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01

Tribal medicine formulation (TMF) used by Tribal Practitioner's for Foot and Mouth disease in Animals from Buldana district, Maharashtra

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Abstract:-

In the present investigation, an attempt has been made to appraise the ethno-veterinary medicinal plants of Buldana district. Plants are utilized extensively as raw drugs for many formulations in traditional system of medicine. Our country has a long tradition of using herbal products for livestock. This indigenous traditional knowledge of medicinal plant of various ethnic communities, where it has been transmitted orally for centuries n fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture. The paper deals with the selected 10 ethno veterinary medicinal plants which is used in treatment of foot and mouth diseases in animals from Buldana district.

Key words:- Ethno veterinary medicinal plants. Tribal medicine formulation.

Introduction:-

The nature has provided an absolute resource of remedies to cure the several ailments of mankind as well as Animal health care. Ancient human beings were closely associated with other animals especially the domesticated ones and with the plants those were found in and around their close vicinity as well as with other plants which were used for their daily necessities like food, shelter, clothing and medicines. Ancient man has discovered natural products to satisfy his needs including relief from his personal ailments as well as fellow domestic animals. This has been tested through lapse of time and later on these findings were transmitted to the succeeding generation through the words of mouth very little of this knowledge has recorded so far and it seems that these valuable time tested findings are on the verge of extinction. A part of such knowledge is tried to be retrieved in this paper.

Methodology:

I. Tribes and culture:-

This tribal 'Bhil' lives in the tropical evergreen forest association with nature referring to the dense thickets they inhabit. The tribal are involved in collecting of non timber forest products like honey, lichens, soapnut etc.

II. Interaction with tribal healers:-

The indigenous information of the community herbalists tribal practitioners, other rural traditional hearers and the ethnomedicinal plant drugs (EMP) practiced for medicinal utility were collected through extensive base line survey with tribal medicinal men (TMM) followed by personal interviews and semi-structured questionnaire prepared for documentation of traditional knowledge.

III. Collection of Ethno-medicinal plant drugs:-

The plants of ethno-veterinary significance were collected in vegetative as well as blooming condition, simultaneously jotting down the vernacular names. The morphotaxonomical description of each plant taxon was done and identified with the help of different floras. The collected data arrange with alphabetically name of plant, local name, parts used and mode of Administration.

Observation:-

Foot and mouth disease

Mouth disease attacks the animals when they eat newly raised grass on the ground in the month of June. The symptom of the disease is that animals gets boils inside and outside the mouth. The boils appear on the tongue also. These boils look red and froth comes out of the animals' mouth. Sometimes blood might come out from the wounds caused by

the boils outside the mouth, boils are visible in the corners of the lips due to this animal cannot eat fodder properly and it becomes foot disease attacks the cattle, when the animals walk in water, mud, through wet places etc symptoms of the disease is that the animal cannot walk properly

Sr. No	Plant Name	Local name	Family	Plant Parts used	Mode of Administrations
1.	*Annona squamosal* L	Sitaphal	Annonaceae	Leaves	Decoction of leaves after warming mixed with alum and dropped over the injuries.
2.	*Azadirachta indica* A. Juss	Kadunimb	Meliaceae	Leaves	Decoction of leaves given to animals .
3.	*Barleria prionitis* L.	Kalekoranti	Acanthaceae	Leaves	Paste of leaves applied to inter digital space in animals .
4.	*Calatropis Procera* (Ait) R	Rui	Asclepiadaceae	Leaves from leaves	Latex from leaves in the treatment of food and mouth disease.
5.	*Clerodendrum multiflorum* (Burm)f	Aarni	Verbenaceae	Latex	Decoction of leaves dropped on injuries.
6.	*Diospyros melanoxylan Roxbe. L.*	*Tendu*	*Ebenaceae*	*Fruit*	Paste of roasted fruits is applied over the mouth and hooves of animals.
7.	*Ficus religosa L.*	Pimpal	Moraceae	Bark	Bark boiled in water for 30 minutes and lukewarm leachare is applied on the affected hoofs during foot and mouth disease .
8.	*Gloriosa superba.* L.	Kal-lavi	Liliaceae	Tubers	Tubers crushed with water applied over toes of the cattle.
9.	*Pueraria tuberosa* Roxb. ex-Wild	Bhuilcohala	Fabaceae	Tubers	Powdered tubers mixed with water given twice daily to cure foot diseases of animals.
10	*Semecarpus anacardium* L.	Biba	Anacardiaceae	Fruits	Fruits sand witched in Jawar bread are fed to animals.

Discussion:-

The present study embodies the information on 10 ethno veterinary medicinal plants and mode of administration employed by different herbal healers inhabiting in herbal healers. The foot and mouth disease in animals is incurable but practioners used the Ethnomedicinal plants for the treatment.

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02

UV-Visible, FTIR and NMR spectroscopic analysis of *Ampelocissus latifolia* (Roxb.) Planch

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Abstract:

Present investigation was carried to make an UV-Vis, FTIR and NMR spectroscopic analysis of *Ampelocissus latifolia* (Roxb.) Planch tuberous root. Petroleum ether extract was examined under UV and Visible light for proximate analysis. UV-VIS spectrum profile of petroleum ether extract of *Ampelocissus latifolia* tuberous root showed the peaks at 425 nm, 490 nm and 600 nm with absorption of 1.569, 1.144 and 0.626 respectively.

FTIR method was performed by using Perkin Elmer (RX1) Spectrophotometer and characteristic peaks were detected. FTIR spectrum was confirmed the presence of alcohols, aromatic compounds, alkanes, aldehydes, ketones, alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halides in petroleum ether extract. ^{1}H NMR spectrum of *Ampelocissus latifolia* tuberous root powder ethanol extract indicate the nature of proton as -CO-N-H, C=C-H, CH-F, CH-O-H, C-OH, CH-COOH and -CH_3 at chemical shift values 8.1456, 6.6449, 4.8353, 3.7820-3.1182, 2.5096, 2.1699, 1.2299-1.0598 ppm respectively. Result of present study strongly support the presence of various bioactive compounds and hence used by traditional practioners to cure various ailments.

Key words: UV-Vis, FTIR, NMR and *Ampelocissus latifolia.*

Introduction

Medicinal plants are considerably useful and essential for human survival. They contain variety of active constituents that are used for the treatment of many human diseases, in health care troubles [1]. Ancient Indian literature incorporates an extremely wide range of definition of medicinal plants and considers 'all plant parts to be potential sources of medicinal substances'[2]. Hence at present, researchers and organization has been engaged in determination of traditional remedies as an alternative source of medicine [3].

Large number of medicinal plants and the active constituents obtained from them has shown a various remedial activities [4]. Spectroscopic methods such as UV-Vis and FTIR are required either separately or in combination for simple, cost-effective and rapid detection of phytoconstituents [5, 6, 7]. NMR spectroscopy has also been utilized for the identification of unknown compounds from complex mixture [8]. *Ampelocissus latifolia* (Roxb.) Planch belongs to family Vitaceae, used to treat various ailments in ethno medicine practices such as antidote, dyspepsia, gout, tuberculosis and as a health tonic [9]. Hence present investigation was carried out by using UV-Vis, FTIR and NMR spectroscopic techniques which may provide an imminent use of selected plant as traditional medicine.

Materials and methods:

Collection of plant materials:

Whole plant of *Ampelocissus latifolia* was collected from Wasali forest area of Buldana District, Maharashtra, India. Plant was identified by using various floras [10, 11] and also from experts of this region. Tuberous roots were

collected, thoroughly washed with distilled water; shade dried and then grinds into fine powdered by using mechanical grinder.

Successive solvent extraction:

The grinded fine tuberous root powder of *Ampelocissus latifolia* was subjected to successive solvent extraction by using petroleum ether and ethanol by soxhlet apparatus. About 20 gm of tuberous root powder was successively extracted with 250 ml of petroleum ether and ethanol for 8 hrs separately. Petroleum ether and ethanol extract was filtered through Whatman No. 1 filter paper and filtrate was collected (crude extract), these extracts were concentrated, solidified and used for further studies.

Ultraviolet-Visible (UV-VIS) spectroscopy:

Petroleum ether extract of selected plant was examined under UV and Visible light for immediate investigation. This extract was centrifuged at 3000 rpm for 10 minutes and filtered through filter paper (Whatman No.1) under high pressure of vacuum pump. The sample was diluted to 1:10 by using same solvents. Extract was scanned in the wavelength range from 190-1100 nm using EQUIP-TRONICS (EQ-826) spectrophotometer and peaks were detected.

