers		
1	Red cotton bug	*Dysdercuscingulatus*	Feed on developing and mature seeds, stain the lint to typical Yellow color, reddish nymphs seen in aggregations around developing and open bolls, affected seed yield low oil.
2	Dusky cotton bug	*Oxycarenushyalipennis*	Associated with ripe seeds, all stages characterized by a powerful smell, discolour the lint if crushed, affected seed yield low oil

From above samples I have taken photographs of jassids, aphids, red cotton bug and dusky cotton bug from steriozoom microscope. Further remain samples photograph was taken from internet sources because they are not taken clearly in steriozoom microscope due to some clarity problem. (Photo source: www.google.com)

DISCUSSION

Pest problem is one of the major constraints for achieving higher production in agricultures crops. India losses about 3o% o f its crops due to pest and disease each year. Agriculture has been facing the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields. Pests are constantly being introduced to new areas either naturally or accidentally, or, in some cases, organisms that are intentionally introduced become pests. Global trade has resulted in increased numbers of invasive non-native pest species being introduced to new areas. Controlling these invasive species presents an unparalleled challenge worldwide. (Salma et al. 2011).

In present study a total eight species of cotton pest collected from cotton cultivated fields. From cotton crop filed I have collected pink bollworms, spotted bollworms, sucking pest like jassids, aphids, thrips, whiteflies, and strainers like red cotton bug and dusky cotton bug.

Many host records periods of abandon growth of pest and feeding behavior under normal condition. All the life stages apart from eggs and pupae cause damages through direct feeding, inserting there stylet into leaf veins and extracting nourishment from phloem sap. As a biproduct of feeding, honey due is excreted and that alone can be a second major source of damage.

Larvae cause damage by consuming foliage. Young larvae initially consume leaf tissue from one side from leaving the opposite epidermal layer intact. By the second and third instar larvae began to make holes on leaves and eat eged of leafs inwards.

Spotted bollworms can damage to cotton crop by Boremark in main shoot, dried and withered away shoot, twining of main stem due to axillary monopodia, feeding holes in flower buds and bolls blocked by excrement.

Pink bollworms damage cotton crop by “Rosetted” bloom, pink larvae inside developing bolls with interloculi movement.

Jassids can damage cotton crop .by Affected leaves curl downwards, turn yellowish, then to brownish before drying and shedding, “hopper burn” stunts young plants.

Aphids can damage cotton crop by Leaf crumpling and downward curling of leaves, sticky cotton due to deposits of honey dew on open bolls.

Thrips can damage cotton crop by Leaves of seedlings become wrinkled and distorted with while shiny patches, older crop presents rusty appearance from a distance.

Whiteflies can damage cotton crop by Upward curling of leaves, reduced plant vigour, lint contamination with honey dew and associated fungi, transmission of leaf curl virus disease

Red cotton bug can damage cotton crop by Feed on developing and mature seeds, stain the lint to typical yellow colour, reddish nymphs seen in aggregations around developing and open bolls, affected seed yield low oil.

Dusky cotton bug cotton crop by Associated with ripe seeds, all stages characterized by a powerful smell, discolour the lint if crushed, affected seed yield low oil (Vennila et al., 2000).

CONCLUSION AND SUMMARY

In present study during July 2016 to February 2017 I have collected eight species samples from the cultivated cotton fields from Akot region area. In Akot region from the different sides like Popatkhed, Akola road, Vai area I have collected samples from cotton field like Bollworms, sucking pest, stainers, from the eight month of study periods at randomly.

In bollworms I have collected spotted bolloworms, pink bollworms. In sucking pest I have found Jassids, Aphids, thrips, and whiteflies. In stainers I have found red cotton bug and dusky cotton bug. The bollworms can damage cotton filed by damaging bolls of cotton crop, in case of jassids the affected leaves curl

downwards, turn yellowish, then to brownish before drying and sheeding. Aphids can attack on leaf, the leaf crumpling and downwards curling of leaves, sticky cotton due to deposits of honey dew on open bolls is the main symptoms of ahpids. Thrips can cause leaves of seedling become wrinkled and distorted with while shiny patches, older crop presents rusty appearance from a distance. Whiteflies can have attacked on leaves. upward curling of leaves, reduced palntvigour, lint contamination with honey dew and associated fungi, transmission of leaf curl virus disease.

The stainers like red cotton bug and dusky cotton bug when attack on cotton crop can cause the damage by feed on developing and mature seeds stain the lint to typical yellow color, reddish nymph seen in aggregation around developing and open bolls. Associated with ripe seeds, all stages characterized by a powerful smell, discolor the lint if crushed, affected seeds yield low oil. This causes of damage done by above mentioned stainers of cotton crop respectively.

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

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RESEARCH ARTICLE

Survey of Infertility in Pcos Patients in Females of Vidarbha Region, M.S. India

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ABSTRACT

Polycystic ovary syndrome (PCOS) is common endocrine disorder in women of reproductive age and occurs amongst all races and nationalities. This syndrome is characterized by chronic anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovary, commonly leading to infertility. In the present study, total 216 females were surveyed and analyzed as the percentage of PCOS and non PCOS females with fertility, infertility and obesity. Total 18.98% females had PCOS out of them 24.39% were infertile and 75.60% were fertile. In concern to obesity, total 19.44% women's had obese out of them 33.33% with PCOS and 66.66% without PCOS. Finally we may conclude that PCOS condition is one of the major clinical factors causing infertility and rarely by obesity.

Key words: PCOS, obesity, fertility, infertility females, Vidarbha region.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic heterogeneous disorder, with a likely genetic origin, influenced by environmental factors such as nutrition and physical activity (Carlos *et al*., (2012). It is characterized by chronic anovulation and hyperandrogenism and can be clinically expressed with infertility, oligo or amenorrhea, hirsutism or androgen dependent alopecia and acne. Additionally, PCOS seems to be associated with obesity and metabolic abnormalities such as insulin resistance and dyslipidemia. PCOS is the most common hormonal disorders among women of reproductive age, and is leading cause of infertility (Boomsma *et al*., 2008). Women with PCOS are at increased risk of anovulation and infertility; in the absence of anovulation, the risk of infertility is uncertain.

Up to 50 percent of women affected with PCOS are obese, a condition that has been found to increase the magnitude of underlying insulin resistance (Legro and Dunaif, 1997). Obesity tends to be less of a problem in women with PCOS in the adolescent population (Reaven, 1988). Obesity has also been linked to increased androgen production and

hirsutism (Balen *et al*., 1995). It has been proposed that women with PCOS might be at an increased risk for eating disorders given the propensity for obesity in PCOS. Obesity and, specifically, central obesity, is a common feature of PCOS that worsens the phenotype (Gambineri *et al.,* 2002). The prevalence of depression in PCOS is high (Trent *et al .,* 2002; Hahn *et al.,* 2005). Depressive symptoms and mood disorders are common in most obese patients (Dixon *et al .,* 2003)

MATERIALS AND METHODS

Study population

A total 216 females were identified randomly visited to different Gynaecological, dermatological Hospitals, beauty parlours and inhabiting in civil areas of Amravati region. We included diagnosed females for PCOS in Gynaecological hospitals. The study group diagnosed with PCOS according to the Rotterdam ESHRE/ASRM consensus (Rotterdam, 2003). Female's were assessed by history and physical examination (name, age, address, occupation), a detailed examination was conducted and noted the height and weight of all females. Data were collected from clinical and anthropometric variables, body mass index (BMI) and a demographic questionnaire inquiring about age, education, occupation, and duration of illness.

BMI was calculated as weight (*kg*)/ height*2* (*m*).
In present study, we also included different parameters like age, pelvis sonography. Informed consent was sought from them and data entered into pre-structured standard pro forma.

These women were further divided into two groups according to their body mass index (BMI);
Group A- obese and overweight -BMI>23 and Group B- non-obese (normal weight and lean) -BMI<23 (Choo , 2002)

RESULTS AND DISCUSSION

We used revised 2003 Rotterdam consensus as diagnostic criteria of PCOS, in which diagnosis of PCOS was based on clinical characteristics in combination with ultrasonography. In the present study, we reported percentage of PCOS and non PCOS womens along with infertility and obese clinical features. Out of total 216 females 18.98% were suffered from PCOS problem and out of the total PCOS females 24.39% females were infertile, 75.60 % females were fertile and 34.14% females were obese. Pfeifer and Kives (2009) have also mentioned infertility as one of the long term sequelae of PCOS.

In the present data, we also found that the total obese females were 19.44% .Out of total obese females 33.33% had PCOS and 66.66% females were without PCOS. Many researchers (Fruzzetti *et al.,* 2008; Diamanti- Kandarakis *et al.,* 2007; Berneis *et al.,* 2007; Cupisti *et al.,* 2008) believe that obesity is more prevalent in women suffering from PCOS. According to Angioni *et al.,* (2008), a high proportion of women with PCOS are obese. Polycystic ovarian syndrome is a multisystem disorder closely associated with obesity. Lim *et al.,* (2012) concluded that women with PCOS had a greater risk of overweight, obesity and central obesity. Jajoo and Angik (2013) also said that PCOS patients, irrespective of weight are at risk of infertility.

Table 1 Showed the percentage of PCOS in association with and without infertility and Obesity.

Total patients observed	216	
Total married womens	45(20.83%)	
Total unmarried womens	171(79.16%)	
PCOS Patients	Total PCOS	41(18.98%0
	PCOS with infertility	10(24.39%)
	PCOS with fertility	31(75.60%)
	PCOS with obesity	14(34.14%)
	PCOS without obesity	27(65.85%)
Obesity patients	Total obesity patients	42(19.44%)
	Obesity with PCOS	14(33.33%)
	Obesity without PCOS	28(66.66%)
Normal without PCOD	175(81%)	

CONCLUSION

High percentage infertility has been observed in a survey group of PCOS females. PCOS is one of the clinical characteristics causing infertility. It may be caused by ovulatory dysfunction and prolonged periods of anovulation. Present study data also showed percentage PCOS with and without obesity, it may be due to metabolic disorder. It is also one of the clinical factors for causing obesity and their association with PCOS. Finally we may conclude that PCOS condition is one of the major clinical factors causing infertility and rarely by obesity.

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

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RESEARCH ARTICLE

Ostracods Diversity of Gorja Lake of Bhadrawati, District Chandrapur (M.S.), India

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ABSTRACT

The Gorja Lake is principal fresh water body located in Gorja village of Bhadrawati tahsil in Chandrapur district of Maharashtra state. Bhadrawati is a tahsil place and it is 25 km north side of Chandrapur and 125 km south east side from Nagpur. It is situated at about 211 m above the mean sea level. The Ostracods were studied from June 2014 to May 2016 during this period total 2 species of Ostracods were found in sample three sites A, B and C of Gorja Lake.

Keywords: Gorja lake, Ostracods diversity.

INTRODUCTION

Ostracods commonaly known as seed shrimps and also Ostracods class of a Crustacea and found in a wide variety of aquatic habitats. It is bivalve and appear like small seeds.The body of Ostracod is laterally compressed and protected by a bivalve like such as chitinous or calcareous valve or "shell". Ostracod occurs in both water standing as well as running water. The Ostracods this organisms very good food for the fishes and aquatic organisms (Tonapi, 1980).

Gorja lake is 10 km south side from Bhadrawati tahsil at about 198 m above mean sea level and is at 79°05'48''E longitude and 20°05'59'' N latitude.Gorja Lake receives the water from the surrounding catchment areas during the monsoon period. The area of Gorja Lake is spread over 300 acres. The depth of water is 35 feet during the monsoon and 12 feet during the summer season. The water of this lake is primary used for washing, bathing, fishing activities, agriculture and other domestic purpose but now it is at a transitional state with respect to degradation

MATERIALS AND METHODS

Sample for planktonic study were collected monthly from three sites of lake. The samples were collected in the morning hours between 8.30 a.m. to 10.30 a.m. 50 Lt. of water sample was filtrated through the plankton net made of bolting silk number 25 with mesh size 64 lime the collected samples were allowed to settle down by adding Lugol's Iodine. Normally sedimentation requires 24 hrs. after which supernatant was removed and concentrate was made up to 50 ml. depending the number of plankton and preserved in 5% formalin for further studies.

For the quantitative study the concentrated sample was shaken and immediately one drop of sample was taken on a clear micro side with the help of standard dropper the whole drop was then carefully covered with the cover glass and observed. Plankton identification up to genera and whenever possible upto species level was classified according to keys given by Prescott (1954), Edmonsonic (1959), Sehgal (1983), Adoni (1985), and APHA (1985) and standard analysis was undertaken as per Zar (2005).

Quantitative study of plankton was done by Sedgwick – Rafter Cell method

Sedgwick – Rafter Cell Method

The Sedgwick – Rafter Cell is a special kind of slide similar to the Haemocytometer. The cell has a 50 mm x 20 mm × 10 mm rectangular cavity that holds 1 ml. sample. The cell is moved in horizontal direction on the stage of an inverted microscope and plankton species encountered in the field are enumerated. A number of replicate samples are enumerated to calculate plankton/lit.

Plankton (units/lit) = $n \times c/v$

Where, n = number of plankton in 1ml
c = Volume of Concentrate
v = Volume of sample in lit.

RESULTS AND DISCUSSION

According to Kedar, (2002) reported 7 species belong to Ostracods in Rishi lake and Yedshi lake of Washim District of Maharashtra. Pawar and Pulle, (2005) observed 4 species of Ostracods from Petwadaj dam of Nanded, Maharashtra. Jayabhaye and Madalapure, (2006) recordrd 3 species of Ostracods in Parola dam of Hingoli. Sakhre and Joshi, (2006) observed 4 species of Ostracods in Yeldari reservoir. Ansari and Raja, (2007) founded only one species belong to Ostracods in two freshwater bodies of Aligarh, Uttar Pradesh. Rajan, *et.al.,* (2007) observed 3 species of Ostracods in three polluted water bodies of Virudhunagar District, Tamilnadu.

In the present investigation, 2 species were reported at all the three sampling sites A, B and C of the lake under study during 2014-2015 and 2015-2016.

In site A, during 2014-15 , 2 species were recorded among which *Cypris sp.* (44 no./lit) and *Stenocypris sp.* (44 no./lit). In side A, during 2015-16, 2 species were recorded *Stenocypris sp.* (42 no./lit) and *Cypris sp.* (40 no./lit).

In site B, during 2014-15, 2 species were recorded *Stenocypris sp*. (44 no./lit) and *Cypris sp.* (36 no./lit). In side B, during 2015-16, 2 species were recorded *Stenocypris sp.* (34 no./lit) and *Cypris sp.* (29 no./lit).
In site C, during 2014-15, 2 species were recorded *Cypris sp.* (29 no./lit) and *Stenocypris sp.* (24 no./lit). In side C, during 2015-16, 2 species were recorded *Cypris* (22 no./lit) and *Stenocypris sp.* (21 no./lit). Amoung the different species in side A, Cypris sp. was dominant by Ostracods, Stenocypris sp. In side B, Stenocypris sp.was dominant by Ostracods, Cypris sp. In side C, Cypris sp. was dominant by Ostracods, Stenocypris sp.

Bhagat, *et.al.,* (2010) recorded 5 species of Astracoda in Ambadi irrigation dam of District Akola. Kumar, *et. al.,* (2011) observed one genera each of Ostracods of a Varasda wetland system of Rajkot District, Gujarat.

Table 1: Yearly variation of Ostracods from sites of Gorja Lake during year 2014-15

	Year	A	B	C	Total
Ostracods	**2014-15**	7.33 ± 5.66	6.67 ± 4.64	4.42 ± 3.62	6.14 ± 0.84
	2015-16	6.83 ± 5.18	5.25 ± 3.79	3.58 ± 3.17	5.22 ± 0.84
	2014-16	7.08 ± 4.79	5.96 ± 4.21	4.00 ± 3.38	5.68 ± 0.58

Shashikant Sitre and Mahendra Thakare, (2013) observed ostracods by one species in Balaji temple tank of Chimur city of Chandrapur District (M.S.). Balakrishna, *et. al.,* (2013) reported 2 species of Ostracods at Dharmasagar lake of Dharamsagar of Warangal District, Andhra Pradesh. Kamble and Mudkhede, (2013) observed only *Cypirus* in Ambadi reservoir of taluka Kinwat, Maharashtra. Jaiswal, *et. al.,* (2014) reported two species of Ostracods were distributed in a freshwater of Rangavali Dam in Navapur, District Nandurbar (M.S.). Gunwant Gadekar, (2014) founded 3 species of Ostracods in Pangdi lake of Gondia of District Gondia, Maharashtra. Gunwant Gajanan Sontakke and Satish Mokashe, (2014) observed 2 species of Ostracods in Dekhu reservoir from Aurangabad, Maharashtra.

In the present investigation the Ostracods density is a maximum during the winter and minimum during the monsoon season. Patil, (2008) recorded the maximum population of Ostracods during the summer season and minimum during the monsoon season. Pejawar and Gurao, (2008) observed them only during monsoon and stated that these are pollution sensitive species. Nirmal Kumar, *et. al.,* (2011) founded the maximum population of Ostracods during the summer season and minimum during the monsoon season of a Varasda wetland system Gadekar, (2014) observed maximum Ostracods population were reported in summer, in March month while minimum in monsoon season, i.e. in July month in Pangdi lake of Gondia of District Gondia, Maharashtra. Shashikant Sitre, (2014) reported maximum Ostracoda population were observed in summer months and minimum in rainy season in Sunkadin Naik lake of Nagpur city (M.S.).

CONCLUSION:

In the present investigation, the maximum Ostracods during the winter season is probably due to availability of suitable food and favorable temperature and minimum density in monsoon season which could be due to dilution of water resulting in fewer nutrients and due to reduction of transparency and dissolved oxygen

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

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RESEARCH ARTICLE

Investigation of peripherial blood smear with RBC morphology of Iron deficient anemia

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ABSTRACT

Iron deficiency anemia is one of the world's most widespread health problems especially among children. Approximately 40% of children are anemic across various countries. Iron deficient anemia leads to weakness poor physical growth & compromised immune system decreasing the ability tofight infections and increasing morbidity and is also thought to impair cognitive performance and delay phycho motor development. Recent microeconomic estimates suggest that the impact on school participation and learning anemiacould also be central to understanding the intergenerational transmission of poverty. Peripherial blood smear investigation reveled the shape of different blood cells from affected persons.

Key words : Iron Deficiency Anemia, Peripheral blood smear.