Fourier Transform Infrared Spectroscopy (FTIR):

Dried powder obtained from petroleum ether extracts of tuberous root of *Ampelocissus latifolia* was used for FTIR analysis. Dried 10 mg of extract powdered was mixed with Potassium Bromide (KBr) salt and encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. An infrared spectrum of solid was recorded in scan range from 4400-450 cm^{-1} on a FTIR spectrophotometer, Perkin Elmer Spectrum (RX1) with a resolution of 1 cm^{-1} using KBr pellet method. The results obtained were plotted against wave number versus percentage transmittance.

Fourier Transform - Nuclear Magnetic Resonance (FT-NMR) Spectroscopy (400 MHZ):

Dried powder obtained from ethanol extract of tuberous root of *Ampelocissus latifolia* was used for NMR analysis. Sophisticated multinuclear Avance-II Bruker 400 FT-NMR spectrometer was used for ^{1}H NMR spectroscopic analysis. The instrument was equipped with automatic sample changer. Deuterated dimethyl sulphoxide (DMSO) was used as solvent and the chemical shifts were determined. FT-NMR spectroscopic analysis was carried out at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh.

Results and Discussion:

Qualitative UV-VIS spectrum profile of *Ampelocissus latifolia* tuberous root powdered for petroleum ether extract was selected at wavelength range from 400 to 600 nm due to sharpness of peak and proper baseline (fig. 1). The profile was shown peak at 425 nm, 490 nm and 600 nm with absorption of 1.569, 1.144 and 0.626 respectively (Table no.1).

Fig. 1: UV-Visible spectrum of petroleum ether extract of tuberous root of *Ampelocissus latifolia*

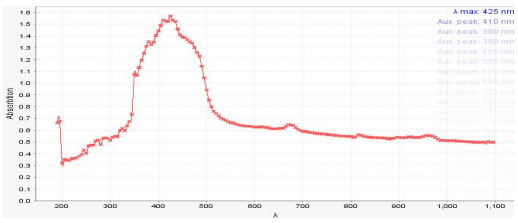

Table 1: UV-VIS peak values of Petroleum ether extract of *Ampelocissus latifolia* tuberous root

Sr. No.	Extract	Wavelength (nm)	Absorption value
1	Petroleum ether	425	1.569
		490	1.144
		600	0.626

FTIR spectroscopic analysis of *Ampelocissus latifolia* tuberous root was carried out by using petroleum ether extract (fig. 2). It was revealed the presence of alcohols, aromatic compounds, alkanes, aldehydes, ketones,

alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halides (table no. 2). Similar work was also carried out earlier by Pednekar and Raman, (2013) and Raman *et al*., (2014) also support the presence of various functional groups reported during present study.

Fig. 2: FTIR spectrum of petroleum ether extract of *Ampelocissus latifolia* tuberous root

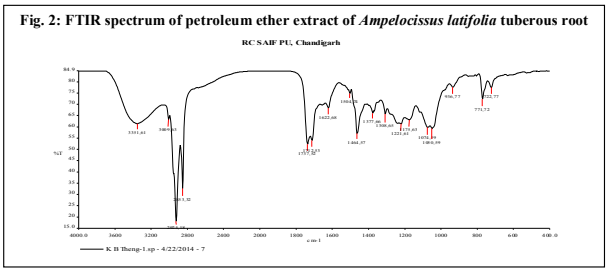

Table 2: FTIR peak values of *Ampelocissus latifolia* tuberous root powdered in petroleum ether extract

Sr. No.	Wave Number cm^{-1}	Bond	Functional group Assignment	Group Frequency cm^{-1}
1	3351,61	O-H stretch	H-bonded alcohols	3200-3600
2	3009, 63	-C-H stretch	Aromatic compound	3000-3150
3	2925,18	C-H stretch	alkanes	2850-2970
4	2853,32	C-H stretch	alkanes	2850-2970
5	1737,52	C=O stretch	aldehydes	1725-1740
6	1712,53	C=O stretch	ketones	1705-1725
7	1622,28	C=C stretch	alkenes	1600-1680
8	1504, 74	N-O asymmetric stretch	nitro compounds	1475-1550
9	1464,57	C-H bend	alkanes	1340-1470
10	1377,66	C-H bend	alkanes	1340-1470
11	1308,65	N-O stretch	nitro compounds	1300-1370
12	1221,61	C-N stretch	amines, amides	1180-1360
13	1175,63	C-O stretch	alcohols, carboxylic acids, ethers, esters	1000-1320
14	1074,59	C-F stretch	Alkyl halides	1000-1150
15	1050,59	C-N stretch	primary amines	1020-1090
16	936,77	=C-H bend	alkenes	650-1000
17	771,72	=C-H bend	alkenes	650-1000
18	722,77	C-H bend	alkanes	720-725

^{1}H NMR spectroscopic analysis of *Ampelocissus latifolia* was carried out by using ethanol extracted powder of tuberous root. The ^{1}H NMR spectrum was obtained as represented in figure 3.

^{1}H NMR spectrum of *Ampelocissus latifolia* was compared with the reference chart (Pavia *et al*., 2007) and probable functional groups present in ethanol extracted powdered of tuberous root was determined as represented in table 3.

Singlet peak appeared at chemical shift (?) 8.1456 ppm was indicated the presence of hydrogen atom attached to nitrogen of an amide (-CO-N-H). The appearance of peak at ? 6.6449 ppm was revealed that hydrogen atom attached to double bonded carbon atom (C=C-H). Peak appeared at ? 4.8353 ppm was shown that hydrogen atom attached to same carbon with halide as adjucent atom (CH-F). Multiplate peak appeared in range of ? 3.7820- 3.1182 ppm was indicated that hydrogen with carbon atom attached to the adjacent oxygen atom (CH-O-H) in deshielded environment due to electronegativity of oxygen atom.

Singlet peak appeared at ? 2.5096 was indicated the presence of hydroxy group (C-OH) in more deshielded environment. Chemical shift value 2.1699 ppm was confirme the presence of hydrogen atom adjacent to carbonyl group (CH-COOH). Apperance of multiplate peak in range of ? 1.2299- 1.0598 ppm was shown presence of -CH_3 group (Table no. 3).

Fig. 3: ^{1}H NMR spectrum of *Ampelocissus latifolia* tuberous root

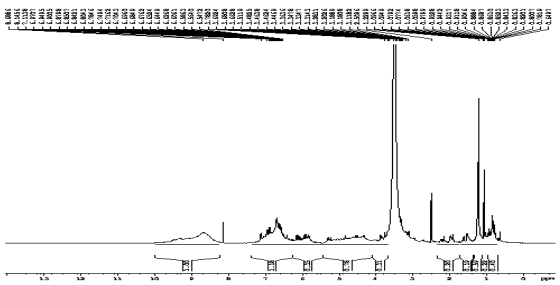

Table 3: ^{1}H NMR data and their assignment from *Ampelocissus latifolia* tuberous root powder extract

Sr. No.	Chemical shift (δ ppm)	Nature of proton
1	8.1456	-CO-N-H
2	6.6449	C=C-H
3	4.8353	CH-F
4	3.7820- 3.1182	CH-O-H
5	2.5096	C-OH
6	2.1699	CH-COOH
7	1.2299-1.0598	$-CH_3$

Conclusion:

Analysis of *Ampelocissus latifolia* tuberous root by using UV-Vis, FTIR and NMR spectroscopy supported that it acted as important source of phytoconstituents. FTIR spectroscopy is used to determine various functional groups present in sample and as a tool to confirm the identity of a particular compound. ^{1}H NMR spectroscopy is used to find out the nature of hydrogen atom present in compound and also to find out connectivity of hydrogen atom. This selected plant can be used by traditional practioners for various medicinal purposes. Hence, further advanced analytical techniques are required for the structural elucidation and identification of compounds.

Acknowledgement:

We would like to thanks Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh for providing FTIR and NMR facilities.

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03

"OCCURRENCE OF PEDIASTRUM SPECIES FROM KHADAKPURNA RESERVOIR IN BULDANA, DISTRICT– MAHARASHTRA, INDIA".

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Abstract

The present investigation deals with the study of genus *Pediastrum* from Khadakpurna reservoir, Khadakpurna Reservoir is constructed on Khadakpurna River of Godavari basin near Garkhed Village, Deulgaon Raja Tehsil in Buldana District, State Maharashtra. Water samples were collected monthly from four different locations, during August - 2013 to July -2015, during this period three different forms of species have been collected that are 1. *Pediastrum ovatum* (Ehr.) A. Braun 2. *Pediastrum tetras* (Her.) Ralfs Var. *excisum* (Rabenh.) Hansgirg. 3. *Pediastrum simplex* Meyen Var. *duodenarium* (Bailey) Rabenhorst.

Key words: *Pediastrum species, Morphology, Khadakpurna reservoir.*

Introduction

Algae range from small, unicellular organism to multi-cellular organism, some with fairly complex and differentiated form. Algae are usually found in damp places or bodies of water and thus are common in terrestrial as well as aquatic environments. *Pediastrum* is a widely distributed genus of green algae characteristically consisting of disc-shaped colonies or coenobia, assembled from at least four interconnecting cells. Several species of the genus *Pediastrum* frequently occur in eutrophic fresh water bodies (Komarek & Jankovska, 2001). The algae have been an interesting group for investigation because of their very primitive nature and a world-wide in distribution, which is due to their capability to exist under most varied environmental conditions. Chlorococcales algae are mostly unicellular or may form colonies of a definite shape (Prescott, 1962). Fresh water green algae (Chlorophyta) are characterized as the largest and most varied algal group. The fresh water green algae have great diversity in their cellular organization, morphological structure and reproductive process than that of any other algae (Bold *et al,* 1978).

Materials, methods and study areas.

Water samples were collected from four different locations L-1 Takarkhed, L-2 Garkheda, L-3 Singaon Jha and L-4 Gavhan regularly at monthly intervals using clean sterilized plastic bottles. Samples of fresh water algae were collected with the help of phytoplankton net mesh and preserved in 4% Formalin. Khadakpurna Reservoir is constructed on Khadakpurna River of Godavari basin near Garkhed Village, Deulgaon Raja Tehsil in Buldana District, State Maharashtra, India. It is situated at 20° 9' 30" N and 76 4' 30" E, only 60 km away from district place. The dam is about 2160 meters in length with catchment area of 5133.18 sq km. The project is having 160.606 mcum capacity of water storage, which includes 93.404 mcum live, and 67.202 mcum dead water stocks. Collected algal sample were studied in department of Botany G.S. College, Khamgaon, District Buldana (M.S.).

Results and Discussion

The morphological characteristics of the three forms of species including genus *Pediastrum* from Khadakpurna reservoir were observed which are described as follows.

Systematic Position

Kingdom : Plantae
Division : Chlorophyta
Class : Chlorophyceae
Order : Chlorococcales
Family : Hydrodictyaceae
Genus : *Pediastrum*

1. *Pediastrum ovatum* (Ehr.) A. Braun. Plate - 1, F - 1, 2 & 3

Phillipose, M.T. 1967, p. 115, Fig. 37, a, b & f.

Colonies usually 4-8-16- (rarely 32-) celled, with the cells arranged in a ring round a central space or with one or more interior cells and number of marginal cells, perforate or almost imperforate, perforation being small. Cells plumper than in *P.simplex var duodenarium* with outer side of peripheral and often central cells convex. Cell wall smooth or ornamented. Colony is 76.20 µm in diameter and cells are 25.7 µm long, 12.8 µm broad Location - L-1, L-2, L-3.

2. *Pediastrum tetras* (Her.) Ralfs Var. *excisum* (Rabenh.) Hansgirg. Plate - 1, F - 4

Phillipose, M.T. 1967, p. 129, Fig. 45, f.

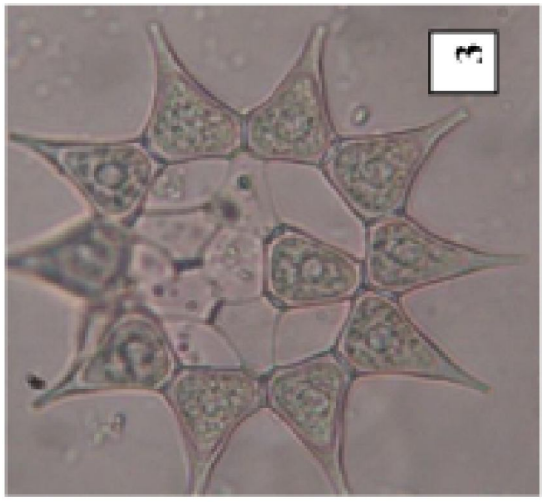
Colonies rectangular, oval, or circular of 4-8-16 (-32) cells without intercellular spaces. Marginal cells divided in to two lobes by a deep linear to cuneate incision on the outer side reaching to the middle of the cell. Each lobe truncate, slightly emarginated, or further divided in to two lobes. Inner cells 4-6 sided with a single linear incision. Colony rectangular, 4 celled, lobes are deeply concave. Cells 10.5 µm broad and long, colony is 21 µm long. Location – L-2, L-3.

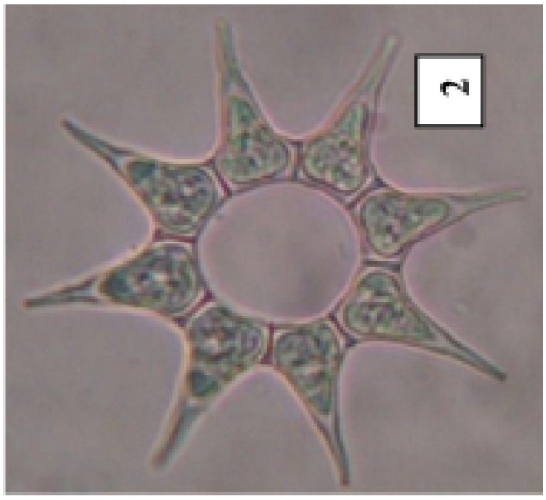
3. *Pediastrum simplex* Meyen Var. *duodenarium* (Bailey) Rabenhorst. Plate-1, F - 5 & 6

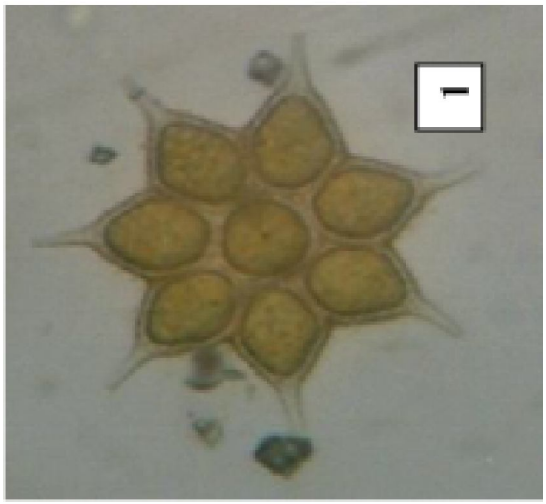
Phillipose, M.T. 1967, p. 115, Fig. 36, f & g.

Colonies of 4-8-32-64-128 (usually 8-16-32) cells. Differs from the type in having large intercellular spaces or a single central space with the cells arranged in a ring at the periphery. Inner face of marginal cells concave, outer face prolonged in to a single delicately tapering process. Sides of marginal cells also concave or nearly straight. Interior cells similar to marginal cells but with shorter processes. Cell wall smooth or finally punctate. Cells. Location – L-2, L-3, L-4.