INTRODUCTION

Although much is known about iron metabolism, the health consequences of iron deficiency continue to be a subject of re-search and debate. This is partly because in many regions of the world iron supplements are the standard of care for individuals with anaemia. Most trials of iron supplementation have measured haemoglobin concentration as the primary outcome. There is a relatively small body of clinical trials of iron repletion to humans with functional iron deficiency (i.e. iron deficiency severe enough to affect erythropoiesis) with pregnancy outcomes or mortality as primary objectives. There is surprisingly little evidence to either support or refute a causal link be-tween iron deficiency and these important adverse health outcomes. As processes like this comparative risk assessment (CRA) bring to light the overall weakness of evidence either supporting or refuting the re-lationship, new research priorities may emerge.

MATERIALS AND METHODS

- Disposable latex gloves (Use non-latex, e.g. nitrile or vinyl, if the employee and/or client has a latex allergy).
- 70% isopropyl alcohol
- Cotton balls or gauze
- Blood lancets for finger puncture (capable of making a puncture to the depth of 1.5 mm)
- E. Blood lancet designed for heel sticks on infants and premature babies, to a depth of less than 2.0 mm (e.g. BD Quikheel™ Lan-cet).
- F. Puncture resistant sharp's containers
- G. Band Aids (optional)
- H. Appropriate microcuvettes or tubes for micro sampling
- I. Disinfectant (freshly prepared 10% household bleach) for bench tops.

Procedure:

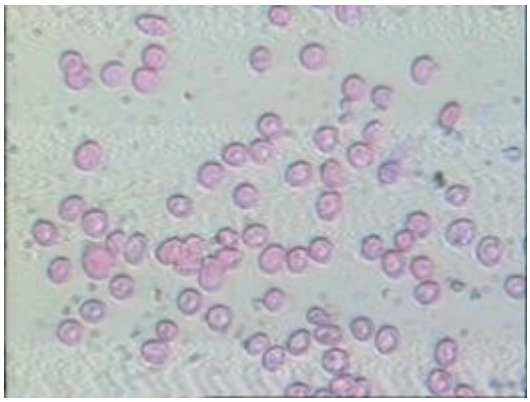
1) Small drop of blood (with or without anticoagulant) was place on new slide.
2) Push forward the spreader with a quick, smooth and single move-ment so as to make 2-3 cm long smears with convex edge.
3) Smear was dried quickly and stained the slides by using Leishman stain and methanol was used as a fixative.
4) Permanent slide was prepaired by covering with cover slip.

RESULTS AND DISCUSSION

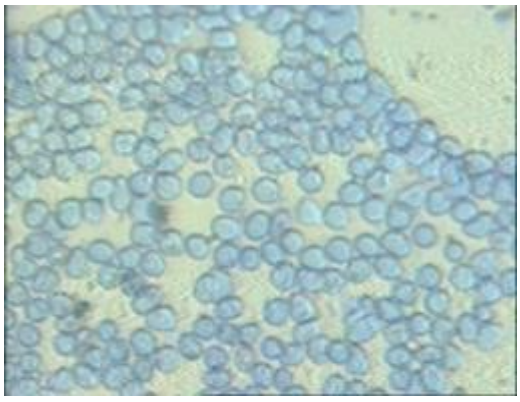
Peripheral Blood Smear Examination

The examination starts with a macroscopic view to evaluate the quality of the smear based on overall appearance. The microscopic analysis begins on lower power (10X), primarily to assess cellular dis-tribution, staining quality, and to select an area where the RBCs are barely touching each other. This area is used to conduct a complete assessment of the cellular elements on higher magnification. All of the detailed analysis of the cellular elements on higher magnification. All of the detailed analysis of the cellular elements is performed using oil immersion. This final microscopic examination was perform at 50X and 100 X oil immersion and includes.

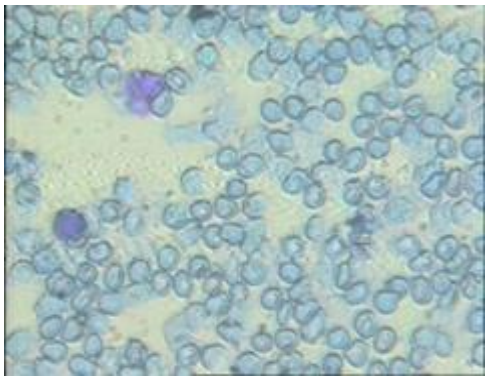
- A WBC differential
- The identification of abnormal and peculiar leukocytes.
- Assessment of RBC morphology
- The number and morphology of the platelets
- The identification of intra- and extra-cellular elements.
- Assessment of any organisms present.
- Following criteria was used to examine the peripheral blood smear of anemic patients.
- Size
- Shape
- Color
- Inclusions
- Peculiarities
- Relationships



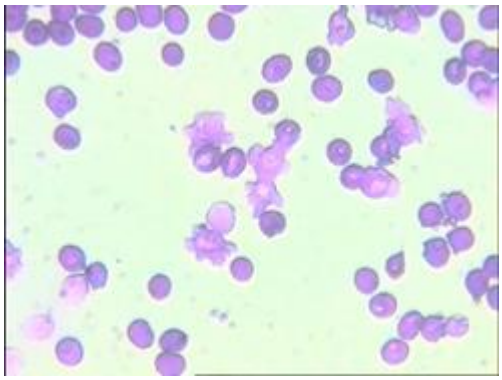
Sample 1: Peripheral blood smear shows that fragmented red blood cell. Frag-mented cells are seen. Specific terms, depending on the shape, in-clude schistocyte, acanthocyte, spur cells, and burr cells.



Sample 2: This microphotograph depicts polychromasia. Referring to the blue-gray color of the red cell. Peripheral blood smear also showing micro-cytic, poikilocytosis including elliptical and elongated RBCs



Sample 3: The arrowed cells are anisocytes, target cell and tear drop cell also clearly seen. Microcytsosis also depicts. A micro--cyte is a small red blood cell, having a diameter of less than 7 um.



Sample 4 : Peripheral blood smear shows an platelet aggregation, vacuoles in RBCs. Microcytic, hypochromic, anisocytosis and poikilocytosis also observed.

Analysis of Peripheral Blood Smear :

Peripheral blood smear examination showed different size and shape of RBCs including schistocyte, acanthocyte, spur cells, tear dorp cell and burr cells. Microcytosis also depict. Red blood cell count showed lower values in 57% of patients studied. The haemoglobin level was found to be below normal. The haematocrit value was also found to be below normal. However the white blood cell count was consistently elevated in the population affected with IDA. Hemolytic ane-mia causes change in red cell morphology may identify the cause of erythrocyte destruction (eg, the presence of bite cells points to a Heinz body hemolytic anemia) and the ultimate diagnosis (eg, ox-idant damage to the red cell secondary to drugs) Iron Deficiency anemia also causes Thrombocytopenia – Distinguishing between in-creased platelet consumption and reduced platelet production can often be made through review of platelet size. Anisocytosis - vari-able sizes of red blood cells may indicate anemia; RBCs smaller than 7 µm are referred to as microcytes and RBCs larger than 7 µm are called macrocytes. Poikilocytosis - various shapes of red cells; these may include echinocytes, acanthocytes, elliptocytes, keratocytes, rouleaux, sickle cells, target cells, teardrop cells, and shistocytes. As part of a blood smear evaluation, a manual WBC differential is performed. Typically, at least 100 WBCs are found, counted, and categorized according to type. The percentage of each type is calculated. In addition, the appearance (morphology) and stage of development of the WBCs are noted. White blood cells have a nucleus surrounded by cytoplasm. All WBCs are derived from bone marrow stem cells. In the marrow, they differentiate into two groups: myelocytic and lymphoid cells. They mature into five distinct types of WBCs.

Those with granules in their cytoplasm are also called 'granulocytes' and include:

- Neutrophils (10-18 µm) are cells that have cytoplasm with pink or purple granules. They compose the majority of WBCs in a healthy adult. They are involved in the defense against infections.
- Eosinophils (10-15 µm) are easily recognized in stained smears with their large, red-orange granules. Generally low in number (1-3%), they most often increase in number in individuals with allergie s and parasitic infections.
- Basophils (10-15 µm) have large, black granules and are the least often seen type of WBC (1%).

The non-granulocytes include:

Monocytes are usually the largest of the WBCs (12-20 µm) and are often referred to as scavenger cells (phagocytes). They can ingest particles such as cellular debris, bacteria, or other insoluble particles.

Lymphocytes are smaller in size (10-12 µm) and have a homogeneous cytoplasm and a smooth, round nucleus. One type of lymphocyte, the B-cell, is responsible for the production of antibodie s (immunoglobulins). All these abnormalities are seen.

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

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RESEARCH ARTICLE

Zinc induced histopathological and biochemical anomalies in the liver of fish *Ophiocephalus punctatus*

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ABSTRACT

The continuous discharge of effluent containing heavy metals and their compounds at an unprecedented and constantly increasing rate, even below permissible level from various industries into aquatic bodies may result in accumulation and subsequent magnification up to dangerous level because of their toxicity, water solubility and non- degradable qualities. Their tendency to accumulate in the organism and in undergoing food chain amplification, causing disorder in the aquatic ecosystem with deterious effect on biolife in unnaturally high concentration. The present study deals with the toxicity of zinc ($ZnSO_4$), as a component of industrial waste and its effect on liver of fish *Ophiocephalus punctatus*. The toxicity of zinc even at sublethal level causes histopathological disorganization of hepatocytes. The estimated protein concentration increased, whereas, glycogen and lipid content were found to be reduced in the liver during the exposure periods. These adverse effects of zinc toxicity reduce functional capacity of liver and caused reduction in nutritive value of fish significantly.

Key words: *Ophiocephalus punctatus*, zinc, liver, protein, glycogen, lipid.

INTRODUCTION

Zinc in traces is essential to sustain biological processes such as optimum body growth, development, reproduction and as immune stimulant. Its presence is essential for smooth working of various important enzymes like DNA and RNA polymerase, reverse transcriptase, alcohol dehydrogenase, sarbitol dehydrogenase, glucose -6- dehydrogenase etc. Its deficiency leads to retardation of growth, chronic renal disease, oligospermia, cessation of estrous and menstrual cycle in mammals. Zinc is an essential and beneficial element in human metabolism.

Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations. (Ho, 2004). When fishes are exposed to great elevate level of metal in polluted aquatic ecosystem, they tends to take these metals up from their direct environment (Hoo *et al.*, 2004;

Charjan and Kulkarni, 2013). These heavy metal toxicants are accumulated in the fish through general body surface which affect severally their life support system at molecular biochemical levels. Once these toxic substance enters into body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders (Arasta *et al*., 1999). Liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals (Dural *et al*., 2006; Agah *et al*., 2009). Liver is an important organ of detoxification where break down of toxic substances is carried out by the endoplasmic reticulum of hepatocytes due to which the hepatic cells are damaged severely and became disorganized (Bhatkar, 2011).

MATERIALS AND METHODS

The fish, *Ophiocephalus punctatus* (Bloch), a common air breathing fresh water teleost, which are locally priced as food fish and abundant in various lakes near Amravati (Maharashtra state) were used in the present study. Fish weighing 20-25 gm and between 10-12 cm in length were purchased from local fish market. The fish were treated with 0.1 % $KMnO_4$ solution for 1 to 2 minutes to clear any dermal infection. They were maintained under laboratory condition in aquarium for acclimatizing them for seven days. They were fed with commercial feed. The water in the aquarium was changed daily to remove detritus.

a. ***Water used*** - Water used through out experiment was aged tap water. The physiochemical parameters of aged tap water were determined periodically (Table 1) as per standard method for examination of water and waste water (APHA, 1998). The same water also served a control medium throughout the experiment.

Table 1: Physiochemical properties of water used to keep fish, *Ophiocephalus punctatus*.

Sr. No.	Parameter	Range
1	PH	7.4 ± 0.5
2	Temperature	$25^0C \pm 2^0C$
3	Dissolved oxygen	6.3 mg / l
4	Total hardness	65 – 90 mg / l

b. **Test Toxicant** - Zinc sulphate ($ZnSO_4$), a salt of zinc was a toxicant for present study.

c. ***Bio assay Study*** - To study effect of toxicant $ZnSO_4$ on liver, LC_{50} was determined for 24 hours, it was found to be 20.5 mg / l. The sublethal concentration of 6 mg of $ZnSO_4$ /l of water was selected. For histopathological and biochemical study fish were taken at 7 days, 14 days, 21 days and 28 days.

d. ***Histopathological Studies*** - For histopathological study, tissue (liver) were fixed in aqueous Bouin's fluid. After proper fixation tissue were washed with running tap water, then dehydrated in different grades of alcohol, cleaned in xylene and finally paraffin blocks were prepared. Sections cut at 5µ were stained with haematoxylin, eosin stain.

e. ***Biochemical Studies*** - Protein, glycogen and lipids contents of liver were estimated in 7, 14, 21 and 28 days exposed fishes.

RESULTS AND DISCUSSION

Histological study appears to be a very sensitive parameter in determining cellular changes occurs in target organ like liver. Liver of *Ophiocephalus punctatus* consists of polygonal hepatic cells (Fig. 1).

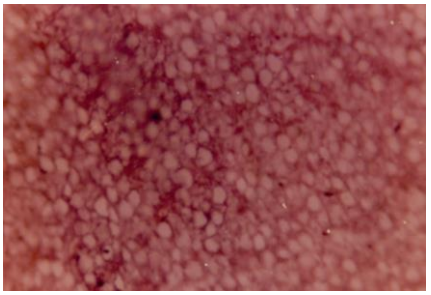

Fig. 1

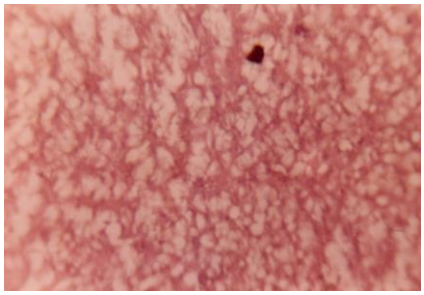

Fig. 2

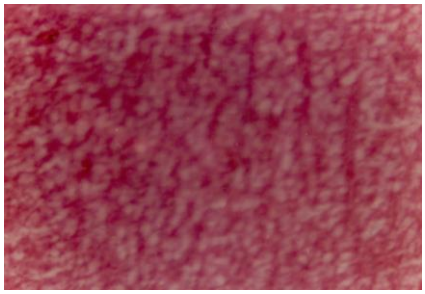

Fig. 3

Table 2 - The results of biochemical estimation of protein, glycogen and lipid in liver tissue of the *Ophiocephalus punctatus.*

Biochemical estimation	Control	Experimental			
		7 days	14 days	21 days	28 days
Protein	171.12 ± 2.5	183±3.5 (+7.03)	176.6± 5.2 (+ 3.22)	188.19 ± 4.1 (+ 9.97)	191.81 ± 1.6 (+ 12.09)
Glycogen	148.15± 1.6	116.18 ± 1.8 (−21.57)	90.10± 2.8 (−39.18)	62.24 ± 1.5 (−57.98)	58.72 ± 1.4 (−60.36)
lipid	0.18 ± 0.05	0.17 ± 0.01 (−5.55)	0.15 ± 0.02 (−16.66)	0.12 ± 0.02 (−33.33)	0.09 ± 0.01 (−50.00)

Each value (μg / mg wet tissue) is the mean of 5 estimations ($\pm SD$).
Values in parenthesis are percent change over control.

It also shows the sinusoids and sections of bile ducts and blood capillaries. Though the liver has no direct contact with the toxicant water, it is indirectly affected through its contact with blood.

After seven days of exposure, the nuclei of the hepatic cells appeared prominent and large (Fig. 2). Dark granular patches were observed in between the hepatocytes. Shrinkage of blood vessels, clumped erythrocytes and widely separated bile canaliculi were noticed. After twenty eight days of exposure, perilobular and centrilobular cirrhosis, cellular necrosis, proliferation of bile duct epithelial cells leading to the formation of new bile canaliculi were seen (Fig. 3).

Because of drastic changes in the liver histology after treatment of sublethal concentration of zinc sulphate, disorder and imbalance in the metabolic state of fish might have caused and hence that reflected in biochemical changes (Table 2).

In the present investigation, an increase in protein content was observed in liver of experimental fish. Liver exhibited 12.09% increase in protein after 28 days of exposure. It might be due to stimulation of protein synthesis to form detoxification enzymes. A significant decrease in glycogen and lipid content was noticed. After 28 days, the glycogen drastically reduced by 60.36% and lipid by 50.00%. This might be due to increased glycogenolysis to meet the excess energy demands imposed by the severe anaerobic stress of zinc intoxication. Among the fish organs, the liver and kidney appear to have a significantly higher tendency for the accumulation of most of the metals (Rauf *et al*., 2009). Kumar *et al*. (2015) recorded a maximum of 151.12% elevation in zinc accumulation in liver of fish *Channa punctatus* exposed to 10mg/l concentration of $ZnSO_4$ for 28 days, over controls. Several degenerative changes in liver of fish, *Channa punctatus* due to acute toxicity of zinc sulphate were also observed by Avinashe and Farheen (2017).

Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Saxena *et al*., 2009). Through the food chain ultimately this metal-contaminated food reaches to the body of human beings. Long-term intake of Zn (150 – 2000 mg/day) induces sideroblastic anemia, leukopenia and hypochromic microcytic anaemia (Simon-Hettich *et al*., 2001) and may face greater risk of health problems.

CONCLUSION

Thus, the toxicity of $ZnSO_4$ even at sublethal level may damage liver tissue to reduce functional capacity of liver and reduce nutritive value of the fish significantly.

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

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RESEARCH ARTICLE

Microbial study of indoor air quality from various schools of Amravati City, Maharashtra, India

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ABSTRACT

Air pollution is one of the most growing problems of our World. Indoor air quality issues are not new to India. Presence of bacteria in indoor air causes a serious health problem for human beings. Determination of various groups of indoor microorganisms is necessary for both to estimate the health hazards & to create standards for indoor air quality control. Unhygienic environment conditions of schools indicator of air quality which shows the adverse effect on school children of all ages. The present study was carried out to determine the presence of bacteria in indoor air of some Z.P. & AMC schools of Amravati city, Maharashtra (India). This was investigated through air quality sampling by open plate technique. The air sample are collected from class room, staffroom, washroom & corridor of some ZP & MNP school during spring season (March-April). Then the microflora was isolated & identified on the basis of cultural characteristic. The overall most common identified bacteria are gram positive bacteria such as *Baciilus* spp*., Micrococcus* spp*., Streptococcus* spp*., Staphylococcus* spp. The gram positive bacteria were found to be higher than gram negative bacteria, with shows adverse impact in the form of skin infection, throat infection, illnesses, diarrhea, neonatal meningitis, mucosa, urine infection & some chronic diseases, which are observed in school children.