PLATE – 1







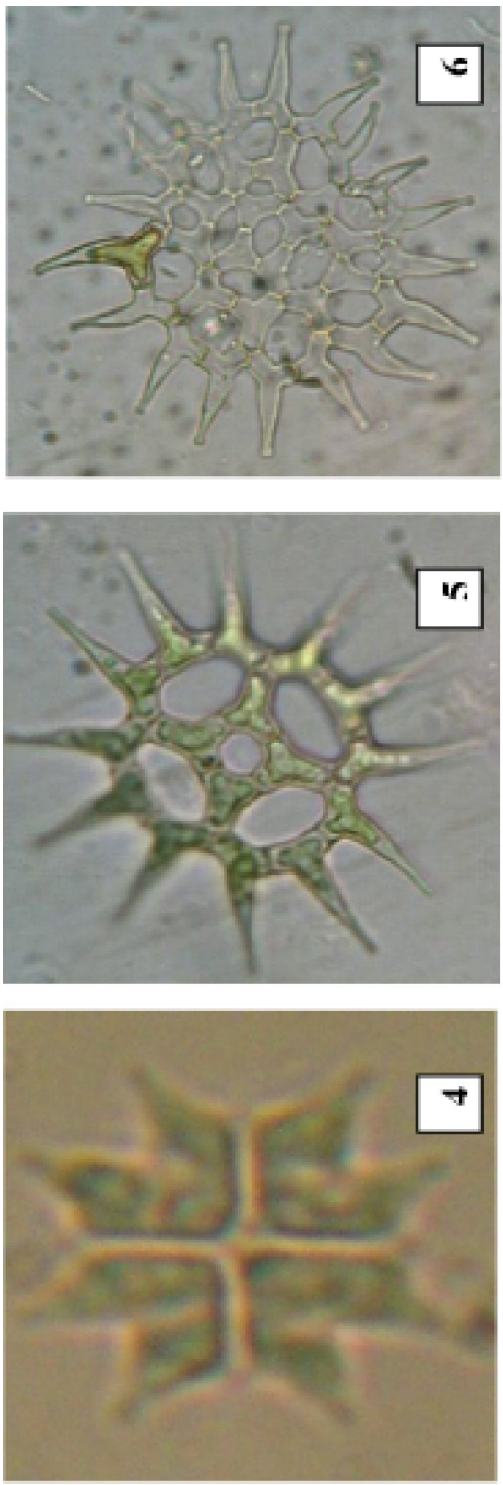


Fig – 1, 2 & 3 *Pediastrum ovatum* (Ehr.) A. Braun 4. *Pediastrum tetras* (Her.) Ralfs Var. *excisum* (Rabenh.) Hansgirg. F. 5 & 6. *Pediastrum simplex* Meyen Var. *duodenarium* (Bailey) Rabenhorst.

Conclusion

"Pediastrum is a Pearl of Water". Temperature and light important to growth and development of phytoplankton's. Prescott G. W. (1984) reported that for fresh water the optimum temperature is between 20- 25. An increase and decrease in temperature may affect the growth of phytoplankton. Present investigation includes three species of *Pediastrum* found predominantly in post monsoon and summer season. Due to contamination of fresh water in monsoon season *pediastrum* species does not observed.

Acknowledgement:

I would like to thanks Principal Dr. D. S. Talwankar and Head, Department of Botany, G. S. Science, Arts and Commerce College, Khamgaon, Dist. – Buldana, (M.S) for providing laboratory facilities.

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04

Numerical Taxonomy of *Hibiscus*

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Abstract

Numerical taxonomy of 8 different species of Hibiscus were studied from herbarium specimen deposited at BSI, Western circle, Pune. The measurement of quantitative characters were recorede by using scale in cms. The data was analysed for Principal component analysis (PCA) and cluster analysis. The results of both leads to delimitation and closed relation of the taxa. Correlation between all quantitative characters showed that internode is the delimiting facter and petiole length and leaf length are close to each ofther.Analysis refers to clsuter analysis and dendrogram on the basis of farthest neighbour mean characters difference abd constrained clustering strategy exhibits dissimilarities in between the species. Eigen value table showed that Internode is actually the main facter and leaf length can also delimit the taxa. Value of scree plot is above one. Internode and leaf length have eigen values above 1.In the morphometric studies it has been observed by the most allied species are H. vitifolius and H. cannabinus. Both are closely related and H. micranthus is the most diverse from the other group

Key words- Numercal taxonomy, Hibiscus, PCA, Cluster analysis

Introduction

Hibiscus is heterogenous genus of the family Malvaceae. (Fryxell, 1997). the genus Hibiscus is repre- sented by over 200 species of trees, shrubs and herbs, widely distributed in tropical and subtropical regions (Bailey, 1950; Bates,1965,Beers and Howie, 1992). Hibiscus is medicinally important. Flowers,roots and buds are used in burning sensation, skin disease and constipation. (Ivan, 1999; Kirtikar and Basu, 1999; Pullaiah, 2006). The flower extract is used internally in the treatment of heavy and painful menstruation. Veneral diseases and to promote hair growth (Burkill, 1995).Promote stages of cancer development (Sharma and Sultana, 2004). In systematics, morphological data is very important. The vegetative characters are significant in plant systematics. Vegetative characters always show variation. This variation can be analysed bya a gaps between taxa which reflect evolutionary arrangement begun through morphological changes.((Otte and Endler,1989).Many taxonomist made their contribution on numerical taxonomy. Deshmukh, (2011), Deshmukh & et.al. (2012), Deshmukh & et.al. (2013), and Puhuaand Ohashi,(2010), Puhua & et. Al. (2010),Sonibare & et.al.(2004).

Material and Methods

Herbarium of 8 different species like *H. Canescens, H.schizopetalus, H. ficulneus, H.rosasinensis, H. vitifolius, H. cannabinus, H. cancellatus, H. micranthus* were selected for taking observations on the characters like length of internode, leaf length, breadth of leaf at three part of a leaf.(base, middle and apex) and petiole length. Measurements were taken for six characters quantitative characters. Mean values were calculated and processed for principal component analysis and cluster analysis Similarity matrix and tolearance of Eigen values caluclated by keeping standardized data. Eigen values and variance of the characters were calculated and scree plot graph plotted. (Kovach, (1999). The objectives of all the caluclations was to determine the chacter that constributed strongly to the delimitation of the taxa and their close relationship on the basis of cluster analysis..

Result and Discussion

Morphometric studies af any taxa is based on Principal Component Analysis and cluster

analysis.(Soladoye et al., 2010). The study was carried out for 8 species of Hibiscus for 6 characters.

Table 1. Voucher specimen of Herbarium

Fig 2. Dendrogram showing clusters

Table 3. Text Dendrogram

Fig 4. Scree plot on the basis of eigen value percentage.

Table 5. Mean and stdandard deviation of quantitative characters

Table 6. Similarity matrix

Table 7. Eigen value percentage

Tabe 8.Variance in quantitative characters

Table 9. Euclidean distance

Dendrogram exhibit 7 nodes.

Most allied species are H. vitifolius and H. cannabinus. Both are closely related and H. micranthus is the most diverse from the other group.

Node 1. – It is formed by 2 species namely *H. vitifolius* . Both are closely related with each other. It shows least dissimilarities between both the species.

H. cannabinus. They are closely related with each other.

Node 2.- It is formed by 3 species namely *H. rosa-sinensis, H. vitifolius, H. cannabinus*

Node 3- It is formed by *H. ficulneus* , include 3 species namely *H. rosa-sinensis, H. vitifolius, H. cannabinus*

Node 4.- It include 2 species namely H. canescens and H.schizopetalus

Node 5.- *H. canescens and H.schizopetalus, H. ficulneus, H. rosa-sinensis, H. vitifolius, H. cannabinus*

Node 6. Comprises of 7 species namely *H. canescens and H.schizopetalus, H. ficulneus, H. rosa-sinensis, H. vitifolius, H. cannabinus and H. cancellatus*.

Node7.- Comprises of 7 species *namely H. canescens and H.schizopetalus, H. ficulneus, H. rosa-sinensis, H. vitifolius, H. cannabinus and H. cancellatus* and *H. micranthus*.

Table1. voucher specimen no.

Species	Voucher specimen No.
H. canescens	95288
H.schizopetalus	2170
H. ficulneus	964
H. rosa-sinensis	1173
H. vitifolius	132833
H. cannabinus	142523
H. cancellatus	-
H. micranthus	-

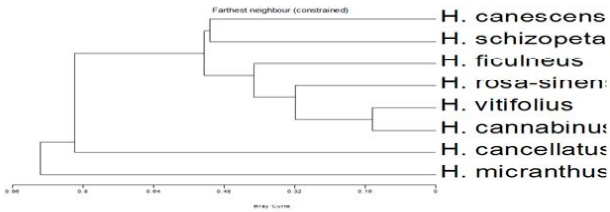

Fig 2. Dendrogram on the basis of Farthest Neighbour, Mean Charater, Differences and Constrained Cluster strategy observed in Quantitative character of *Hibiscus* Species

				Species				
Node	Group 1	Group 2	Dissimil.	in group				
1	H. vitifolius	H. cannabinus	0.145	2				
2	H. rosa-sinensis	Node 1	0.319	3				
3	H. ficulneus	Node 2	0.413	4				
4	H. canescens	H. schizopetalus	0.513	2				
5	Node 4	Node 3	0.525	6				
6	Node 5	H. cancellatus	0.819	7				
7	Node 6	H. micranthus	0.897	8				