Keywords: Air pollution; Indoor Air Quality of school, Microflora, Analysis.

INTRODUCTION

Atmospheric pollution is one of the most pressing problems of our age. The pollution has now reached advanced level that poses a potential threat to the health & well-being of the population. Human being inhales air every moment. Even most of the microorganisms present in air are harmless but still less than 1% of the airborne bacteria are pathogens. Mostly outdoor air does not contain disease causing pathogen whereas indoor air has to contain disease causing pathogen especially in large gatherings area like educational institute, schools, colleges & universities.

The quality of the indoor environment is not easily controlled or defined and can probably place human occupants at risk (Diriba *et al*., 2014). The indoor air quality is one of the most significant factors affecting the health. The air inhaled by people is abundantly populated with microorganisms called bio aerosol. Bioaerosols represent roughly, all biologically originated aerosols & can be found both indoor & outdoors. The most studied bioaerosols are the airborne bacteria & fungi (Mentege *et al*., 2009).

Indoor air is mostly contaminated by airborne particles including bacteria, fungi, allergens, dusts, mould & yeast. Many bacterial genera are also emitted by indoor sources like food, pet animals, dustbin, indoor plants, dust, flower pot, wood furniture, organic dust, various materials stored in the buildings, and the air inflowing from the ventilation and air conditioning systems. People spend mostly (80-90%) of their time in indoor environments such as office, school & house. Therefore, these indoor environments are more significant for the contribution of the daily pollutant exposure than outdoors. The amount of the pathogenic microorganisms is higher in indoor compared with outdoor air (Dacarro *et al*., 2003.). School is the second most important indoor environment, which is evaluating the quality of indoor air and health components of occupants (Godwin and Batterman 2007). In case of children, a great part of their time is spent at school for studying & working in enclosed spaces every day. Therefore, assessment of this microenvironment are important to evaluate their time-weighted exposure to air pollutants **(**Katiyar, 2013**)** (Hannan *et al*., 2013).

Indoor microflora is reported to be responsible for health problems, especially among children. Bioaerosols are decrease air quality and affect human health, and causing some diseases such as tuberculosis, diphteria, legionellosis, fever, rhinitis, nausea and asthma (Karwowska, 2003).

Poor indoor air quality of school causes illness, can cause acute health symptoms, requiring absence from school, decreasing performance in student. Children are more likely to suffer the consequences of indoor pollutants than adults, because they are still developing physically **(**Bayer and Ashrae, 2001) **.**
The presence of the microbial content in school indoor air is an important factor because it has a direct impact on the physical development, mental health and performance of the students (Naruka and Gaur, 2013).

The aim of this study was to identify and compare the airborne bacterial quality of indoor bacteria present in indoor environment of various ZP & AMC school of Amravati city.

MATERIALS AND METHODS

Sampling site-

Amravati is located at 20.93°N 77.75°E. It has an average elevation of 343 metres (1125 feet). Amravati has a tropical wet and dry climate with hot, dry summers and mild to cool winters. Summer lasts from March to June. The population of Amravati near about 2200057 lac.

In the present study ten schools are selected randomly as sampling sites in Amravati city,from different sites of Amravati. The areas being selected by considering the different sources of pollution in the nearby areas of schools.

School Description

This study was focused on primary school of Zilla Parishad & Amravati Municipal Corporation of Amravati city, Maharashtra, India. Indoor air samples were collected at 10 schools from the city, in Mar 2017. For this study four sampling sites (Class room, Staff room, Corridor, Washroom) from each of the 10 schools were selected according to the different intensity of human activities inside the school building.

Sampling-

Sample was taken from a sampling site by standard settling method. The cultural plate exposure method was adopted for trapping the air borne micro flora. A petri plate with the nutrient agar exposed to air for 15 min & at that time petri plate were setup at a height 1.5m above floor. The time of sampling was kept uniform at all the sites. After sufficient growth of bacterial colony which is identified by colony characteristics, Gram test & IMViC test. The bacterial cultures were identified on the macroscopic (shape, size, color, margin, elevation, opacity consistency & appearance of colony and microscopic (grams staining). Biochemical characterization of recovered isolates were performed according to Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

The study of airborne bacteria in indoor environment of ZP & AMC school of Amravati. A total No.of 10 species of bacteria were observed from all sample sites. From sample sites the identified bacteria species *Micrococcus, Staphylococcus, Streptococcus* & *Bacillus* are predominant. Same result found in study of school in Poland Micrococcus spp. was found in all sample & staphylococcus spp. was the next dominant. (Pastuszka *et al*.,2000) Staphylococcus spp. & Bacillus spp. were found in dominant in school env. of Eldrine. (Avdogdu *et al*.,2005)Bacterial species such as *Micrococcus, Staphylococcus* & *Bacillus* were found predominant indoor environment in Ankara (Mentege *et al*., 2009).

In the study higher concentration of bacteria found in class room & washroom of all school show that this may be due to more human activity or crowding at this location less ventilation. *Micrococcus, Staphylococcus, Streptococcus* & *Bacillus* spp.are found in all V, CR,ST& WR of all school (100 %). *Escherichia* spp.(2.45 %) is mostly found in V, CR&ST. Less frequently founded bacteria are *Klebsiella* (1.47%). *Enterobacter* spp. (1.90 %) are occurred in mostly 90% washroom of all school. Bacteria belonging to Enterococcus geneus are potentially related to various type infection such as urinary tract infection and meningitis (Fisher & Philips, 2009). *Pseudomonades (1.26%) are* found in V of school 1, WR of school 2, 5,7,10. Proteus (0.79%) are found in school 1, 2, 3, 45, 6,8 &10. *Clostridium* (1.58%) found in some V, CR, SR & WR of school.

Bacterial concentration is high in AMC school than ZP school of Amravati. In AMC school Microbial flora of indoor air depend on several factor, including the number & hygienic standard of people present the quality of school infrastructure & mechanical movement with in the enclosed space. Many of the species of bacteria isolated from the buildings are harmless & frequently include members of *Bacillus, Micrococcus* spp that have isolated & can cause problem are Pseudomonas spp

Table 1 : Type of Bacterial species isolated from some school of Amravati city

Sr. No	Types of microorganism isolate	ZP School																				AMC School																	
		School – 1				School – 2				School -3				School- 4				School- 5				School- 6				School- 7				School- 8				School- 9				School-10			
		V	C	S	W	V	C	S	W	V	C	S	W	V	C	S	W	V	C	S	W	V	CR	S	W	V	C	S	W	V	C	S	W	V	C	S	W	V	C	S	W
			R	R	R		R	R	R		R	R	R		R	R	R		R	R	R			R	R		R	R	R		R	R	R		R	R	R		R	R	R
1	*Micrococcus* sp.	7	7	6	8	7	8	6	9	8	9	5	8	7	8	9	6	10	7	6	9	8	9	7	8	6	8	8	11	8	7	9	6	7	12	6	11	10	8	6	9
2	*Staphylococcus aureus*	8	7	6	7	6	8	6	7	6	8	7	4	5	7	8	7	7	8	6	6	7	8	6	7	7	9	5	7	9	10	7	9	7	9	6	9	7	9	6	8
3	*Streptococcus* spp.	4	6	4	4	7	7	4	5	7	8	7	6	7	8	3	5	6	5	5	4	10	7	9	8	7	6	9	7	6	8	5	5	6	10	7	8	8	9	7	8
4	*Escerichia*	2			1		1			1	1	1		2	1	1			2	1			2	1		2		2	1				3	1	1					2
5	*Bacillus* spp.	5	6	5	6	7	5	5	6	7	8	7	7	9	8	7	8	5	10	7	6	7	8	6	7	5	9	5	6	8	10	7	6	6	8	7	9				
6	*Klebsiella*		1	1			1			1	1			2	1			2	1							2	2					1		2	1						
7	*Entrobacter* spp.				3				2			2			1		1			2			3					2					2	1					2		
8	*Pseudomonas*		2								1				1	1		1			1	1			1	2			1		2		2							
9	*Proteus*				1			1			1				1						1				1			1	1					2							
10	*Clostridium*	1	2			1				1				2				1			1				1	1		1		2	3					2				

Table 2: % of Occurrence / Contribution of bacteria in school

Sr. No	Types of microorganism isolate	ZP School		AMC School		Total No of Colony in all school	% of Occurrence/ Contributions in all school
		Total No of Colony	% of Occurrence/ Contributions	Total No of Colony	% of Occurrence/ Contributions		
	Micrococcus sp.	152	26.25	163	23.90	315	24.98
	Staphylococcus aureus	130	22.45	155	22.72	285	22.60
	Streptococcus spp.	109	18.82	155	22.72	264	20.93
	Escerichia	13	2.24	18	2.63	31	2.45
	Bacillus spp.	134	23.14	142	20.82	276	21.88
	Klebsiella	9	1.55	11	1.61	20	1.58
	Entrobacter spp.	11	1.89	13	1.90	24	1.90
	Pseudomonas	7	1.20	9	1.31	16	1.26
	Proteus	5	0.86	5	0.73	10	0.79
	Clostridium	9	1.55	11	1.61	20	1.58

CONCLUSIONS

In particular, exposure to the most prevalent bacteria detected in the present study such as E-coli & Enterobacter has been strongly associated urinary trac disease like urine infection, kidney infection. Due to the presence of above mentioned bacterial species in school indoor environment causes health impact in children & staff are irritation of skin, rashes, urinary tract infection & dirrhia, , irritation of eyes, nasal congestion, fever. In conclusion the present study suggests that the ZP & AMC school of Amt. Preventive measure for cleanliness & carried out regular air monitoring at indoor & outdoor environment of school. Monitoring of airborne bacteria can be useful in prevention of bacterial allergic diseases.

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

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RESEARCH ARTICLE

Role of Physicochemical Parameter in Soil Quality of Amravati District

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ABSTRACT

India is an agrarian country. Most of the people are dependent on farming. It has severe issues related farming. Vidarbaha region is major cultivator of agriculture land. It's farming and productivity affects due to the imbalance of nature, irregular rainfall, use of pesticide and fertilizers. Different agricultural practices leads to the excessive use of chemical fertilizer, which leads to depletion of soil quality and biomagnifications. To acquaint the soil quality, soil testing. Soil testing is the best tool to understand the productivity of soil which is directly related with its physicochemical properties. The objectives of the study is to determine the its physicochemical properties of the soil sample collected from the different agricultural lands of Amravati district. The study of physico chemical characteristics of the soil is based on various parameters like temperature, $P^{H,}$ EC, Moisture, NPK. It is observed that different conclusions are observed in different parameters due to different soil quality. The study will be helpful to the farmers for the management of nutrient balance of their soil to increase the crop yield.

Key words: Soil quality, Soil analysis, Physicochemical parameter and NPK.

INTRODUCTION

Soil is an important component of life support system. Unfortunately, it has been overused over the country. There is different physical, chemical and biological processes which build up the soil layer over a long period of time. Soil is a medium for plant growth. Its physical, chemical and biological properties determine the degree of workability, suitability to specific crop varieties, physical and chemical capacities as well as productivity (Thakre *et al.,* 2012). Agriculture is an art of raising plants from the soil and is one of the most economical factors for human beings (Wagh and Sayyed, 2013). Due to the continuous cultivation of same crop also adds to soil loss and also reduced level of humus or accumulation of salts in soil thus results into its degradation.

The soil condition is of great importance because it is a universal medium for plant growth, which supplies essentials nutrients to the plants (Narkhede *et al.,* 2011). But according to Bell and Dell the optimum plant growth and crop yield depends not only on the total amount of nutrients present in the soil at a particular time but also on their availability which in turn is controlled by physicochemical properties (Bell and Dell, 2008). The macronutrients are N, P and K. These three elements are those most rapidly removed from the soil by plants. Therefore, many commercial plant fertilizers supply these three essential elements. In a recent decade to increase the yield and production cultivable plant more and more organic and inorganic fertilizers have been added to natural soil. But due to continuous and excess use of such fertilizers, the primary constituents status in soil is being changed (Kamble *et al.,* 2013).

All agricultural production and development of forest depends upon physicochemical parameters of the soil used for it (Kekane *et al.,* 2015). pH and conductivity of the soil highly affect plant growth (Fauzie *et al.,* 2015). The absorption of nutrients is depending on the moisture of the soil (Tale and Ingole,2015). Soil temperature plays an important role in germination of seeds. The change of temperature will have an impact on the growth of biomass and activity of the microorganisms (Naranjo *et al.,* 2004). N,P,K ratio is an important indicator in crop production that identifies balanced and unbalanced fertilization. Hence, balanced fertilizer applications are important for high crop yield (John *et al.,* 2010).

This study will also help in suggesting the farmers about the proper supply of nutrients to increase productivity of the soil and also for healthy growth of the crops which will be beneficial for the human race and for the future generations.

MATERIALS AND METHODS

About study area:

The study area of the chosen topic will be Amravati District. The district is situated between $20^0$32' and $21^0$46' north latitudes and $76^0$37' and $78^0$27'east longitudes. The district occupies an area of 12,235km^2. The district consists of six sub-divisions, which are further divided into 14 talukas. The climate of this district is hot and dry. The average rainfall 700-800 mm. The maximum temperature during summer goes up to 46^0c while the minimum temperature during winter drops to 5^0c – 9^0c. Like the rest of vidarbha, the economy in Amravati is mainly depend on agriculture. The main crop pattern of Amravati district is cotton, wheat, sugarcane, green chilies, oranges etc.

Soil sampling:

The soil samples are collected from 14 different locations. Collected soil samples were air dried. Then they are ground and passed through 2 mm sieved and stored for subsequent physical and chemical analysis.

Physicochemical analysis:

The collected soil sample will be analyzed for various physicochemical parameters such as temperature, moisture, pH, conductivity and NPK. (All the parameters will be analyzed by Standard laboratory procedure and Standard parameter manual.)

- The soil temperature was taken by soil thermometer at the time of sample collection.
- Moisture content of the soil samples were calculated by oven drying method.
- The pH of soil samples was taken by using pH meter in 1:5 soil water suspension.
- The electrical conductivity of a soil samples was determined by using electrical conductivity.
- Available nitrogen was estimated by Kjeldhal method.
- Available phosphorus was determine by using spectrophotometer.

Available potassium estimated by using flame photometer as per the standard method.

RESULTS AND DISCUSSION

Temperature: The temperature of soil samples was found in ranges of 28^0c to 35^0c. Maximum temperature was found from the Daryapur site and minimum temperature was found from the Chikhaldara site.

Moisture: Moisture content values were observed from 2.1% to 15.8% for samples. The lower moisture content value was recorded from the Morshi site and higher moisture content value was recorded from the Chandur Bazar site.

pH: pH of soil sample was found in the ranges of 6.8 to 8.8. Maximum pH was found at Daryapur and minimum pH was found at Chikhaldara and Dharni.

Table 1: Physico-chemical parameters of different soil sample.

Sample Stations	Temp (⁰C)	Moisture (%)	pH	EC (µS)	N (mg/kg)	P (mg/kg)	K (mg/kg)
Amravati	32	8.2	7.6	0.66	115.0	12.4	167.6
Chandur Bz.	33	15.8	8.1	0.57	152.1	41.6	150.2
Morshi	34	2.1	7.9	0.46	90.2	23.4	123.8
Warud	32	11.3	8.0	0.52	255.1	42.9	365.2
Tiosa	30	12.5	8.1	0.56	243.2	44.8	284.3
Achalpur	32	6.0	7.9	0.58	153.1	16.6	250.6
Chikhaldara	28	4.2	6.8	0.46	85.8	9.6	70.9
Dharni	29	3.0	6.8	0.44	94.3	8.2	50.6
Anjangaon	34	3.8	8.0	0.45	140.0	15.6	115.2
Daryapur	35	10.7	8.8	0.87	116.2	33.4	100.8
Bhatkuli	33	8.7	7.8	0.70	43.5	32.7	90.2
Chandur Rly	32	6.6	8.1	0.76	71.8	5.4	300.2
DhamangaonRly	31	6.3	8.0	0.51	104.2	19.5	70.0
Nandgaon Kh.	32	14.6	8.1	0.54	48.1	24.6	200.1

Electrical conductivity: The EC values ranged from 0.44µS to 0.87µS. Lower EC value was observed 0.44 in Dharni site and higher EC value was observed 0.87 in Daryapur site.

Available nitrogen: Nitrogen content in soil sample ranged from the 43.5 to 255.1 mg/kg. The lower value of N was reported from the Bhatkuli site and higher value of N was reported from the Warud site.

Available phosphorus: Phosphorus content in soil sample ranged from the 5.4 to 44.8mg/kg. The lower value of P was reported from the Chandur Rly site and higher value of P was reported from the Tiosa site.

Available potassium: Potassium contents in soil sample ranged from the 50.6 to 365.2mg/kg. The lower value of K was reported from the Dharni site and higher value of K was reported from the Warud site.

In the course of this study 14 soil sample of different region of Amravati district were examined. The result and the parameters ae similar to that of varus earlier researchers. Bhat shows the physicochemical factors of different agro ecological condition of Chandur Bazar, Amravati District (2), whereas Chaudhari and Jichkar investigates study of nutrients from soil in Warud takuka of Amravati district (3) and Tippat and Pachkhade determine the physicochemical analysis of cropland from Takerkhrda Shambhu, of Amravati district (12) all these data presented similar prophile between these parameter.

The present study shows the soil pH is slightly alkaline in nature and electrical conductivity shows the soluble salts in soil sample. Somewhere the NPK values results in to low range and somewhere high. Generally, we observed that, in irrigated area the NPK values are high. It is due to the continuous irrigation and higher supply of the NPK fertilizer into the agriculture field.

CONCLUSION

The selected topic is having socio-environmental value. The present work is useful to the society. It determines the physicochemical parameter of soil, which are very useful to the farmers and society. From this they can know the soil status. Today, use of fertilizers and pesticides are necessary in agriculture technology to obtain high crop yield. That's why farmers should be identified the structure and chemical content of the soil and then the most appropriate type of fertilizers and pesticides should get selected.

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

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RESEARCH ARTICLE

Statistical Analysis of Air Quality as PM_{10} and PM $_{2.5}$ of Amravati city, Maharashtra, India

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Jane Manisha, Ingole Sangita and Lunge HS (2017) Statistical Analysis of Air Quality as PM_{10} and PM $_{2.5}$ of Amravati city, Maharashtra, India, *Int. J. of. Life Sciences*, Special Issue, A8: 159-162.

ABSTRACT

This paper is dealing with the Statistical Analysis of Air Quality of Amravati city. The present work is carried out during the year 2013-2014 with respect to NOx, SOx PM_{10} and PM $_{2.5}$. The paper is based on seasonal comparative study of PM_{10} & PM $_{2.5}$ of Amravati city. Exposure to pollutants such as airborne particulate matter, oxides of sulphur, and oxides of nitrogen has been associated with increases in mortality and hospital admissions due to respiratory and cardiovascular disease. Suspended particulates matter in ambient air of four stations in Amravati city was collected using a fine dust sampling technique. Attention was focused on the various sources of Amravati city. The statistical calculation is carried out with respect to coefficient of variation and obtained results are compared with sampling sites. The sampling stations are divided into three areas. Sampling is carried out two days in week. The concentration of suspended particulates matter PM $_{2.5}$ max. 65 µg/m^3 ^{at} MIDC Amravati & PM_{10} max. 177 µg/m^3 at Rajakamal square Amravati as commercial area. During whole year coefficient of variation showing higher variation in concentration recorded at S.S.S.C. Amravatit. 16.78% for $PM_{10.}$ the conc. of $PM_{2.5}$ is more consistent in MIDC, Amravati i.e 15.27% more variation are observed at Shri Shivaji Science College Amt.23.73% & Rajkamal 15.36%.

Key words: Air, Air Pollution Analysis, PM_{10}, PM $_{2.5}$

INTRODUCTION

Air pollution could also be defined as “any atmospheric conditions in which substance are present in the high concentration enough higher than their normal ambient level to provide a measurable effect on, animals, vegetation or materials” (Seinfeld, 1986). According to the section 2(b) of air (prevention and control of pollution) act, 1981 ‘air pollution’ has been defined as ‘the presence in the atmosphere of any air pollutant.’ As per section 2(a) of air (prevention and control of pollution) ‘air pollution’ act, 1981 had been called as ‘any solid, liquid or gaseous substance present in the atmosphere in such concentration as tend to be injurious to human beings or living creatures or environment’ (NAAQMS 2011).

The emissions are due to the several non-ideal processes taking place in real combustion and that are harmful to the surroundings and human health (Timoney, 2005).

Air supplies us with oxygen, which is crucial for our bodies to live. Adults breathe in on 10-20 cubic meters of air a day. Youngsters breathe almost double that amount because they are smaller, and their metabolic process systems are still maturing (Sood 2012).

Environmental issues studied in Iran, urban air pollution is one among the serious Environmental source, mostly stationary, industrial and domestic fuel combustion, motorized vehicles emissions and ineffective environmental rules (Mansouri, 2011).

Natural sources smoke that comes from volcanoes, methane, dust, pollen, spores and anthropogenic activities like Power Plants, and Biomass Burning, Fuel Adulteration, Construction Activity, Vehicle Emission and Traffic Congestion (Kumar, 1999). According to Hrdlikova (2008) Quality of air is one amongst the essential of indicators of the quality of the environment. Internal combustion unit, the prime movers for vehicle emit hydrocarbons, carbon monoxide, lead, oxides of nitrogen, road and tire dust "A" human carcinogen studied by Balashanmugam (2012) Bhadhwar *et al*. (2006). The clean air act needs "Environmental Protection Act" set national ambient air quality standards for common air pollutant are such as (NO_2), (O_3), (CO), ($PM_{2.5}$ and PM_{10}), (SO_2), and (H_2S). These are called as "criteria pollutants"

PM_{10}

less than 1-2 um diameter get deposited within the alveolar region of the lung wherever the absorption of trace element is 60-80 % (Sirvastava, 2002)

$PM_{2.5}$

Worldwide Short-term exposure at elevated concentrations will considerably contribute to heart disease (Aaron, 2005). In 2011 study all over that traffic exhaust is that the single most serious cause of heart attack (7.4%) in the public. (Nawrot, 2011).

Particulate pollution new borne overseas that floats into Canada, united Mexican states and also the US account for premature death such year (Bina Rani, Upma Singh 2011)

MATERIALS AND METHODS

Determination Particulate Matter (PM_{10} & $PM_{2.5}$) Gravimetric Method

Sampler: Ambient fine dust Sampler with size selective inlet for $PM_{10,}$ $PM_{2.5}$ and automatic volumetric flow control. Fine Dust sampler (Instrumex IPM-FDS) is used for sampling.

RESULTS AND DISCUSSION

$RSPM_{10}$ (Respirable Suspended Particulate Matter Less Than 10 µg/m^3)

Coefficient of variation during 2013 & 2014 ($RSPM_{10}$)

Coefficient of variation is calculated for selected sampling station for each pollutant to make the comparison between seasons. For $RSPM_{10}$ the Amravati city S.S.S.C.22.51% varied drastically as compared to Rajkamal 13.10%, MIDC-Nandgao peth14.40%, MIDC-Amravati17.85%, & rural places at Morshi more consistent 11% than the warud 18.57%, paratwada 16.99% & dharni 16% during whole year. During whole year from coefficient of variation it was found that the higher variation in concentration recorded at S.S.S.C. Amravati. 16.78% more variations were observed than the MIDC, Nandgao peth 12.26%, MIDC, Amravati13.39%, Rajkamal was more consistent sampling site 11.84%. Whereas in rural areas Dharni was more varied 20.70% than the Warud 16.59%, Paratwada15.13% and Moshi 10.34% was more homogeneous than other places recorded (Table 1 & Fig. 1.)

$RSPM_{2.5}$ (Respirable Suspended Particulate Matter Less Than 2.5 µg/m3)

Coefficient of variation during 2013 & 2014

From $RSPM_{2.5}$ all samples calculated by coefficient of variation it was found that the S.S.S.C. sampling site 24.67% more variation was observed than the MIDC, Amt.17.96, Rajkamal 19.37% and MIDC, Nangaopeth 19% recorded in Table 2 & fig.2.

During 2014 MIDC, Amravati 15.27% was more consistent than the S.S.S.C. Amravati. 23.73%, Rajkamal 15.36% and MIDC, Nandgao peth 16.97%. Change in the temperature pattern due to Festival and wind speed.

Table 1: Average Concentration of RSPM$_{10}$ µg/m^3 Amravati City during 2013 & 2014

Sampling station	2013			2014		
	Mean	Standard Deviation	Coefficient of Variation	Mean	Standard Deviation	Coefficient of Variation
SSSC, Amravati	84.85	19.10	22.51	90.87	15.25	16.78
Rajkamal Square, Amravati	125.04	16.38	13.10	124.37	14.73	11.84
MIDC Area, Amravati	104.60	18.67	17.85	109.16	14.62	13.39
MIDC, Nandagaon peth	98	14.11	14.40	100.08	12.27	12.26

Table 2: Average Concentration of RSPM $_{2.5}$ µg/m^3 Amravati City during 2013 & 2014

Sampling station	2013			2014		
	Mean	Standard Deviation	Coefficient of Variation	Mean	Standard Deviation	Coefficient of Variation
SSSC, Amravati	40	9.87	24.67	40.75	11.49	28.21
Rajkamal Square, Amravati	50.5	9.78	19.37	55.41	7.46	13.46
MIDC Area, Amravati	51.08	9.17	17.96	54.66	7.36	13.47
MIDC, Nandagaon peth	42.33	8.01	18.93	44.83	7.87	17.55

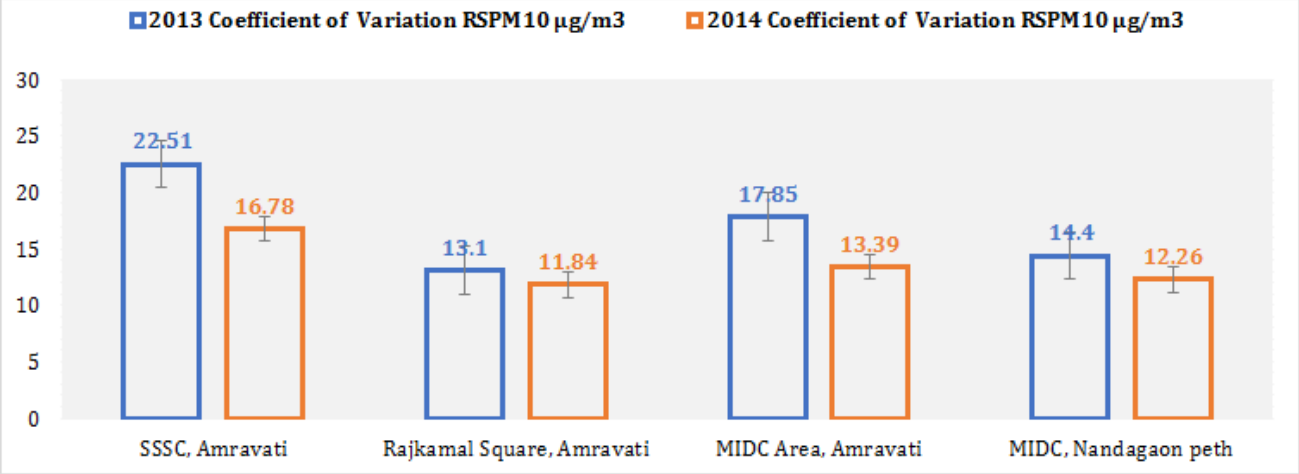

Fig. 1. Coefficient of Variation of RSPM10 µg/m3 Amravati City during 2013 & 2014

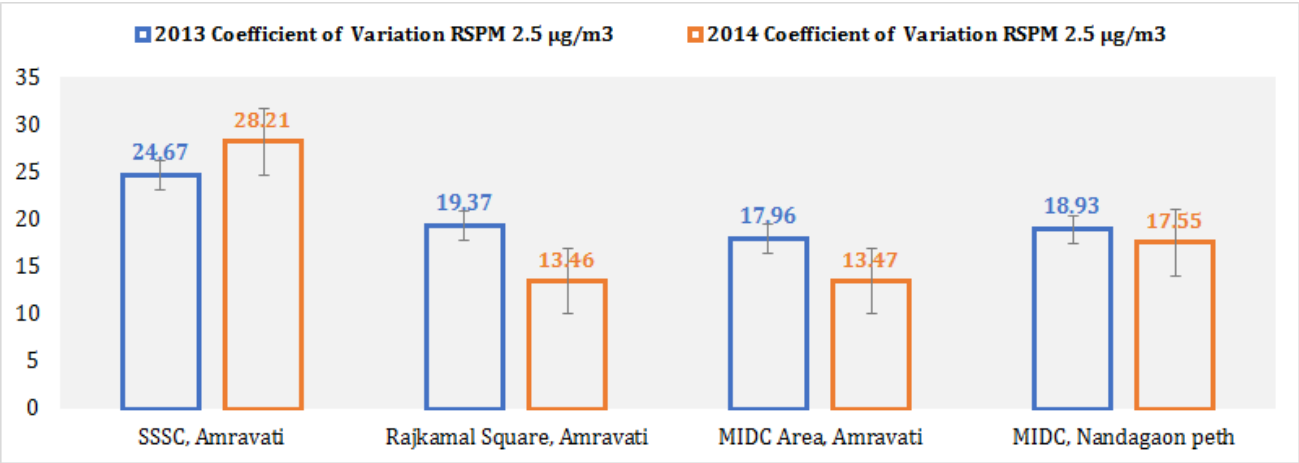

Fig. 2. Coefficient of Variation of RSPM 2.5 µg/m3 Amravati City during 2013 & 2014

CONCLUSION

- From all four sampling stations, maximum $RSPM_{10}$ value is found 136µg/m^3 for (Commercial area) and 100µg/m^3 for Morshi during winter season 2013 whereas in 2014 it is 139µg/m3 for Industrial area.
- During summer 2013 maximum concentration of $RSPM_{10}$ is 117 µg/m^3 in May recorded for Partwada and 155µg/m^3 found for Commercial area whereas it is 157 µg/m^3, for Industrial area during same season 2014.
- Maximum concentration of $RSPM_{10}$ is 131µg/m^3 from Commercial area during Rainy season 2013 whereas maximum value is134 µg/m^3, for Industrial area Amravati, during 2014
- During Monsoon Season 2013 the maximum value of $RSPM_{10}$ 177 µg/$m^3$is found from Commercial area and maximum 125µg/m^3 for Warud whereas in 2014 it is 150µg/m^3, for Amravati Industrial area.
- In winter season 2013 analysis it is found that the $PM_{2.5}$ for MIDC, Amravati it is maximum 54 µg/m^3whereas 2014 (64 µg/m^3) for MIDC, Amravati.
- During summer 2013 at Rajkamal, it is found that $PM_{2.5}$ maximum value noted 64 µg/m^3whereas it is 65µg/$m^3$for Commercial area during 2014.
- In Rainy Season 2013 it is found that $PM_{2.5}$ maximum concentration 58 µg/$m^3$for MIDC, Amravati whereas 2014 it is 58 µg/$m^3$for Commercial areas.
- The sample of 4 sites show $PM_{2.5}$ maximum 60 µg/$m^3$is record for MIDC, Amravati during Post Monsoon Season 2013 & in 2014 maximum concentration is $PM_{2.5}$ 60 µg/m^3Amravati (Industrial area) The statistical calculation is carried out with respect to coefficient of variation and obtained results are compared with sampling sites.
- The $RSPM_{10}$ maximum variation in CV 22.51% for S.S.S.C. Amravati in 2013 where as in 2014 it is 16.78% MIDC, Nandgaopeth.
- In 2013 $RSPM_{2.5}$ show maximum variation in CV 24.67% for S.S.S.C. Amravati. Whereas in 2014 maximum variation are recorded 25.56 % commercial area

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

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RESEARCH ARTICLE

Heavy metals analysis of vegetables irrigated on Amaba Nala, Amravati, MS, India.

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ABSTRACT

Vegetables are important ingredients of human diets, that contains essential nutrients and trace elements. For the development of human life heavy metals are essentials. Contaminated vegetables may produce health effect on human life due to excess of heavy metals. Propose study investigate the level of six different heavy metals which are toxic (Cr, Cd, Ni, Fe, Zn, Cu) in different vegetables irrigated from waste water of Amba Nala flowing through Amravati city of Maharashtra state, India. Heavy metals were extracted by extraction method using concentrated HNO3, atomic adsorption spectrophotometer was used to analyze the level of heavy metals. Heavy metals were evaluated from waste water and from selected seasonal vegetables. The contents of heavy metals in waste water was found very high than vegetables crossing the permissible limit. Seasonal variation in heavy metals are also discussed.

Keywords : Heavy Metals, Waste Water, Vegetables.

INTRODUCTION

Waste water irrigation resulted in the significant mixing of heavy metals contents of agricultural land (Mapanda et al., 2005). Thus long term waste water irrigation leads to build up of heavy metals in soil and food crop (Khan et al 2008). The polluted effluents water is found to be reach not only in organic water and nutrients but also heavy metals like Cd, Cr, Fe, Ni, Zn, and Cu etc. that finally reach to soil through irrigation and leads to food chain contamination as crop and vegetables absorb them from the soil. Heavy metals are not easily biodegradable and leads to their accumulation in human vital organs causing various diseases, (Ward et al., 1995). The use of domestic waste water for irrigation practice is common on Amba Nalain adjoining villages of Amravati. Therefore an attempt has been made to focus sever pollution problem of soil and water quality in vegetables. Some vegetables like tomato, brinjal, cabbage and spinach are tested for their toxicity in relation to human health.

MATERIALS AND METHODS

In the present study heavy metals in vegetables like tomato, brinjal, cabbage and spinach are analyzed, similarly waste water sample used for irrigation are tested for their qualitive and quantitative estimation.

Wastewater and vegetables Sampling

100gm of edible and leafy parts of different vegetables are collected from the field of sampling sites. Small pieces of sample are dried, grinded, and mix homogenously. I gm of sample weighing on Metalic elements are ubiquitous in the environment. Some trace elements are significant in nutrition, either for their essential nature or their toxicity.

RESULTS AND DISCUSSION

Metalic elements are ubiquitous in the environment. Some trace elements are significant in nutrition, either for their essential nature or their toxicity. In the present study quantitative analysis of heavy metals in wastewater and in vegetables on seasonal basis are shown in table – 1 and 2 (Fig – 1 and2) for rainy season and winter season respectively.

Heavy metal concentration found higher in sewage water this is in agreement with Rattan et al (2005). However, seasonal trend showed lower concentration of heavy metal during rainy days as compared to winter. It my be due to dilution effect. Waste water used for irrigation showed higher concentration in both the season when compared with standard values, FAO, 1985. Heavy metal concentration varied among different vegetables (Fig 1 and 2) which may be attributed to variation in heavy metal concentration (Vousta et al, 1996). In the present investigation concentration of metals were compared with permissible limits of Indian Standard (Awasthi, 2000) and safe limits given by WHO/FAO, (2007). It was found that concentration were higher in all the studied vegetables.

It has been studied that higher concentration of copper toxicity above the safe level reveals the vegetables are contaminated with copper and are toxic to cosumer (Xiong and Wang, 2005; Asdeo and Loonker, 2011).

Concentration of Cu was found in order Spinach>Brinjal>Cabbage>Tomato for rainy season (Table-1) and Spinach>Tomato>Cabbage>Brinjal during winter months.

Zinc accumulation was found to maximum crossed standard and safe limit WHO/FAO and FAO. Maximum level of tolerance of zinc for human health is 20 mg/kg according to Chinese Department of preventive Medicine (1995). Acute exposure of zinc can cause tachycardia,

Table 1 : Quantitative analysis of heavy metal in waste water and in vegetables during rainy season.

Sr. No.	Elements mg/1	Standard values	Waste water value mg/1	Tomato	Brinjal	Cabbage	Spinach
1	Cu	0.05	3.085	0.122	0.651	0.611	1.712
2	Fe	0.1	2.512	1.225	1.266	0.699	2.094
3	Cd	0.01	1.395	0.025	0.072	0.044	0.091
4	Cr	0.05	0.013	0.013	0.032	0.019	0.41
5	Zn	5.00	1.924	1.02	1.098	0.756	1.624
6	Ni	0.75	0.571	0.11	0.011	0.075	0.019

Table2 : Quantitative analysis of heavy metal in waste water and in vegetables during winter season.