Text-based graphs are best viewed with a fixed pitch font (e.g. Courier New or Fixedsys).

```
                +------------------------------------ H. canescens
              +|
              |+------------------------------------ H. schizopetalus
  +-----------------|
  |           |     +------------------------------- H. ficulneus
  |         +-------|
  |           |     +---------------------- H. rosa-sinensis
  |         +-------|
  |           |     +---------- H. vitifolius
  |         +-----------|
  |                 +---------- H. cannabinus
  +---|
  |   +------------------------------------------------- H. cancellatus
```

Table 3- Text dendrogram showing the relationship of 8 species of Hibisucs based on 6 morphological characters

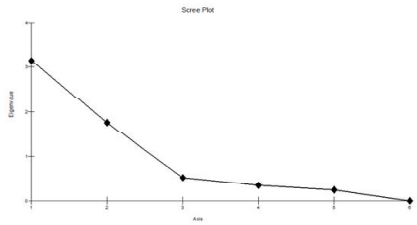

Fig-4 Scree plot for the 6 morphological characters used in the morphometric study of *Hibiscus* species

	H. canescens	H. schizopetalus	H. ficulneus	H. rosa-sinensis	H. vitifolius	H. cannabinus	H. cancellatus	H. micranthus
H. canescens	1							
H. schizopetalus	0.487436	1						
H. ficulneus	0.917634	0.474732	1					
H. rosa-sinensis	0.636307	0.710892	0.587417	1				
H. vitifolius	0.764383	0.685013	0.74803	0.786974	1			
H. cannabinus	0.729176	0.677155	0.713141	0.680919	0.855454	1		
H. cancellatus	0.47307	0.185334	0.462922	0.321219	0.31444	0.295172	1	
H. micranthus	0.526898	0.617941	0.513514	0.548804	0.62388	0.608206	0.111363	1

Table- 5. Showing the mean and the standard deviation among the selected species of genus *Hibiscus*

Table 6.

Similarity matrix based on correlation coefficient

Similarity matrix					
	Internode	Leaf L	Petiole L	Base	Middle	Apex
Internode	1					
Leaf L	-0.825	1				
Petiole L	-0.469	0.043	1			
Base	-0.562	0.193	0.745	1		
Middle	-0.688	0.51	0.251	0.306	1	
Apex	-0.643	0.618	-0.194	-0.085	0.408	1
	Internode	Leaf L	Petiole L	Base	Middle	Apex

Eigenvalues							
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6
Eigenvalues	3.142	1.761	0.517	0.339	0.24	0
Percentage	52.366	29.358	8.614	5.656	4.005	0
Cum. Percentage	52.366	81.725	90.339	95.995	100	100

Table 7. Eigen values

PC	1	2	3	4	5	6
Eigenvalue	206.419	12.5007	1.29869	0.643619	0.159148	0.015376
% variance	93.387	5.6555	0.58754	0.29118	0.072001	0.006956
Eig 2.5%	5.23E-31	2.72E-31	1.35E-33	9.75E-33	7.04E-35	2.90E-36
Eig 97.5%	99.065	90.627	12.083	6.0736	1.3671	0.09335

Table 8.Variance in quantitative characters

Apex	13.43078	40.78242	11.41578	3.305222	9.595104	0	
Middle	6.50172	35.3055	3.978015	7.177347	0	9.595104	
Base	10.85334	40.0347	8.823431	0	7.177347	3.305222	
Petiole L	6.518288	35.39325	0	8.823431	3.978015	11.41578	
Leaf L	8.377639	0	35.39325	40.0347	35.3055	40.78242	
Internode	0	8.377639	6.518288	10.85334	6.50172	13.43078	
	Internode	Leaf L	Petiole L	Base	Middle	Apex	

Table 9.Euclidean distance

Acknowledgement

We are thankful to the Director, Botanical Survey of India, for providing us the voucher specimens. Pune.

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05

Phytochemical Analysis of *Geodorum densiflorum* (Lam.) Schltr using UV-VIS. & FTIR Technique.

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Abstract:

The present study was carried out to characterized the bioactive constitute present in *Geodorum densiflorum.* Fourier transform infrared (FT-IR) and ultraviolet-visible (UV-VIS) spectrum profile of pseudobulb extract in chloroform was used for analysis. FTIR analysis was used to detect the characteristic peak values and their functional groups. FTIR analysis of pseudobulb powder extract in chloroform revealed the presence of alcohols, phenols, aldehydes, ketones, alkanes, aliphatic compounds, aromatic amines, alkenes, aromatics and sulfides. UV-VIS profile of pseudobulb powder extract in chloroform showed the peaks at wavelength 286 nm, 321 nm and 411 nm with the absorption 0.39, 0.44 and 1.68 respectively. So the present investigation provides evidences that pseudobulb of *Geodorum densiflorum* contain bioactive constituents which could be of interest for the development of new drug.

Key Words: UV-Vis, FTIR spectrum, pseudobulb, chloroform

Introduction:

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (1). Natural products as standardized plant extracts provide unlimited opportunities for the new drug (2). This field has been growing and includes diverse subjects as negotiation of power based on medicinal plant knowledge (3). Phytochemical evaluation of plant material is important as it relates to the nature and extent of therapeutic action possible with its use (4). Fourier Transform Infrared spectroscopy (FTIR) gives a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an advance method to characterize and identified functional groups (5). Ultraviolet-visible spectrophotometry (UV-Vis) related to the spectroscopy of photons in the UV-Visible region. UV-Visible spectroscopy uses light in the visible ranges or its neighboring ranges. The colour of the chemicals involved directly affects the absorption in the visible ranges (6).

Materials and methods:

Collection and extraction of plant material:

The fresh pseudobulb material of *Geodorum densiflorum* was collected from Amba Barwa forest, Jalgaon Jamod tehsil, district Buldhana (M.S.). Pseudobulb material was washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in airtight bottles. About 25 gm powdered plant material weighed accurately and extracted in Soxhlet apparatus by using chloroform as solvent.

Spectroscopic analysis:

About 10 mg pure solute obtained after evaporation of solvent was used for in fourier transform infrared spectroscopic analysis. The dried 10 mg powdered extract was mixed with KBr salt and encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powder sample of each plant specimen was loaded by using a Perkin Elmer Spectrum RX1, FT/IR spectrometer, with wave number from 4400 to 450 cm^{-1} having a nominal resolution of 1 cm^{-1}. For each spectrum 64 runs were collected and averaged. Sample was

placed in sample chamber and spectra were taken ATR mode. Results were plotted against wave number verses percent transmittance.

Geodorum densiflorum pseudobulb extract in chloroform was examined under UV and visible light for immediate investigation. Plant sample extract was centrifuged at 3000 rpm for 10 minutes and filtered through filter paper (Whatman No.1) under high pressure of vacuum pump. The sample was diluted to 1:10 by using same solvents. The extract was scanned in the wavelength range from 190- 1100 nm using EQUIP- TRONICS (EQ-826) and the peaks were detected.

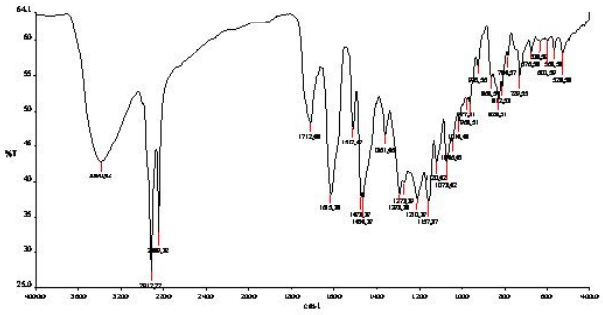
Result and Discussion:

The FTIR spectrum was used to identify the functional group of different phytoconstituents based on the peak values in region of infrared radiation (4400- 450 cm^{-1}), present in chloroform extracts of pseudobulb powder.

Table 1: FTIR spectral peak value and functional groups obtained for chloroform extract of *Geodorum densiflorum* pseudobulb.