Sr. No.	Elements mg/1	Standard values	Waste water value mg/1	Tomato	Brinjal	Cabbage	Spinach
1	Cu	0.05	6.243	2.01	1.851	1.990	2.01
2	Fe	0.1	6.173	1.275	1.266	1.06	1.07
3	Cd	0.01	2.016	0.999	0.786	0.751	0.780
4	Cr	0.05	0.897	0.872	0.210	0.220	0.75
5	Zn	5.00	7.266	3.220	2.280	3.02	4.02
6	Ni	0.75	1.433	1.001	1.05	1.07	1.75

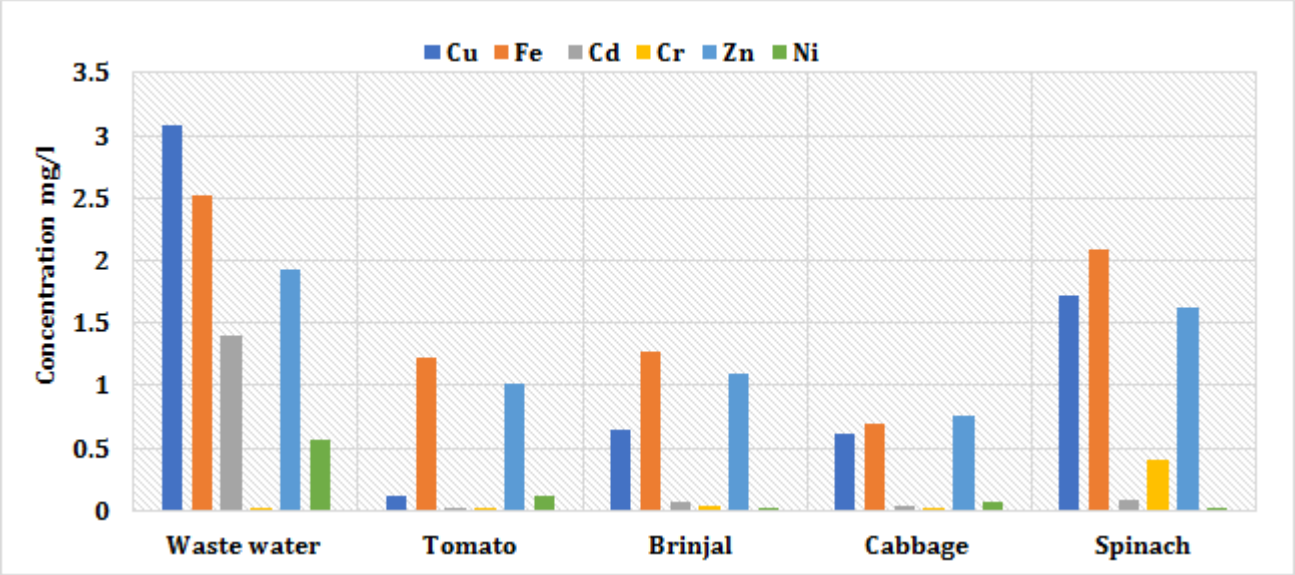

Fig. 1: Quantitative analysis of heavy metal in waste water and in vegetable during rainy season

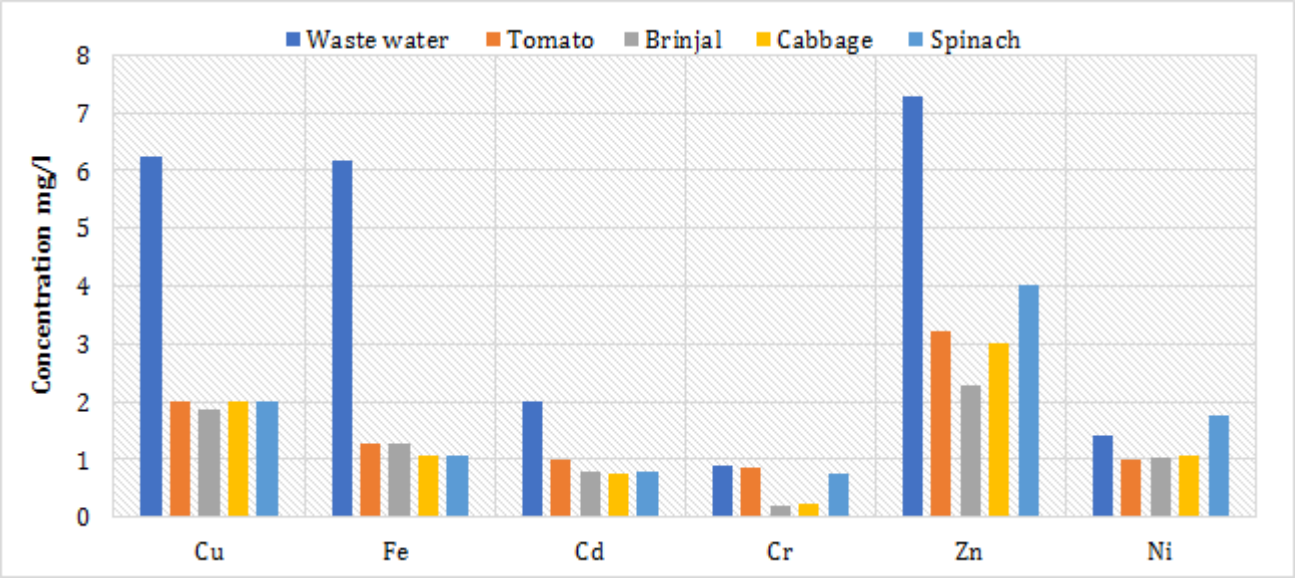

Fig. 2: Quantitative analysis of heavy metal in waste water and in vegetable during winter season

Vascular shock, dyspeptic nausea, vomiting, pancreatic disorder, diarrhea and damage of hepatic parenchyma (Salqueiro etal, 2000).

As the level of cadmium exceed the limit prescribed by FAO and WHO/FAO, consumer must be prevented from their use.

Table – 1 and2 gives the comparative concentration of iron uptake in vegetable matter is may field higher than standard values. Concentration potential was maximum except cabbage (0.699 mg/gm).

Level of nickel and chromium during rainy season was observed within permissible limit except spinach 0.41 mg/Gm. However, the results in winter months was found higher than standard values and safe limit. The concentration of different metals in vegetable samples has been compared with natural concentration and Indian standard in Table – 1and 2 , Fig. 1 and2 . It was seen that level of each Metals in vegetables were many field higher than the natural concentration and Indian standards. Overall observation on coparision showed that metals in irrigation water cause severe impact on vegetable produce and such vegetables should not be consumed at all.

CONCLUSION

Heavy metal shows toxic potential which causes adverse effect on human health. Hence it has made attention of scientist and common people towards the harmful effect of metals. Thus regular monitoring of heavy metal contamination in vegetables grown at waste water irrigated area is necessary and consumption of contaminated vegetables should be avoided in order to reduce the health risk. The waste water treatment technology should involve step to remove metals causing risk to human health.

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

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RESEARCH ARTICLE

Exploring the antifungal potential of spider web protein

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ABSTRACT

Spider dragline silk is an outstanding material made up of unique protein, spidroins. It contains Pyrolidin and potassium hydrogen phosphate as well as potassium nitrate that help to maintain pH of about 4 making it acidic and thus protect it from fungi and bacteria. This property of spider web is being studied here in this study by testing against certain pathogenic fungi of plants and animals. In this study spider webs were extracted using different solvents such as methanol, ethanol, acetone and chloroform. These extracts were screened for antifungal activity using turbid metric method. Ten different fungi as *Trichoderma, Fusarium, Penicillium, Rhizopus, Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus terrus, Colletotrichum capsci, Alternaria alternata*, and *Cereospora species* were tested against increasing concentration of spider web protein. Study reveals that web protein is extracted best in acetone and showed maximum antifungal activity against Aspergillus fumigatus.

Keywords: Spider Web Protein, Antifungal, Spidroins, Dragline silk.

INTRODUCTION

Fungi are amazing organisms because they possess an ability of using any surface for growth. They cause infections in plants, humans, and animals as well as economic losses to farmers through infections in harvested crops. Since last two decades, the cases of fungal infections especially in immunocompromised patients have increased drastically (Claude and Selitrennikoff (2001). Remarkable advances in medicine save the patient's life making them prone to fungal infections. Very few or no drugs are available for treatment against fungal infections. Antifungal proteins, peptides and their derivatives obtain from natural substances show great potential as promising therapeutic agents (Bansod and Rai (2008). Fungi are extensively diverse group of microorganisms. Hence much investigation is required to develop a series of novel but specific drugs against particular members of group of fungi.

Spider silk is one of the most versatile materials in nature. It has a great strength and flexibility. Spider use silk for a variety of different purposes. Spider silk possess supreme mechanical properties. Hence it proves to be wonderful for producers requiring finer sutures, such as ocular,

neurological and cosmetic surgeries. Silk fiber possesses tremendous potential as biomaterials for wound dressings, artificial ligaments, tendons, tissue scaffolds, microcapsules and other applications. Specialized proteins are the main constituents of spider silk. Three main proteins are found in spider silk. Pyrolidin which is very hygroscopic in nature, Pyrolidin which prevents the web from drying out, Potassium hydrogen phosphate is very acidic and acts as a deterrent to bacteria and fungi (Termonia, 1994). Spider silk is one of the strongest fibers on earth (Blackledge and Hayashi, 2006). Now-a-days bandages, pads are being created by using spider web material that speed healing and prevent scarring. Antibiotic properties are naturally associated with spider webs that facilitate wound healing and cell regeneration, mammalian neuronal regeneration or regeneration of the neurons of the retina (Allmeling et al., 2006). Bundles of spider silk has also been used to graft severed nerves when nothing else has shown so effective.

The purpose of this investigation is to determine if spider webs exhibit antifungal properties. We tried to explore the potential Spider web against fungi.

MATERIALS AND METHODS

Most of the data (sample) for the following study was collected during July to September and December to January 2012 to 2014Melghat forests Amravati district of Maharashtra, India. Web samples of the spider were collected between 0800 and 1700 h IST on windless rainless days at 21 to 38 ^{0}C. Relative humidity ranged from 68% to 98%. (Bera et al., 2002) Experiments were run on 54 funnel webs, 67 orb webs, 55 sheet webs, 82 tangle webs and 26 irregular webs that had been constructed by 14 different species of spider. In each case the spider webs were collected from 20 sites of Melghat with sterilized scissors and forceps, webs were cut at four corners and transferred in saline bottles. Utmost care was taken towards fresh sample collection.

2.1 Extraction of spider web protein

Webs were collected in sterilized glass bottles containing 5ml of saline each. Webs were washed with deionised distilled water & air dried for 2 days in petriplates. 5mg of spider webs in micro centrifuge tubes were measured and dissolved in hydrochloric acid (0.1N),Acetic acid(0.1N) (50:50v:v) and neutralized with sodium hydroxide(0.1N) (Chakraborty and Das, 2009). Different solvents for better extraction of protein from web samples were screened using 1ml of ethanol, methanol, acetone and chloroform (and distilled water), teasing with dissecting needle and Centrifuged this at 250 rpm for 2hrs at 27^{o}C .solutions of extracted proteins were now used for antifungal test.

2.2 Assay of spider web protein for antifungal activity by turbid metric method

Antifungal activity of spider web protein was tested by incubating fungal cultures in presence and absence of silk and measuring mycelial growth using photo spectrometry (Varaprasad, 2009). Master plates of known fungi i.e. .*Aspergillus flavus, Aspergillus fumigatus, Aspergillus terrus, Rhizopus oryzae, Penicillium species, Fusarium species, Trichoderma species,* were collected from Ramakrishna Bajaj Agriculture College, Pipri, Dist. Wardha, Maharashtra, India. Fresh culture was then prepared from stock culture by inoculating and incubating Potato Dextrose Broth (Hi-Media) with loopful of fungal colony for three days at 27^{0}C. 5 ml of potato dextrose broth in a sterile 20 ml universal tube was inoculated with 20µl of culture and spider web was inoculated with increasing concentration extracted individually in four different solvents namely ethanol, methanol, acetone and chloroform (20 to 120 µl). Simultaneously a control was kept without spider web protein. All the tubes were incubated for 48 hrs at 27^{o}C and rotated on orbital shaker at 150 rpm for 5 min before taking the absorbance at 550 nm (Wright and Goodacre, 2012) using UV-Visible Spectrophotometer. Light absorbance is expected to be proportional to fungal density in the medium with absorbance increasing with fungal density. Assay was performed in triplicates to assure the findings.

RESULTS AND DISCUSSION

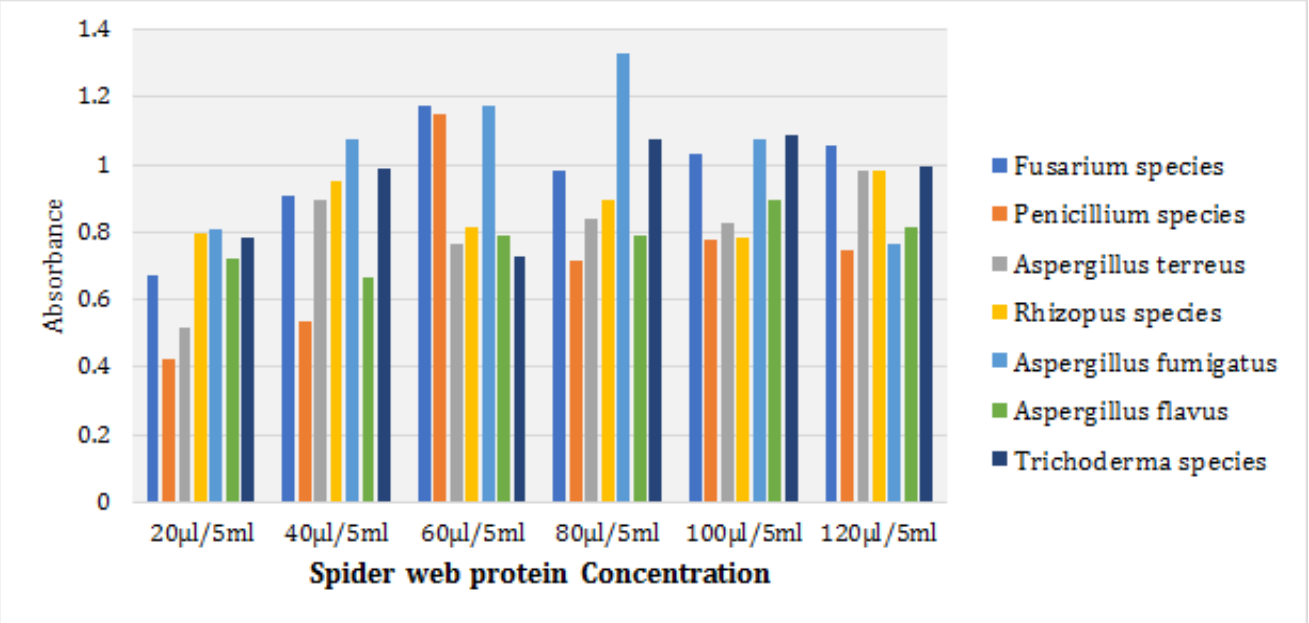
Natural materials have historically been a source of novel antimicrobial agents. These results provide robust support for the hypothesis that the extracted protein of spider silk has a significant spectrum of antifungal activity against pathogenic fungi of plants, animals and human beings when compared with standard Itraconazole. This is the first study of its kind evaluating the effect of web protein on fungi of medical

Table. 1. Antifungal activity of spider web protein against different fungi

Concentration (µl/ml)		Fungus tested						
		Aspergillus terrus	*A. fumigatus*	*A. flavus*	*Rhizopus oryzae*	*Fusarium species*	*Penicillium species*	*Trichoderma species*
20	**E**	0.627	0.662	0.555	0.684	0.392	0.568	0.815
	M	0.647	0.966	0.835	0.794	0.583	0.358	0.985
	C	0.582	0.498	0.528	0.495	0.352	0.488	0.448
	A	0.514	0.807	0.721	0.799	0.669	0.426	0.782
40	**E**	0.788	0.745	0.577	0.798	0.448	0.407	0.874
	M	0.865	0.633	0.585	0.583	0.583	0.430	0.692
	C	0.693	0.528	0.482	0.448	0.901	0.469	0.828
	A	0.893	1.077	0.664	0.954	0.907	0.536	0.986
60	**E**	0.676	0.633	0.627	0.753	0.520	0.447	0.680
	M	0.781	0.743	0.594	0.561	0.795	0.556	0.682
	C	0.815	0.515	0.418	0.548	0.953	1.027	0.498
	A	0.766	1.176	0.790	0.812	0.176	1.148	0.725
80	**E**	0.795	0.789	0.764	0.886	0.578	0.459	0.847
	M	0.830	0.607	0.683	0.523	0.656	0.500	0.939
	C	0.802	0.440	0.578	0.512	0.661	0.453	0.456
	A	0.837	1.329	0.791	0.898	0.981	0.715	1.074
100	**E**	0.488	0.528	0.685	0.797	0.638	0.360	0.844
	M	0.762	0.674	0.780	0.763	0.505	0.503	0.785
	C	0.826	1.074	0.898	0.783	0.029	0.778	1.090
	A	0.804	0.549	0.780	0.532	0.948	0.520	0.567
120	**E**	0.550	0.764	0.601	0.787	0.797	0.416	1.040
	M	0.896	0.869	0.699	0.673	0.705	0.669	0.869
	C	0.740	0.668	0.763	0.628	0.959	0.583	0.697
	A	0.983	0.764	0.813	0.983	1.056	0.748	0.994

E = Ethanol;M= Methanol;C= Chloroform;A= acetone

Comparative Graph showing Antifungal Activity of Spider Web Protein *Against Fusarium spp., Penicillium spp. , Aspergillus terreus , Rhiopus oryzae, Aspergillus fumigatus, Aspergillus flavus, Trichoderma species.(Extraction in Acetone)*



as well as agricultural relevance. In the present study the antifungal activity of spider web protein was tested in vitro against 7 known fungi namely *Rhizopus oryzae, Penicillium spp., Fusarium spp. ,Trichoderma spp, Aspergillus fumigatus*, *Aspergillus flavus, Aspergillus terrus,* . Moreover use of biocontrol agents decreases the application of chemical pesticides, which have a great impact on the environment, humans and animals. Acetone was found to be the best solvent for web protein. The study revealed that acetone has greater extraction value and greater antifungal Activity against all the tested species of fungi. The web protein showed higher Antifungal activity against Aspergillus fumigates than other tested fungi. Thus web protein could be use as pharmaceutical agent for the treatment of human and animal fungal diseases.

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

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RESEARCH ARTICLE

Study on the Heteroceran Lepidoptera (Moth's) Biodiversity in Dnyanganga Wildlife Sanctuary, Buldhana District, Maharashtra, India

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ABSTRACT

Heteroceran Lepidoptera(moth's) are common pests and damage leaves, stems, flowers and fruits. A study was carried in Dnyanganga wildlife sanctuary, Buldhana district, Maharashtra during 2012-14. Total 42 species were found belonging to family Sphingidae, Noctuidae etc. The species were collected to get the biodiversity. The Dnyanganga wildlife sanctuary is situated 8 km from Buldhana and 20 km from Khamgaon town near the Dnyanganga river of Maharashtra, India. The sanctuary has good deciduous dense forest and containing different kinds of trees. Lot of research work has been done in the fields of biodiversity but rarely found in the area of Dnyanganga wildlife sanctuary therefore the present topic is selected for the study.

Key words: Heteroceran, Lepidoptera, Biodiversity, Dnyanganga wildlife sanctuary, Buldhana, Maharashtra.

INTRODUCTION

Moths (Heterocera) represent one of the most heterogenous groups among insects. There are about 1,27,000 species of moths from all over the world and of these over 5,000 species are reported from India (13). ZSI (14) reveals the new record of 48 species of moths pertaining to genera and families and reported total 313 species/sub species of moths belonging to 221 genera and 25 families from central India.

The Dnyanganga wildlife sanctuary was declared in January 1998 in accordance with the State governments. It is situated 8 km from Buldhana and 20 km from Khamgaon town in Maharashtra, India. Geometrically, the sanctuary is at $20^0$25' to $20^0$40'N and $76^0$15' to $76^0$30'E at height of 360 to 600 meters from sea level.

In Dnyanganga wildlife sanctuary, 25 species of butterflies belonging to five different families were recorded (4) but the exact status of moths(Heterocera) of Dnyanganga wildlife sanctuary is not clearly known. Study on moths of the said sanctuary is not found. Hence the present topic is selected for the study.

MATERIALS AND METHODS

1. Collection of moths

Moths were observed, captured, identified and released immediately at the spot of capture. Insect net and light trap method was also used for capturing moths. Many species of moths were photographed in Dnyanganga wildlife sanctuary.

2. Identification of moths

The identification of moths were carried out in PGTD of Zoology, SGBAU Amravati using *Moths of India* (12), *A concise guide to butterflies and moths*(3) and available literature(7,8,9,10,11), Bell and Scott(1) and other published literature.

The dead species, many of them not in good condition were kept in wooden box for future study.

RESULTS AND DISCUSSION

In present study, a total 42 species of moths belonging to nine families were recorded from Dnyanganga wildlife sanctuary, Buldhana district, Maharashtra, India. Among the nine families, Noctuidae and Eribidae dominated the list with 10 species. Sphingdiae with 6 species, Saturniidae, Crambidae, Geometridae and Arctiidae with 3 species each and Lymantridae, Lasiocampidae have 2 species each. It is found that 2 species very common, 19 species common, 16 species rare and 5 species are very rare in occurrence.

Table 1: List of Moth species and their occurence

Sr No	Family	Sub family	Name of species	Occurence
1	Noctuidae	Catocalinae	*Spirama retorta*	VR
		Catocalinae	*Trigonodes hyppasia*	R
		Calpinae	*Sphingomorpha Chlorea*	VR
		Catocalinae	*Ophiusa tirrhaca*	C
		Catocalinae	*Ophiusa algira*	VR
		Aganainae	*Asota Caricae*	R
			Grammodes geometrica	C
		Catocalinae	*Remigia frigalis*	C
		Catocalinae	*Remigid undata*	R
		Hadeninae	*Spodoptera litura*	R
2	Erebidae	Erebinae	*Ercheia cyllaria*	C
			Utetheisa lotrix	R
		Scoliopteryginae	*Cosmophila fulvida*	C
		Aganainae	*Asota ficus*	R
		Calpinae	*Eudocima meterna*	R
			Erebus caprimulgus	VR
		Catocalinae	*Ophiusa tirrhaca*	R
			Grammodes geometrica	R
		Erebinae	*Caenurgina erechtea*	C
			Phytometra rhodarialis	R
3	Sphingidae	Macroglossinae	*Theretra alecto*	C
		Macroglossinae	*Daphnia nerii*	C
			Nephele hespera	VR
		Sphinginae	*Acherontia styx*	R
		Sphinginae	*Agrius sp*	R
			Agrius convolvuli	C
4	Saturniidae	Saturniinae	*Actias selene*	C
		Saturniinae	*Antheraea mylitta*	C
			Saturnia povonia	R
			Euproctis apicallis	VC

Table 1: Continued...

Sr No	Family	Sub family	Name of species	Occurence
5	Lymantridae		*Euproctis lunata*	VC
6	Lasiocampidae	Lasiocampinae	*Estigena pardalis*	R
			Streblote dorsalis	R
7	Crambidae	Spilomelinae	*Cirrhochrista brizolis*	C
		Spilomelinae	*Diaphania indica*	C
		Spilomelinae	*Spoladea recurvalis*	C
8	Geometridae	Geometrinae	*Maxates sp*	C
			Hyposidra talaca	C
			Ascotis selenaria	R
9	Arctiidae	Arctiinae	*Creatonous gangis*	R
		Syntominae	*Amata passalis*	C
		Arctiinae	*Pericallia ricini*	C

Abbreviations- **R**: Rare, **VR**: Very rare, **C**: Common, **VC**: Very common

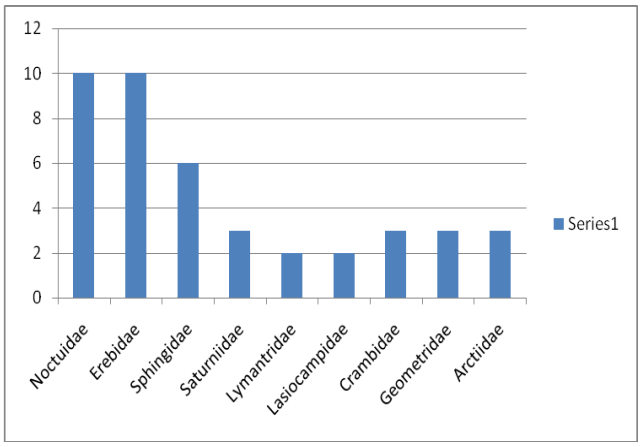

Fig. Family wise distribution of species

Based on survey which was carried out in the present study from 2012-2015 from Dnyanganga wildlife sanctuary Buldhana district, Maharashtra, India, a total of 42 species belonging to 9 families were found. It was observed that number of moth species belonging Noctuidae and Erebidae was found more than other families like Sphingdae, Saturniidae, Crambidae, Arctiidae, Geometridae and Lasiocampidae.

In same order ZSI (15) reported the moth fauna of Jabalpur which was represented by 42 species belonging to 38 genera under 6 families. Also total 41 species from 12 families have been identified in and around Amravati city (5). Many of the species were reported throughout India from various families viz. *Amsacta lineola* in West Bengal (6) of family Acrtiidae, *Acherontia styx*, *Acherontia lachesis*, *Deilephila nerii* and *Theretra alecto* by Bell and Scott(1) of family *Sphingidae; Diaphania indica* in West Bengal by Bhattacharya(2).

Moths are easily affected by slightest disturbances in climate and also by pollution. A sudden variance in the abundance or decline in moth population is often a clear indicator of climatic upheavals increased levels of pollutants in environment.

CONCLUSION

While studying the Heteroceran Lepidoptera(moths) biodiversity in Dnyanganga wildlife sanctuary, Buldhana district, Maharashtra, India, a total 42 species belonging to 9 families were recorded in the present work. Among these members, family Noctuidae and Erebidae were predominant in the collection. Heavy deforestation, habitat destruction and Climatic up down, there were drastic change in the and decline in the diversity of moths of Dnyanganga wildlife sanctuary. Further future study will help to assess moth abundance, species diversity and its preventive measures to prevent adverse effect in species diversity in Lepidoptera.

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

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RESEARCH ARTICLE

Knowledge, Awareness and Opinion related to Sickle Cell Disease amongst the college students of Nagpur, Maharashtra, India

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ABSTRACT

This study was designed to investigate the awareness about Sickle Cell Disease (SCD) and its clinical manifestations, treatment and populations at risk amongst the college students of Nagpur. This study was also used to ascertain knowledge related to the disease in carriers (HbAA) and sufferers (HbSS). The total 1258 students from different streams such as science, commerce and arts were enrolled. All participants were assessed on their general knowledge of the disease. The questionnaire containing the questions on origin, prevalence, incidences, life expectancy, painful crisis, counseling methods, treatments, management, attitude and behavior towards SCD were provided to all participants. The results of this study clearly demonstrated that, only 177 (14.06 %) subjects were found to be known about this disorder. Out of them, 62 (35.02%) individuals had appreciable knowledge, 89 (50.28%) had limited knowledge and 26 (14.68%) had marginal knowledge of SCD. The 9.03% (n=16) respondents were known about need of blood transfusion and 14.68% (n=26) participants had knowledge of interrelation between sickle cell anemia and the disease jaundice be an important clinical complaint in SCD; 32.20% (n=37) respondents known to have about intimate relationship between sickle cell disease and pain crisis. Majority of respondents (113/177) believed that, people should have knowledge and awareness about the disease.

Key words: Sickle cell disease, Carrier, Sufferer, Nagpur, Maharashtra

INTRODUCTION

Due to a variety of reasons, sickle cell anemia (SCA) became one of the most studied genetic alterations affecting mankind. The condition could either lead to natural resistance to major infectious diseases such as malaria among heterozygous individuals or to a debilitating disease that could leads to early death of the homozygous carriers of the gene. The sickle cell trait is known to be confined or occurs in higher frequencies in particular affected populations in the tropics and therefore it recognize

as one of the most classical population specific markers (Lanclos *et al*.,1991, Oner *et al*.,1992, Goncalves *et al*.,1994).

The African continent has been regarded as the epicenter of sickle cell disease with an annual estimated number of 200,000 new born affected by sickle anemia (Diallo *et al*.,2002; Ohene-Frempong and Nkrumah, 1994). This constitutes 66.6 % of the children born with the hemoglobin disorders in the whole world. It is estimated that, every year 15,000 children are born with sickle cell disease in Ghana (Kwaku, 2005) and over 80% of these children die before they celebrate their fifth birth day. In India, the extensive surveys performed by the Anthropological Survey of India estimate an average sickle cell trait frequency of 15% across the states of Orissa, Madhya Pradesh and Maharashtra which, with the estimated population of 300 million people, implies that, there may be more cases of sickle cell disease born in India than in Africa. In Maharashtra especially in Vidarbha region, Deore and Zade (2013) noticed alarming and consistent frequency of this disease in all districts. Many other authors also had same observations (Deshmukh *et al*.,2006).

The college students of 18 years and older were selected for this study, although they are usually in relationships that may eventually lead to marriage in future, so issue of pre-marital screening may be of concern, as this may be affected by existing knowledge and attitude to SCD. This is central to prevention efforts since the disease is preventable. Therefore, understanding and knowledge about sickle cell inheritance, its health and reproductive health implications as well as behavior towards individual with SCD is an important regarding limiting the spread of the diseases.

MATERIALS AND METHODS

The participants for this study were college students selected from the Nagpur city of Maharashtra, India. A total of 1258 subjects were enrolled from undergraduate (UG) and postgraduate (PG) classes of science, arts and commerce faculty randomly. The sickle cell disease questionnaire was distributed to all participants along with consent form. The consent clearly informed that this investigation is voluntary and they can leave the study anytime. The name will not be disclosed and participants would not be placed at any risk following the completion of the research work. The filling of questionnaire was completed under the supervision of professors. The questionnaire was containing the questions on origin, prevalence, incidences, life expectancy, painful crisis, counseling methods, treatments and management attitude and behavior towards SCD in present circumstances in India. The opinion of respondents on the prospects of married individuals with sickle cell traits having children with sickle cell disease was also sought. To avoid confusion, terminologies were explained to respondents during the data collection processes. The data retrieved from the questionnaire was analyzed by using software SPSS and p-value ≤0.05 was considered as statistically significant.

RESULTS

A total of 1258 subjects were enrolled, of them 1003 (79.72%) subjects were associated to UG and 255 (20.27%) with PG classes. Majority of students (n=512) participated were from science faculty followed by arts (n=385) and commerce faculty (n=361). In terms of the categories mentioned in different schedules of Indian constitution, 773 (61.44%) students were belonging to Hindu religion, 372 (29.57%) were Buddhist, 86 (6.83%) were Muslims and 27 (2.14%) were Christian.

The results of this study demonstrated that, 177 (14.06 %) subjects were found to be known about this disorder. Out of them 62 (35.02%) individuals had appreciable knowledge, 89 (50.28%) had limited knowledge and remaining 26 (14.68%) only known that sickle cell anemia is a disease. The clinical symptoms are concerned, 13 (7.34%) students observed to known about few important symptoms such as body pain, chest pain and joint pain. 8 (4.15%) participants found to known about joint pain and anemia. The 8 (4.15%) participants were aware about a need of blood transfusion and only two participants had knowledge of jaundice to be one of the important sign of SCD.

The treatments and management point of view, 3.38% (n=6) respondents had found to have limited knowledge. They rarely knew about bone marrow transplant and gene therapy. Majority of the respondents unknown about its inherited nature and

some of them had misconception that the SCD transmitted due to eating of flesh of dead animals. Major source of information includes text books, friends, family members, health professionals and internet.

Related to behavior towards SCD, majority of respondents (56/177) believed that people should know their genotype for genetic disorders especially the sickle cell carrier and disease individuals.

Table 1: Knowledge about sickle cell disease

Group	Participants	percentile
Are you aware of SCD		
Yes	177	10.25
No	1081	89.74
Sources of information (multiple responses)		
Health professionals	52	29.37
friends	17	9.60
Books	80	45.90
Family	28	15.81
Causes of SCD		
Acquired	64	36.15
Inherited	46	25.98
Don't know	66	37.28
Know someone with SCD		
Yes	24	13.55
No	153	86.44
How is SCD diagnosed		
Blood test	69	38.98
Urine test	31	17.51
Don't know	77	43.50
Measures of preventive measures on SCD		
Genetic counseling	36	20.33
Don't know	141	79.66
What should be done by couple when they discover that their genotype predispose them to having children with SCD		
Discontinue their relationship	08	4.58
Continue with their relationship	139	76.83
Don't know	30	16.94

Table: 2

Group	Participants	Percentile
1. Undergraduate	1003	79.72
2. Post Graduate	255	17.88
Faculty		
1.Science	512	40.69
2. Arts	385	30.60
3. Commerce	361	28.69
Religion		
Hindu	773	61.44
Buddhist	372	29.57
Muslims	86	6.83
Christians	27	2.14

According to 27.90% (n=49) of total respondents, SCD genotype should be considered as an important factor for getting married; the patients should either become unmarried or get married with normal partner. When they asked for their opinion in case of couples both partners having sickle cell disease, 4.58% (n=8) were in favor to be discontinued their relationship, majority of respondents (n=139) wanted to be carry on their relationship by taking special care of them, remaining (n=30) could unable to take any firm decision.

DISCUSSION

Although the participants considered for present investigation were graduate and post graduate students, thus educational level has been thought to influence awareness and attitudes regarding genetic testing. In general, the public is not well informed about genetics, and although better-educated groups appear to be more knowledgeable (MacNew *et al*. 2010; Priest 2000). However this study shown very limited participants found to be known about the sickle cell disease. These findings were exactly opposite to the findings by different authors from other countries where they obtained majority to be known about SCD (Qwolabi *et al*. 2011; Animasahun and Akitoye 2009; Okwi *et al*. 2009). For instance, the studies of school and undergraduate students in Nigeria revealed that more than 80% of respondents claimed to be heard about this disease. The differences may be attributed because in this study, the respondents were general student whereas in aforementioned studies the participants were sickle cell anemic person or carriers.

These results clearly demonstrate that, still majority of Indian population is unaware about the sickle cell anemia. Interestingly many studies have been observed alarming frequency of this disease in Maharashtra (Deore and Zade, 2013; Deshmukh *et al.*, 2006). Limited awareness of knowledge of genetic disorders may lead to the implications of genetic risks among people. The lower level of awareness of the current study may be due to the fact that, they either did not exposed to opportunities such as mass media which could widen their knowledge base about genetic diseases especially sickle cell anemia. Second, the disease is included only in the syllabus of human genetics therefore only students of life sciences get benefitted. Third, most of the Indians unfortunately read the literature that will help to get them jobs.

Moreover, in terms of inheritance patterns majority of students did not know the inheritance pattern, however few respondents in arts (n=6) and commerce faculty (n=2) and good mob of science faculty especially students belongs to life sciences had number of respondents (n=27) knowing about inheritance of SCD. Interestingly this disease included in the syllabus of genetics at UG level, thus that could be the reason behind the good response by science faculty students. Many students had misconception that the SCD is transmitted through the eating habits. But related to transmission, there is no association between eating habits and genetic diseases (Urade, 2012). The reason could be attributed to their attitudes towards the genetic diseases. Related the knowledge of inheritance pattern the results of this study are in confirmation with study conducted by Boyd *et al* (2005), although investigation carried out by Dyson (1997) demonstrates that 1/4th subjects were answered the question related genetic inheritance of sickle cell anemia.

In conclusion, Cultural beliefs, attitudes, and behaviors, including spiritual faith and religious practices have been associated with openness and response to genetic testing for disease (Hughes *et al*. 2003; Lannin *et al*. 1998; Schwartz *et al*. 2000) in India. Literacy and poverty are thought to be the prime hazards for limited knowledge and awareness especially about genetic diseases. Thus, the governmental and non-governmental organizations should be focus on raising knowledge and awareness of genetic diseases such as sickle cell disease by using mass media. Health workers are required to work in association with educational institutions (Sunday *et al.,* 2013).