Peak value (in cm^{-1})	Functional group	Bond	Group frequency (in cm^{-1})
3390,42	Hydrogen bonded alcohols, phenols	O-H stretching	3600- 3200
2917,27	Alkanes	-CHO stretch	2970- 2850
2849,32	Aldehyde	C-H stretch	2860- 2800
1712,48	Ketones	C=O stretch	1725- 1705
1615,38	Alkenes	C=C stretch	1680- 1600
1512,47	Aromatic ring	C=C stretch	1600- 1450
1473,37	Aliphatic compounds	C-H bend	1475- 1450
1464,37	Alkanes	C-H bend	1475- 1365
1293,38	Aromatic amines	C-N stretch	1335- 1250
1273,39	Aromatic amines	C-N stretch	1335- 1250
1210,37	Alcohols, ethers, carboxylic acids, esters	C-O stretch	1300- 1000
977,51	Alkenes	C-H bend	1000- 650
729,55	Aromatics	C-H 'oop'	900- 675
633,59	Sulfides	C-S	710- 570 (m)

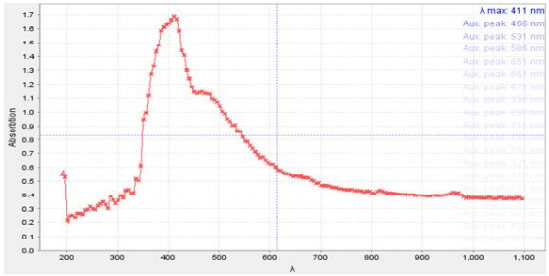
Fig.1: FTIR spectrum of chloroform extract of *Geodorum densiflorum* pseudobulb.



FTIR analysis was used to detect the characteristic peak values and their functional groups. FTIR spectrum of pseudobulb represented in fig. [1] and peak value and functional group in table [1]. FTIR analysis of pseudobulb powder extract in chloroform revealed the presence of alcohols, phenols, aldehydes, ketones, alkanes, aromatic ring, aliphatic compounds, aromatic amines, alkenes and sulfides. Phenols showed antimicrobial, antihelmintic, antiapoptotic and antidiarrhoeal activities (7). The alkanes were found in the plant cuticle and epicuticular wax of many species. They protect the plant against water loss, avoid the leaching of important mineral by rain and protect against microorganism and harmful insects (8). Ketones protect the plants against various herbivorous insects (9). Aldehydes showed defensive response in plants (10).

The high amount of alkenes evolved for the attraction of primarily male bees as pollinator by sensory exploitation (11). Phenylamides are workings of a general defense system in plant against different stresses (12). Sulfides are used as disinfectant and dental creams (13).

Fig. 2: UV-VIS spectrum of chloroform extract of *Geodorum densiflorum* pseudobulb.



The UV-VIS profile of *Geodorum densiflorum* pseudobulb powder in chloroform was selected at a wavelength of 190 to 1100 nm. The peaks were obtained in the range of 200- 500 nm wavelength. The extract showed peaks at the wavelength of 286 nm, 301 nm, 321 nm and 411 nm with absorption at 0.39, 0.44 and 1.68 respectively. The result of UV-VIS analysis of pseudobulb powder extract in chloroform was mentioned in table- [2] and figure- [2].

Table 2: UV-VIS spectrum profile of *Geodorum densiflorum* pseudobulb extract in chloroform.

Wavelength (λ) in nm	286	321	411
Absorbtion	0.39	0.44	1.68

Conclusion: The evaluation concluded that chloroform extract have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute new active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

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06

PHYTOCHEMICAL STUDIES OF CURCUMA AROMATICA SALISB RHIZOME EXTRACT BY HRLC - MS METHOD

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Abstract

Curcuma aromatica Salisb. (Zingiberaceae) is a perennial herb found throughout India, its rhizomes are used to treat bruises, sprains, skin eruptions, infections and to improve complexion. The aim of the present study was to characterize phytochemical constituents from the methanolic rhizomes extract of *Curcuma aromatica* by using High Resolution Liquid Chromatography - Mass Spectroscopy (HRLC-MS) method. The results showed the presence of 7 major abundant metabolites as Fissinolide, 1alpha-hydroxy-23-[3-(1-hydroxy-1-methylethyl) phenyl]-2,22,23, 23-tetradehydro-24,25,26,27-tetranorv, Azithromycin, Hydroxyhydroquinone, Securinine, 27-nor-5b-cholestane-3a,7a,12a,24,25-pentol, OH-Spheroidenone. This report is the first of its kind to analyze the chemical constituents of *C. aromatica* using HRLC-MS.

Key words: *Curcuma aromatica,* phytochemical and HRLC-MS.

Introduction:

C. aromatica belongs to Zingiberaceae, it is commonly known as aromatic turmeric (English), ran halad (Marathi), jangali halad (Hindi), lam yaingang (Manipuri), kasturimanjal (Tamil). Rhizomes of *C. aromatica* Salisb., has found rich in aroma, it also act as stimulant, carminative and tonic. The rhizomes boiled in oil and used externally for bruises and sprain and essential oil of rhizome used in the treatment of early stage of cervix cancer **(Chatterjee and Pakrashi 2001).** Extracts of 95% ethanol of *C. aromatica* showed the repellent activity against *Aedes togoi* on human volunteers and also against *Armigeres subalbatus, Culex quinquefasciatus*, and *Cx. tritaeniorhynchus* under field condition **(Pitasawat *et al.*, 2003).** Different tribes of Cachar district in Assam use rhizome of *C. aromatica* in constipation **(Das *et al.*, 2008).** The skin diseases were cured by the indigenous community of Kanyakumari district (Tamil Nadu) of South India using the rhizomes paste of *C. aromatica* and *Terminalia chebula* seeds **(Kingston *et al.*, 2009),** whereas people of Jalgoan district of Maharashtra use roots of *C. aromatica* for wound healing **(Chopda and Mahajan, 2009).**

Material And Methods

The plant material of *Curcuma aromatica* Salisb., was collected from Sawantwadi, Sindhudurg district of Maharashtra and identified by using floristic literature **(Naik, 1998, Sharma, 1996 and Sabu, 2006).** The voucher herbarium specimen (ASJ 7618) deposited in VH Herbarium, department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Samarth Nagar, Aurangabad.

Preparation of crude extracts

The dried rhizome powder was extracted through Soxhlet using methanol as solvent and heated at 65^0C for 18-24 hours, extract was kept for evaporation and sample was stored in amber coloured bottle for further phytochemical analysis, which carried out using HRLC- MS technique.

HRLC- MS analysis

Instruments and chromatographic conditions

Equipment and conditions for identification of metabolites from an active sub-fraction of methanol extract was carried out at SAIF, IIT, Mumbai. Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min., then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 C, drying gas flow 13 L/min, sheath Gas temp 300, sheath Gas Flow 11, nebulizing gas pressure 35 (psig), fragmentor 175 V, Skimmer 65 V, Octopole RF Peak 750 V, and mass range m/z 50–1000. The resolution was 40,000 FWHM. Metlin database was used to structure confirmation.

Results And Discussion

The results pertaining to HR-MS analysis led to the identification of number of compounds from the HR fractions of the methanolic extract of *Curcuma aromatica* Salisb. These compounds were identified through mass spectrometry attached with HR. The results of the present study were tabulated in **(Table 1).**

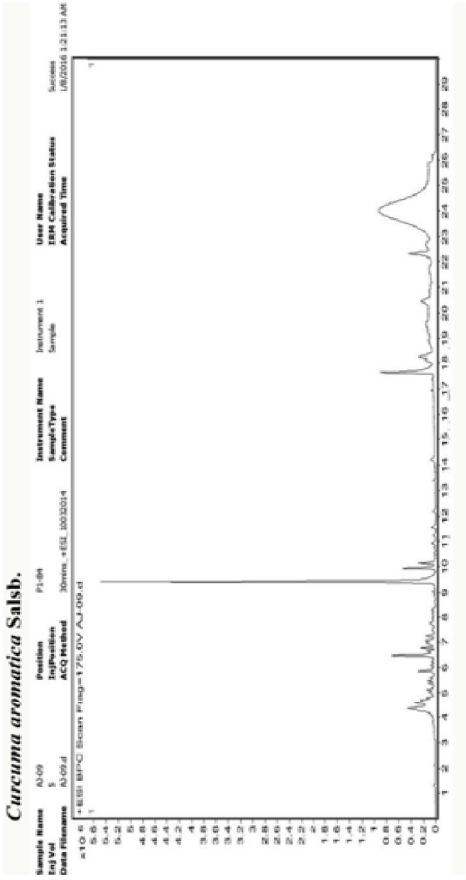
The major abundant metabolites identified in the *C. aromatica* Salisb., methanolic rhizome extract fraction by ESI-QTOF-MS analysis were as Fissinolide,1alpha-hydroxy-23-[3-(1-hydroxy-1-methylethyl) phenyl]-2,22,23,23-tetradehydr-24,25,26,27-tetranorv, Azithromycin, Hydroxyhydroquinone, Securinine, 27-nor-5b-cholestane-3a,7a,12a,24,25-pentol, OH-Spheroidenone. The retention time, m/z value, mass, molecular formula and the DB difference (ppm) of the major 8 abundant metabolites are shown in table, the spectra showed counts versus mass to charge (m/z) ratio **(fig. 1).**

Table 1:

Phytocomponents identified in rhizome extracts of *C. aromatica* Salisb.

Sr. no	Name of the compound	RT	Mass	Formula
1	Fissinolide	4.371	512.2463	$C_{29}H_{36}O_8$
2	Phe Lys Gly	4.715	350.1934	$C_{17}H_{26}N_4O_4$
3	Flurandrenolide	5.146	436.2265	$C_{24}H_{33}FO_6$
4	1alpha-hydroxy-23-[3-(1- hydroxy-1-methylethyl)phenyl]- 22,22,23,23-tetradehydro- 24,25,26,27-tetranorv	5.89	474.3162	$C_{32}H_{42}O_3$
5	Azithromycin	6.481	748.5065	$C_{38}H_{72}N_2O_{12}$
6	Tunaxanthin D	6.481	568.43	$C_{40}H_{56}O_2$
7	Trp Gln Trp	7.249	518.2321	$C_{27}H_{30}N_6O_5$
8	Hydroxyhydroquinone	17.639	126.0342	$C_6H_6O_3$
9	GPCho(0:0/9:0[U])	17.64	398.2322	$C_{17}H_{37}NO_7P$
10	Cys Gly Ser	17.643	265.0741	$C_8H_{15}N_3O_5S$
11	Securinine	17.644	217.1126	$C_{13}H_{15}NO2$
12	Cys Gly Ala	17.644	249.0794	$C_8H_{15}N_3O_4S$
13	3-(a-Naphthoxy)lactic acid	17.646	232.0755	$C_{13}H_{12}O_4$
14	27-nor-5b-cholestane-3a,7a,12a,24,25-pentol	18.09	438.3327	$C_{26}H_{46}O_5$
15	27-nor-5b-cholestane-3a,7a,12a,24,25-pentol	18.265	438.3327	$C_{26}H_{46}O_5$
16	OH-Spheroidenone	22.275	600.4553	$C_{41}H_{60}O_3$
17	Vecuronium	22.323	557.4376	$C_{34}H_{57}N_2O_4$

Fig. 1: A chromatogram of methanolic extract of *C. aromatica* Salisb rhizome



In the present investigation chemical profile of rhizome extract of *C. aromatica* Salisb., using HRMS spectra. The chromatogram showed relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *C. aromatica* Salisb., using HR-MS. In addition to this, the results of the HRMS profile can be used as pharmacognostical tool for the identification of the plant.

Conclusion

The presence of various bioactive compounds i.e. Fissinolide, 1alpha-hydroxy-23- [3-(1-hydroxy-1-methylethyl) phenyl] -2,22,23,23-tetradehydro-24,25,26,27-tetranorv, Securinine, Azithromycin, Hydroxyhydroquinone, 27-nor-5b-cholestane-3a,7a,12a,24,25-pentol, OH-Spheroidenone was inferred by using HRLC-MS technique and showed with different chemical structures. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Acknowledgement

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07

DIE BACK DISEASES OF TREES FROM AURANGABAD, MAHARASHTRA.

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Abstract

In Aurangabad district, trees were surveyed during the years 2008-2011. Trees found affected by various fungal diseases. During the period of present research, several types of diseases were observed on the trees. Die-back and wilt were found as most severe diseases, irrespective of the plant species under attack. During the survey of forest, gardens and orchards 6species were found infected by fungal pathogens causing Die back diseases. The diseases were found to be caused by 6species of fungi viz *Fusarium oxysporum,F. solani*, *Fusarium* sp., *Phomopsis azadirachtae*,*P. amaraii andCurvularia tuberculata*.

Keywords: Aurangabad, Die-back, *Fusarium,* Pathogen.

Introduction:

The trees all over the world are known to suffer from several fungal diseases. These pathogens produce various types of symptoms on the affected plant parts. On a single tree, there may be only one disease at a time or several diseases may occur on a single tree. Several generalizations can be made concerning wilt diseases and the fungi which cause them. Since the casual microbes generally proliferate in the vascular elements, discoloration of these elements and loss of turgor due to impeded water transport are the two most characteristic symptoms. Infection frequently results in a relatively rapid killing of host trees or in the necrosis of large portions of these trees (Smith 1970). In disease die-back progressive death of branches is observed. The drying and dyeing of the affected branch start from the tips. The shrubs and trees suffering from die-back may die or survive indefinitely. The disease may occur on different plant species, irrespective of the age. The disease is reported from the various countries in the year 1900, from Florida, Cuba and West Indian Islands on *Citrus* plants. (Gupta and Sharma 2000). Fungi, which cause diseases of the wilt type, belong to either Ascomycotina or Deuteromycotina. These fungi are usually disseminated from tree to tree by one of three mechanisms; direct infection of roots from the soil, root grafting between infected and uninfected hosts, and insect vector transmission. Even though many wilt-inducing fungi are confined to single-cell types, for example, vessels or tracheids, and can generally only very poorly move through cell walls, most are capable of becoming systemically distributed through the tree in the vascular system (Smith 1970).Die back diseases occurred severly from Karachi, Pakistan(Khanzada *et al.,* 2004). The disease form Junagadh (Gujarat), the incidence varies from variety to variety, ranging from 32 to 96 %. (Bhatt and Jadeja 2010). In 1918, the disease posed a serious threat for growing lime in Britain, Guinea and Trinidad (Fawcett 1936).

Materials And Methods:

A survey of Die back diseases of trees was carried out during the years 2008-2011, In the field, observation were made on few aspects, whether the disease occurs on old or young trees. The disease trees were examined carefully in the field and description was recorded, as suggested by (Rangaswami and Mahadevan 2005). For determining the taxonomic position of host, samples of flowers, fruit, leaves and stem were collected and studied in the laboratory(Naik, V.N. 1998).The causal organism of the affected plant parts was examined immediately after collection from the field. Free hand sections of infected parts were taken and

observed under microscope. Culturing of fungal pathogen, PDA was prepared in the usual way (Aneja 1993). The diseases and pathogens were identified by using relevant literature (Barnett, 1956; Alexopoulos and Beneke, 1962).

Results And Discussions:

1) Acacia nilotica(L.) Del.

Vernacular name: - Babhul

Pathogen: - Fusarium sp.

Locality: - Paithan, Aurangabad, Phulambri

The disease die-back literally means dying from the top to bottom. The disease infects young as well as old trees with young shoots and leaves. The typical symptoms include withering of lower most branches of the tree from top to downwards. The disease is characterized by yellowing and browning of leaves at the tip (Fig.1). Branches of infected tree dries one by one, till the whole plant dies. The leaves from infected branches defoliate giving a barren look to the tree. The infection occurs during the rainy season. Fusarial wilt of *A. nilotica* has been reported earlier by Bagchee (1939).

2) Azadirachta indicaA. Juss.

Vernacular name:-Neem

Pathogen:-Phomopsis azadirachtaeSateesh, Bhat & Devaki.

Locality:-Khulatabad, Gautala, Paithan

The disease is more pronounced during August-December; though it can be observed throughout the year. Disease has been noticed on Neem trees, irrespective age, size and height. Initially, the terminal branches are affected showing twig blight and inflorescence blight (Fig.2). In few cases, infection appears on the top branches, proceeds down affecting the whole tree. As the infection is severe, discoloration of branches, twigs and trunk are observed. In Karnatakathe disease was reported by Fathima *et al.,* (2004). Similar study was done in karnataka Girish and Shankara Bhat (2008).

3) Citrus sinensis(L.) Osbeck

Vernacular name: -Mosambi

Pathogen: - Curvularia tuberculataJain

Locality: - Karmad, Paithan, Kannad

Die- back disease of citrus found to be most prevalent in the gardens and orchards of study area. The characteristic symptoms of the disease were mottling of leaves, defoliation of young branches and dying back of twig from tip downwards resulting in death of tree (Fig.3). In severe cases, yellowing and browning of leaves occur followed by defoliation, giving a barren appearance to the tree. Most of the leaves fall with the onset of summer and die-back of twigs takes place. In certain cases, the tree trunk shows cracks and splitting on it. Earlier this disease was reported by Ramakrishnan (1960), Lele *et al.,* (1968).