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

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RESEARCH ARTICLE

Target identification and drug interaction studies of *Bacillus anthracis*

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ABSTRACT

Target based or structural based drug designing is the rapidly growing area. The explosion of genomic, proteomic and structural information has provided hundreds of new target and opportunity to find new drug lead compounds. Based on burgeoning structural data of *Bacillus anthracis* we focused on anthrax disease. *Bacillus anthracis* is a gram positive, rod shaped bacteria that caused serious infectious anthrax disease. In this study, we have taken 1669 protein sequences from NCBI protein database for which protein structure is available at PDB database. From these 1669 proteins, we have detected 10 druggable and 3 virulence protein sequences using TiD tool. Out of detected virulence protein, we have chosen N5-carboxyaminoimidazole ribonucleotide synthetase as a target. Against this target protein, we have screened Ropinirole and Isatin as a lead molecule for docking studies.

Keywords: *Bacillus anthracis*, drug designing, TiD tool, Chemoinformatics, ligand screening, target identification, Autodock

INTRODUCTION

The oldest isolate of Bacillus anthracis, the causative agent of anthrax, dates back to 1917 (Redmond et al.,1998). Bacillus anthracis, a gram-positive rod shaped bacteria and belongs to the Bacillus cereus group has an extremely monomorphic genome and has high structural similarity with physiological and B. cereus and B. thuringiensis (Pavan et al., 2011). The proteome data is available on The Universal Protein Resource (UniProt) and Protein database at National center for biotechnological information (NCBI) database. UniProt provides a central resource for protein sequences and functional annotation with three database components, each addressing a key need in protein bioinformatics (Wu et al., 2006). Among the total proteome data of B. anthracis, few key proteins are identified as a target. Due to the burgeoning of protein data, there are many proteins are still remaining to explore as a drug target. Insilico approaches are good to explore this data. The target identification is the key step of Insilico drug designing

One of the key protein shortlisted for our study is N 5-carboxyaminoimidazole ribonucleotide synthetase (PurK). Recent research has shown that de novo purine biosynthesis in microbes is different from that in humans. PurK is an enzyme in the purine-biosynthetic pathway that converts 5-aminoimidazole ribonucleotide to N 5-carboxyaminoimidazole ribonucleotide and has been suggested as a potential antimicrobial drug target and it is unique to prokaryotes (Tuntland et al., 2011). The differences in the pathways are centered around the synthesis of 4-carboxyaminoimidazole ribonucleotide (CAIR) which requires the enzyme N(5)-carboxyaminoimidazole ribonucleotide (N(5)-CAIR) synthetase. Humans do not require and have no homologs of this enzyme. In recent study, 2-(2,3-dioxoindol-1-yl)-N,N-diethyl-acetamide compound shows inhibition activity against prokaryots but no inhibition to human bifunctional enzymatic activity (Firestine, S et al., 2009). We have chosen 2-(2,3-dioxoindol-1-yl)-N,N-diethylacetamide compound as a reference molecule for lead screening.

Drug discovery and development is a computational approach which is very intense, lengthy, time-consuming and an interdisciplinary endeavor. In early stages of drug discovery, identification of potential leads with specific interaction to target is very essential and conventionally pharmaceutics adapts wet-lab high-throughput screening (HTS) methods which are high cost and time taking process, an alternative is a computational approach (Cheng et al., 2012). With the advent of genomics, proteomics, metabolomics, Bioinformatics and efficient technologies like combinatorial chemistry, virtual screening, de novo designing and structure-based drug designing have highly revolutionized the process of drug discovery (Lazarova et al., 2008). Present day drug discovery mainly ponders on target based drug designing, which is broadly defined as "single compound acting on a single target to a single disease". Single target based drugs are designed such that lead molecules can promisingly bind to its specific target, reducing the off-target side effects
(Lin et al., 2012).

Chemo-informatics tools present a tremendous potential to advance in silico drug design and discovery, as they serve the integration of information in several levels to enhance the reliability of data outcomes. To name a few, chemical structure similarity searching, data mining/machine learning, panel docking, and bioactivity spectra based algorithms have been routinely and successfully implemented (Katsila et al., 2016). Some examples are the ligand-based interaction fingerprint (LIFt) approach (Cao et al., 2015). in predicting potential targets for small-molecule drugs using physics-based docking and sampling methods and the protein ligand interaction fingerprints (PLIF) method (Eberini et al., 2011). for summarizing interactions between ligands and proteins using a fingerprint scheme.

TiD is a standalone application, which relies on the basic assumption that a protein must be essential for pathogens survival and non-homologous with the host to qualify as a putative target. With an input bacterial proteome, TiD removes paralogous proteins, picks essential ones, and excludes proteins homologous with host organisms. The targets illustrate non-homology with at least 40 out of 84 gut microbes, considered safe for human. TiD classifies proposed targets as known, novel and virulent. Users can perform pathway analysis, choke point analysis, interactome analysis, subcellular localization and functional annotations through web servers cross-referenced with the application. Drug targets identified by TiD for Listeria monocytogenes, Bacillus anthracis, and Pseudomonas aeruginosa have revealed significant overlaps with previous studies (Gupta et al., 2017).

After target identification ligand screening is the next step. For this purpose, we refer ExPASy web server. ExPASy server is the online portal which provides access to scientific databases and software tools (i.e., resources) in different areas of life sciences including proteomics, genomics, phylogeny, systems biology, population genetics, transcriptomics etc. Swiss Similarity is online web based tool for ligand screening using the similarity based method. In this work, two ligand molecules were predicted against a target protein. The last step of drug designing is target ligand interaction studies. The autodock is the best freely available docking software.

Since its release in 1990 (Goodsell et al., 1990), AutoDock has proven to be an effective tool capable of quickly and accurately predicting bound conformations and binding energies of ligands with macromolecular targets (Morris et al., 1998; Huey et al., 2007; Goodsell et al., 1996). In order to allow searching of the large conformational space available to a ligand around a protein, AutoDock uses a grid-

based method to allow rapid evaluation of the binding energy of trial conformation
(Morris et al., 2009).

MATERIAL AND METHODS:

Data Collection

Protein sequence data was collected from NCBI protein database. NCBI database is available at https://www.ncbi.nlm.nih.gov. At NCBI database select protein database and search the query as organism name i.e. *Bacillus anthracis*. After that, the PDB protein sequences were sorted by filtering sequences from filter option and we got 1669 protein sequences. All selected 1669 sequence was downloaded in multiple sequence FASTA file format.

Methodology

The primary aim of this work is to identify the Target proteins from collected *Bacillus anthracis* proteome. Target proteins are those proteins which should be druggable and capable to produce virulence. To find target proteins TiD tool was used which is available at http://bmicnip.in/TiD/. This tool is a standalone tool and no need to do any installation. For this tool, the direct executable file is available only need to manage some data files. Other software's like.Net framework, Python2.7.10, biopython-1.65 for python2.7.10, NCBI BLAST 2.2.31+ were installed.

Following steps were used for target identification using TiD tool.

1. Paralog analysis.
2. Essentiality analysis.
3. Nonhomologous analysis.
4. Target Prioritization.

Paralog analysis:

Paralog analysis was performed CD-HIT at 60% identity to remove redundant paralogous sequences. FASTA file containing 1669 protein sequences were uploaded for this step. Paralog analysis step gives 250 sequences.

Essentiality analysis:

Essentiality analysis looks for pathogen specific essential genes in DEG, CEG and common from both DEG & CEG based on threshold E-values and bit scores.

1. Database of Essential Genes (DEG): It finds essential genes of the pathogen from DEG database.
2. Clustering of Essential Genes (CEG): It finds essential genes of the pathogen from CEG database.
3. Common of DEG and CEG: It finds common essential genes of the pathogen from DEG and CEG database.

In this step common of DEG and CEG analysis were performed. For this step, Essential analysis output file was used. E-value and Bit Score was selected as the default value. The default E-value is 10-5 and Bit Score is 100(same for CEG & Common for DEG and CEG). After common of DEG and CEG analysis, 101 essential gene sequences were obtained.

Nonhomologous analysis:

Nonhomologous analysis helps in identifying non-homolog proteins of pathogenic bacteria in different hosts and gut flora based on threshold E-values and bit scores. The default E-value is 0.005 and Bit Score is NONE. For this step select human as host and 40% threshold value was used for gut flora. After this analysis, 34 protein sequences were obtained.

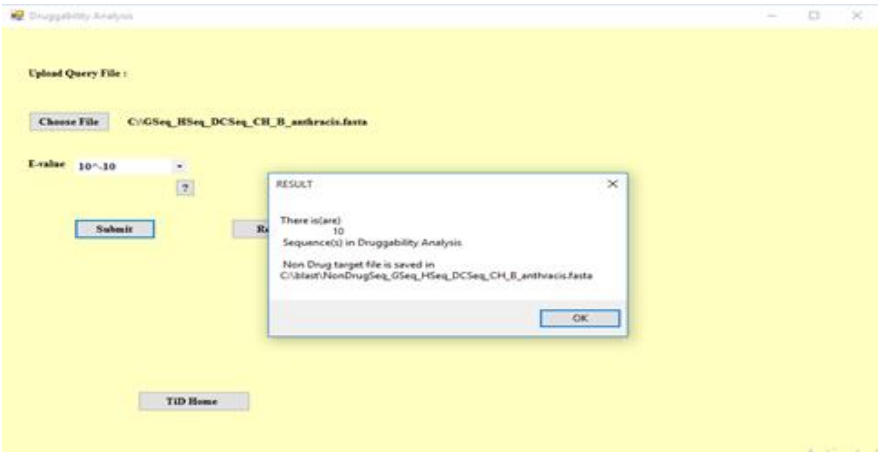

Fig1: Druggability analysis results

Fig2: Identified Druggable Target sequences

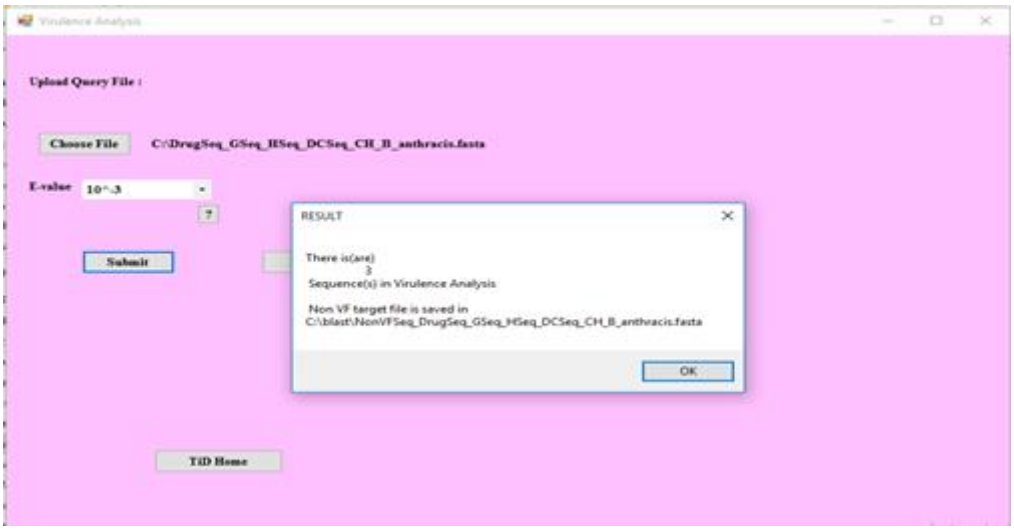

Fig3: Virulence analysis result

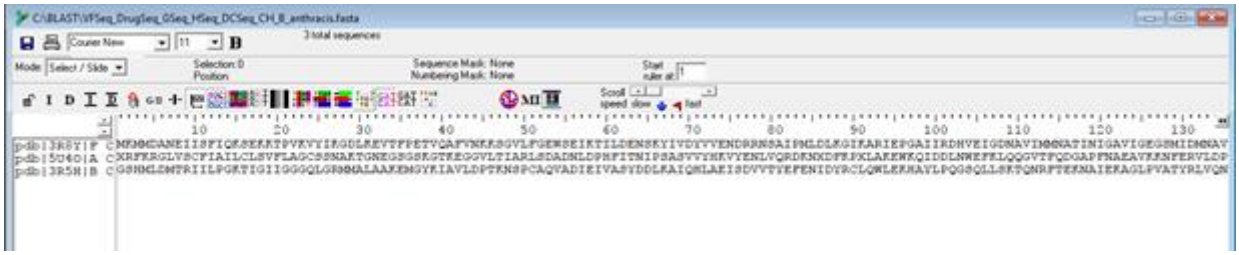

Fig4: Virulence target sequences

Target Prioritization:

Target Prioritization finds homology with druggable target and virulence target. For druggable analysis and virulence target, the default E-value is 10^-10 was used to optimize the result. After analysis 10 druggable and 3, virulence targets was Identified

Docking Studies:

Binding site Prediction:

Among three druggable targets and only one virulent, *N*5-carboxyaminoimidazole ribonucleotide synthetase protein was selected for docking suites. Primarily Protein structure was collected from PDB database and structure file saved as a 3R5H.pdb file. To check the binding site or active site for this protein POCASA tool was used. Using this tool 5 top score binding pockets were identified and top scored pocket coordinates were selected for drug interaction studies.

Protein Preparation:

Downloaded pdb file contained some salts, compounds and water molecules. Protein preparation is the key step in docking study in which all water molecules and other compounds are removed from protein structure file. LidDig online tool was used to remove all salts and

compounds from pdb file. This polish file further cleaned by removing of a water molecule using Auto dock tool.

Lead Identification:

Selected 2-(2,3-dioxoindol-1-yl)-N,N-diethylacetamide compound shows inhibition activity against prokaryots but no inhibition to human bifunctional enzymatic activity. We have chosen 2-(2,3-dioxoindol-1-yl)-N,N-diethylacetamide compound as a reference molecule for lead screening.

Lead compound was identified based on structure similarity search. The 2-(2,3-dioxoindol-1-yl)-N,N-diethylacetamide molecule was screened using ExPaCy's SwissSimilarity tool for which fingerprint algorithm was used against approved and experimental drug database. The highest similar ligand with reference compound was identified and selected for docking. Ropinirole (Approved) and Isatin (Experimental) drug compound were selected based on fingerprint algorithm. Similarity score for these compounds are 0.57 and 0.75 respectively.

Docking Protocol:

The AutoDock suite is used in numerous laboratories: a recent search on PubMed yielded over 1000 citations in the past year. For this work, we used Autodock 4.0 and following protocol was applied to check target ligand interaction.

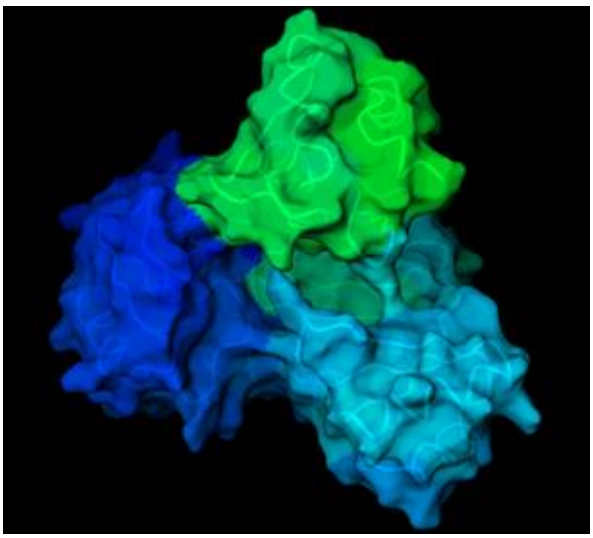

Fig5:N5-carboxyaminoimidazole ribonucleotide synthetase protein cavities

Protocol

1) Ligand preparation

Ligand → input → open (choose lead.pdb)
Ligand → torsion tree → set number of torsion (fever number set to 6)
Ligand → output → save as PDBQT (lead.pdbqt)

2) Protein preparation

Read molecule → open (3R5H.pdb)
Select → select from string (residue HOH*; atom *)
Edit → delete → delete selected atoms
Edit → hydrogen → add (add hydrogen)
Grid → macromolecule → choose (3R5H.pdb)
→ save (3R5H.pdbqt)

3) Grid parameter file

Grid → Grid box (60,60,60) (-12,-8,35) → file → close saving current
Grid → set map types → choose ligand → select ligand
Grid → output → save GPF (3R5H.gpf)

4) Docking parameter file

Docking → macromolecule → set rigid → filename
→ open (3R5H.pdbqt)
Docking → ligand → choose → directly
Docking → search parameters → genetic algorithm → accept
Docking → output → Lamarckian GA → save (lead.dpf)

5) Running docking program in Command prompt.

Autogrid4.exe –p 3R5H.gpf -l 3R5H.glg
Autodock4.exe –p lead.dpf –l lead.dlg

6)

7) Analyzing docking result

Analyze ➔ docking ➔ open (lead.dlg)
Analyze ➔ micromolecule ➔ open (3R5H.pdbqt)
Analyze ➔ conformations ➔ play ranked by energy
Analyze ➔ docking ➔ show interactions

)

RESULTS

In this docking studies best docking poses and score were obtained for Ropinirole and Isatin are shown in fig 6-9. We also compared obtained score of Ropinirole and Isatin with reference compound score and it shows almost similar binding energy score.

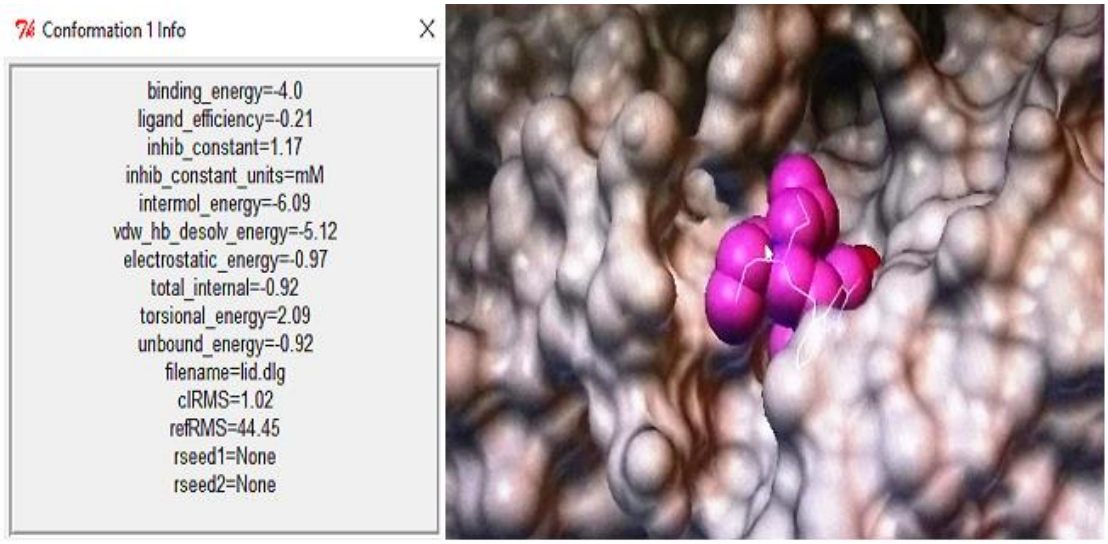

Fig6: Ropinirole - Best Docking pose 1 and docking score

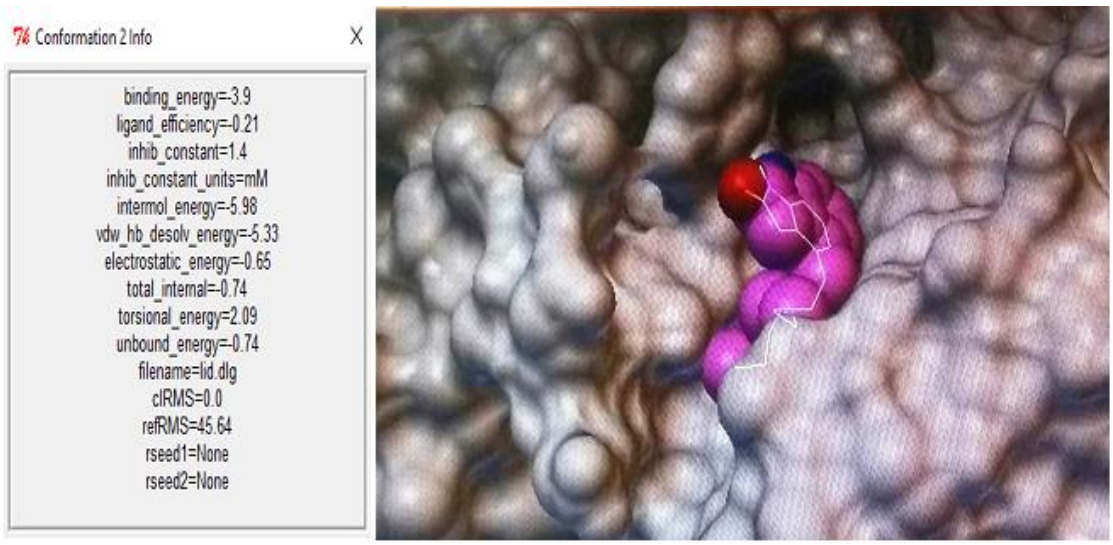

Fig7: Ropinirole - Best Docking pose 2 and docking score

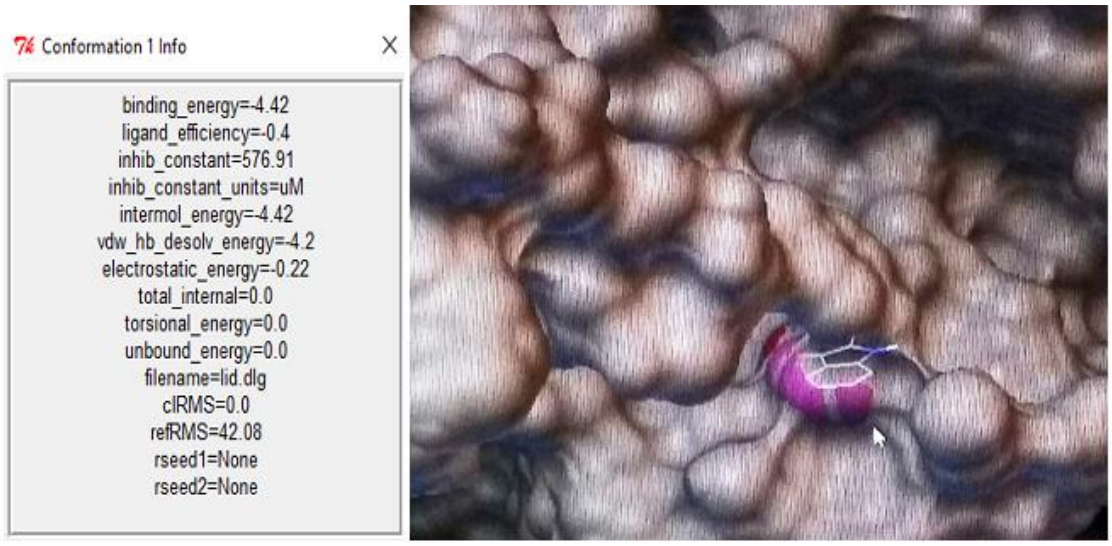

Fig8: Isatin - Best Docking pose 1 and docking score

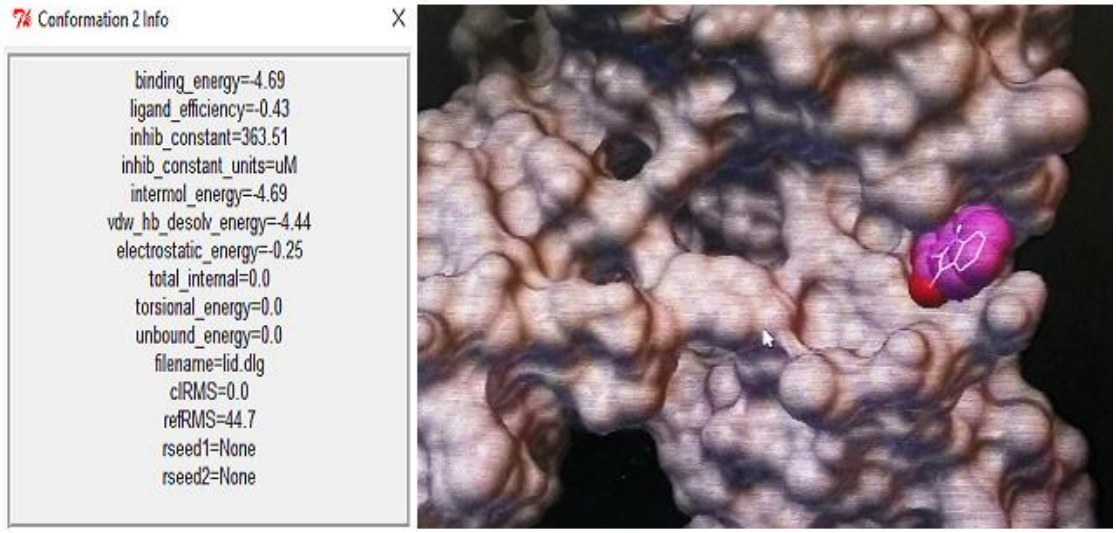

Fig9: Isatin - Best Docking pose 2 and docking score

CONCLUSION

Antibiotic resistance has been a significant increase during the past decade. The increasing frequency of drug resistant bacterial infection as amplified the need for novel antimicrobial agents. Previous study has shown that N 5-carboxyaminoimidazole ribonucleotide synthetase is a key intermediate in purine biosynthesis in bacteria , fungi but not in humans. In our study we indentified N 5-carboxyaminoimidazole ribonucleotide synthetase as a virulent as well as druggable protein using novel approached i.e. TiD. For this target 2-(2,3-dioxoindol-1-yl)-N,N-diethylacetamide compound screened against approved drug and experimental database using fingerprint algorithem. In the screening procedure Ropinirole (approved) and Isatin (experimental) drug were indentify with good similarity score. Further, we have studied interaction of selected target and screened lead compound which results good score with lowest possible binding energy and ligand efficiency. This result shows both Ropinirole and Isatin compounds can be acts as a good antimicrobial drug candidate. The activity of this ligand against target protein can be optimized by wet lab experiment.

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

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RESEARCH REPORT

Skin graft is transplantation of human skin layer for re-pigmentation of vitiligo patches

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ABSTRACT

Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use as per UN Convention on Biological Diversity, Art. 2. The human skin and diseases related to human skin are many. The vitiligo is a commonly found skin disorder in which the skin color is lost and white macules are visible.It is due to lack of melanin formation activity. The melanin decide color of the skin There are many treatment methods for vitiligo. The purpose is to regain original skin color at the sites which are white. The transplantation of skin layer from the unaffected pigmented skin to the affected white skin area. It cannot stop the progression of the disease, but definitely it helps to treat many delicate areas like eyelids, lips which does not show adequate response to medical therapy.

Keywords: Vitiligo, Skin graft, repigmentation,

INTRODUCTION

Vitiligo is a skin disorder in which depigmented macules (white patches) start appearing and progressing. It has high psychosocial impact, particularly in darker skins. With vitiligo, the body's own immune system starts attacking those cells, which is why it's considered an autoimmune disease. Surgical methods become important in cases where medical therapy fails to cause re-pigmentation or in cases of segmental vitiligo where the response to surgery is excellent.

The vitiligo is treated by various methods like phototherapy, herbal medicine therapy, allopathic medicines and homeopathic treatment is also one of the options for patient. The idea of skin graft comes under surgical treatment. In this technique, natural skin colour is obtained by transplanting the skin from unaffected body part to the affected area of skin which has lost colour. The transplanted skin patch helps to achieve the re-pigmentation.

Skin grafting was first described in India in ancient Sanskrit texts around 2500 - 3000 BC as a technique for nasal reconstruction for mutilated

noses. Thin split thickness skin grafts were first introduced in 1872 by Ollier in France, and later by Thiersch in Germany in 1874. Brown in England developed the electric dermatome in 1944, to harvest thin homogenous grafts. In 1947, Haxthausen transplanted thin split thickness skin grafts from normal to vitiliginous skin in three cases, to study the pathogenesis of the disease (Falabell 2007; Savant 2005). Behl (1964) from India was the first to describe the surgical treatment of vitiligo in a large series of 107 patients with thin Thiersch grafts. Falabella (1971) described the suction blister technique for repigmentation of vitiligo and later the miniature punch grafting technique (Falabella, 1971) (Falabella *et al*., 1989) also described the use of *in vitro* cultures of melanocyte-bearing epidermis for the treatment of vitiligo. The use of epidermal suspensions obtained by trypsinization was first reported in 1992 by Gauthier and Surleve-Bazeille [8] and further improved by Olsson and Juhlin, (2002) by adding a melanocyte culture medium, for additional growth. Kahn and Cohen (1996) utilized the motorized dermatome, to obtain ultrathin grafts for vitiligo, and later Kahn *et al.*, (1996) reported the use of a short-pulse carbon dioxide laser, to denude the recipient area. Subsequently, the excimer laser and targeted phototherapy have been developed to treat vitiligo. Thus, surgical treatment of vitiligo has evolved over the centuries, even though the etiology and pathogenesis of vitiligo remain elusive.

Raising of blisters Blisters may be raised using syringes or suction pump and suction cups or a negative pressure cutaneous suction chamber system. Using syringes to raise blisters is the most commonly employed method. The bases of syringes of sizes 10 ml and 20 ml are coated with vaseline and are applied on the donor site. Approximately 20ml to 30ml of air is aspirated using a 50ml syringe and a three-way-cannula. Larger syringes (>20ml) take a longer time to generate a blister, and smaller syringes (<10ml) provide blisters that are difficult to handle. It usually takes 1.5 to 2.5 hours for the development of blisters. A single unilocular non-hemorrhagic blister is the best result. In case of smaller blisters, one can either increase the negative pressure in the syringe by another 5ml or intradermally inject saline into the blister to expand it. Dr. Pandya, the only full-time pigmentary disorders specialist in Texas, has spent more than a decade treating vitiligo patients in the Pigmentation Disorders

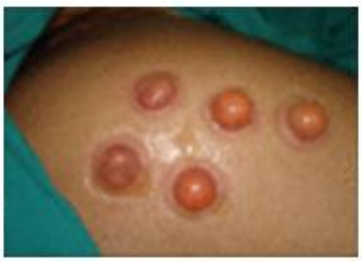

Fig. 1: Raising suction blisters by the syringe method
Fig. 2: Blisters formed after 2 hours of suction.

Clinic at UT Southwestern. Vitiligo affects about 2 million people in the United States. Traditional treatments include phototherapy, requiring different types of machines to shine ultraviolet light on the whole body, localized areas, or the hands and feet. UT Southwestern also uses an excimer laser to treat smaller lesions.

For the transplant procedure, a syringe and a heat lamp to create a small blister graft on the thigh. Thousands of cells are removed from the blister roof and those cells are then applied to the affected area. Suction blister grafting (SBG) is a technique where the pigmented epidermis is harvested from the donor site by using suction to raise a blister which is then transferred to the vitiliginous area.

CONCLUSION

The differentiation and development of the epidermal cells is regulated by the dermis. In surgical techniques like split skin thickness grafting and punch grafting, where both the epidermis and dermis are grafted, the graft retains some of the characteristics of the donor site, hence, the cosmetic outcome may not be an exact match. However, in suction blister grafting, cleavage occurs between the basal cells and the basal lamina of the basement membrane zone and only the epidermal portion of the donor area is grafted. Hence, the graft generally acquires the characteristics of the recipient site, thus leading to a better color match and cosmetic outcome.

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REVIEW ARTICLE

Lonar lake potential remedy site for bioremediation of chromium metal: A Review

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ABSTRACT

Chromium metal is a major contaminate found in industrial effluent. It causes physical discomfort and some life threatening illness including damage to body metabolisms in humans and other organisms. Bioremediation uses microorganisms to break down toxic and hazardous compounds in the environment. Bioremediation is cost-effective and eco-friendly method. Due to its high pH Lonar lake is unique habitat harboring diverse microorganism. In present review, I have tried to focus on chromium bioremediation from various isolate of microorganism from Lonar lake.

Key words: Chromium pollution, bioremediation, Lonar lake.

INTRODUCTION

Chromium metal occurs in several oxidation states ranging from (−II) to (+VI), trivalent and hexavalent states are the most stable states (Nieboer and Jusys 1988). Chromium in hexavalent form is highly soluble and bioavailable (Yadav *et al.* 2005). The chromium is carcinogenic, clastogenic, and teratogenic (Yassi and Nieboer 1988; Chardin *et al.* 2002). Hexavalent chromium, an EPA priority pollutant, is a predominant waste product of several metal finishing, tanning, petroleum refining, and iron and steel industries. It exists primarily as either chromate in basic and neutral environments or as dichromate in acidic environment (Middleton *et al.* 2003). The extensive application of chromium in industries particularly leather tanning industries, dye and pigment manufacturing, wood preservation, textile dyeing, steel and alloy industries to the formation of chromium-contaminated soil and ground water which poses a serious threat to the biological living system particularly to human health.

The conventional metal cleanup technologies like soil removal and land filling, stabilization, physico-chemical extraction, and soil washing (Jeyasingh and Philip 2005) offer a temporary solution by simply immobilizing the contaminant (Pal and Paul 2004). Chemical methods of the reduction of chromate for large-scale decontamination of chromium

are also known (Ackerley *et al*., 2004). But these methods have inherent problem with them. Bioremediation is an economically feasible and technically efficient technology for metal removal/recovery. It can comfortably fit into the metal treatment processes and is eco-friendly in nature (Gupta *et al.*2002). Bioremediation of a refinery sludge containing hydrocarbons in a semi-arid climate using land farming has shown reduced total hydrocarbon content (Marin *et al.,* 2005). Bioremediation is the efficient method in which microorganism is used for the removal of toxic metal from contaminated water. Because the industrial effluent contains high pH, the bacteria were isolated from Lonar Lake provide unique opportunity to be used for bioremediation.

The Lonar Lake, situated in the Buldhana district of Maharashtra State, India, is located at 19° 58'N, 76° 31'E. Lonar Lake is the only meteoritic crater in basaltic rock in the world and thus becomes a unique ecological system be. Preliminary results of fission track dating of shock-melted glass indicate an age of the lake less than 50,000 years (Fredriksson *et al* 1976).It is an almost circular depression, 1830 m across and nearly 150 m deep. The water is very shallow with a maximum depth of the brine about 5 m. Chlorides accounted for 30% w/v of water years (Nandy and Deo 1961). Lonar lake water is alkaline (pH 10.3) and characterized by high concentration of salts (9060 mg/L), chloride (3492 mg/L), salinity (6391 mg /L), alkalinity (3751 mg/L), total hardness (480 mg/L), calcium hardness (118 mg/L), magnesium hardness (361 mg/L), sulphate (21 mg/L), phosphate (0. 44 mg/L), nitrate (3.7 mg/L) and dissolved oxygen (0.0034 mg/L) (Tambekar *et al.,* 2010). The Cr(VI)-reducing bacterial strain MCMB-821 was isolated from the alkaline crater lake of Lonar and was identified as *Burkholderia cepacia. (*Revati Wani *et al*). Reduction, detoxification and possible remediation chromium by using various microorganisms has been the topic of scientific interest for a number of decades. A large number of natural and synthetic organic compounds are biodegradable by microorganisms as part of their normal metabolism for energy and growth. *Pseudomonas aeruginosa* isolated from Lonar Lake showed the potential to reduce, detoxify and possibly remediate chromium effectively and ecofriendly by which it reduces the pollution from water (Tambekar and Adhao). The *Lysinibacillus mangiferihumi* was isolated from Lonar which shown capacity of bioremediation of chromium (Tambekar *et al*. 2016). Another bacterium *Proteus mirabilis* isolated from Lonar Lake found to be highly efficient in using chromium and has potential for bioremediation on polluted sites (Tambekar *et al.,* 2014)

CONCLUSION

Owing to hazards of chromium contamination management of chromium pollution is paramount. Chromium contamination can be managed by chemicals treatment but chemical treatment has its own problems. So, it becomes necessary to focus on biorimidiation of chromium. As most of the industrial effluents has high pH bacteria from alkaline environment can be effectively used for bioremediation of chromium. Lonar being salt lake has high pH and microbes from Lonar Lake have shown it affectivity on chromium management. It is already established that Lonar lake is not less than miracle but with this new knowledge it can also become potential site for microbes for bioremediation.

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

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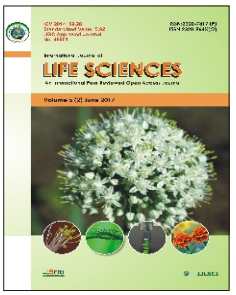

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