4) Dalbergia sissoo Roxb. ex DC.

Vernacular name:-Sheesham

Pathogen:-Fusarium solani(Mart.) App. & Wollenw.

Locality:-Maliwada, Kasabkheda, Garaj

The trees of Sheesham exhibited the symptoms of decline. The top dying of the crown was found to be most prevalent. The top dying starts from the top of the tree and proceeds downwards towards the stem. The leaves become yellow followed by browning and shedding from the top (Fig4). The characteristic symptoms were observed on the trees of all ages and sizes. In severe cases, attacked stem shows black streaks, withering, leaf curling and sometimes plants are killed. This disease was noted during winter season. Earlier from Dehra Dun disease was reported by Bakshi and Singh (1959).

5) Mangifera indica L.

Vernacular name: -Amba

Pathogen:- Phomopsis amaraii Srivastava et.al.

Locality: - Gangapur, Sillod, Kannad

This is one of the most destructive diseases of mango. The disease infects young as well as old trees. Young shoots, leaves and fruits are more pronounced to the infection. The typical symptoms include discoloration and darkening of bark, sometimes accompanied by exudation of gum. The affected leaves turn brown, wither and dry from top to downwards (Fig.5& 6). The branches get infected one by one till the whole tree dies. In severe cases, fruits are also attacked, which fails to attain a

mature size and may drop before ripening. This disease appears during winter season.Die-back of mango has also been observed earlier by Cheema and Dani (1935). Similar symptoms were observed with different pathogen *Botryodiplodia theobromae* as a causal organism of mango die-back. Gupta and Sharma (2000).

6) Psidium guajavaL.

Vernacular name:-Peru

Pathogen:-Fusarium oxysporum (Schlecht. ex.Fr.) emend. Snyd.& Hans.

Locality:-Daultabad, Kagajipura, Khultabad

The symptoms may be quick or fast wilting. The affected plants show leaf yellowing with curling at terminal branches (Fig.7). Later it becomes reddish, consequently shedding of leaves. Fruits of all affected branches remain undeveloped, becomes hard, black and stony. In severe infection, the trees shows wilting and entire tree becomes defoliated and may result in dying. This disease was less severe and observed on few trees during winter season. The disease has been reported earlier by Prasad *et al.,* (1952).

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Die-back: Fig. 1. *Acacia nilotica,*

Fig. 2. *Azadirachta indica*

Fig. 3. *Citrus sinensis,*

Fig. 4. *Dalbergia sissoo*

Die-back: Fig. 5.& 6. *Mangifera indica*

Fig. 7. *Psidium guajava*

08

Studies on some Ethnoveterinary Medicinal Plants from Ajanta region, Maharashtra, (India) –III

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Key words: Ethenoveternary uses, livestock, Medicinal Plants.

Abstract

The folk knowledge of ethno veterinary medicine and its significance has been identified by the rural tribal people over hundreds of years. Present paper deals with the study of some ethno veterinary medicinal plants used for treating livestock from Ajanta region, of Marathwada was undertaker and about thirteen different plant species are being identified from this region.Commonly treated disease were diarrhea, dysentery, indigestion, tympani, pneumonia, render pest and three days sickness. The Ajanta region forest is dry deciduous and mixed forest consisting of a variety of species. Plants are being used for the treatment of various ailments since prehistoric times. Most of the plants occur in wild state, medicinal properties of plants are due to the presence of alkaloids, glycosides, resins, gums, tannin etc. and may be store in roots, leaves, bark, fruits and seeds. The effect of these plants on animal, their remedies and use of such plants by local peoples are being discussed. Extensively survey was carried out during the period 2006 – 2008 of the region to record the traditional knowledge of medicinal plants in the context traditional medicinal practices and healing consideration. In this study it is observed that, aged farmers have more knowledge and experience in remote areas for curing their livestock. The traditional system of treatment is very important systems in poor people.

Introduction

Ajanta region forest is dry deciduous type restricted to satmala ranges. About 148 sq. km. area is covered by dense forest.India is an agricultural country and maximum population living in rural area and their economy mainly depends on agricultural output and hence animal like Cattle, Buffalo, Goat and Sheep have greater role to play in economy and social welfare of country. 75 to 90% rural population of India still depends on herbal medicines for the primary health care of cattle. In chronic disease herbal medicines is very useful. In many villages medicinal herbs are commonly sold along with vegetables and fruits.Some common disease of cattle are Tympani, Render pest, Mouth disease, Mastitis, Hemorrhagic septicemia, T.B., John disease, Bronchichatis, Infertility, Maggot wound, Cataract, Three day sickness, Black curter etc. are cured. In India there is long treatment of herbal medicine like ayurveda, yunani, siddha which is found to be very powerful.

From ancient period farmer, stock raiser maintain livestock in healthy and productive state by indigenously method of treatment which cure disease or prevent epidemic disease in animal, these methods of curing and remedies are time tasted, age old and are a very valuable source of information coming through indigenous knowledge of folk medicines.This traditional knowledge of maintaining livestock is passed by oral tradition in local healers and tribal's is a treasure yet to be discovered. This knowledge is very useful in animal health care needs and livestock development activity.

Material And Methods

For the collection of plants an extensive and intensive field works was undertaken at different places of the forest in different seasons during the period of2006 – 2008. Ethno botanical information was noted on field books and later on recorded on data sheets, for the collection of plant material local

herbalist helps. Very less ethno botanical work has been done in Marathwada region so Ajanta region was selected. Plants were collected, after drying again result confirm from the medicine man. It was found that medicine men confirm same ethno botanical use of plants but in few cases additional use of different disease also recommended by herbalist men. A questionery was develop to describe botanical names, family, vernacular name of plants, part used, mode of drugs preparation and administration, combination with other herbs was recorded.

Cross validation and drug confirmation was done, plants were identified, and their herbarium sheets prepared which are deposited in the herbarium of Moreshwar Art's, Science and Commerce College Bhokardan.

Observation And Results

ETHENO VETERNARY USES:

1). ***Abelmoschusficulneus (L.)*** **wt. and Arn. Ex wt.** Family – Malvaceae, Common name – Ran bhendi.

Roots of the plant are applied on the burns and injuries. Bark powder is mixed with water and this infusion is a remedy on diarrhea or enteritis. Leaves also help in curbingailment like Diarrhea.

2). ***Azadirachtaindica*** **A. Juss**

Family – Meliaceae, Common name – Kadulimb.

A pest of fresh leaves is used for external application in the treatment of rheumatic's pain. The axis of leaves withoutleaflet rubbed like brush on the palate of animal. This remedy use to curing indigestion. The bark of the tree acts as prophylactic against malariya, and is useful in intermittent and other prophylactic fever, and skin disease. Bark of *Azadirachtaindica* and *Acacia nilotica* mixed with water. The pasties applied on wound till complete recovery

3).*Buteamonosperma* (lamk.) Taub.

Family – fabaceae, Common name – Palas.

Decoction of flower is given to the cattle thrice a day for one month for the treatment of Dysurea and paralysis. Roots of the *Buteamonosperma* crushed and mixed with fodder and given to goats for curing Tympani.

4). ***Cassia fistulaL.*** Family – Caesalpiniaceae, Common name – Bahwa.

The paste of pod is given twice a day to cattle to cure indigestion. Leaves are used on haemorrhagicsepticaemia. Stem bark is grind with pepperand garlic and the mixture is given to cure fever. Dried powder of pod given orally to animal for curing Asthama, bronchichatis and pneumonia.

5). Cassia siamea**LamkEnycl.** Family – Caesalpiniaceae, Common name – Kashid, Kassod.

Take a Paste of fresh leaves of this plant are applied externally to horns and mouth parts of animal to cure foot and mouth diseases of the goat.

6). ***Citrus aurantifolia*****(christm.)** Family – Rutaceae, Common name – Limbu, Nimbu.

Leaves of the plant are crushed with water and mixed with sufficient amount of curd, this mixture orally given to animal once or twice a day for curing render pest. Pickle of the fruits along with bread of Jawar given to animal to cure Bronchichatis.

7).*Citrus karna*Raf. Syst. Tell.

Family –Rutaceae, Comman name – idlimbu.

About 100 to 200 ml of fruit juice of this plant is given orally to the cattle to cure kidney stone or retention of urine. While grazing animal may eat metals like iron, binding wire and Pins then fruit juice of *citrus karna* is given 3 to 4 time to animal to dissolve the iron and animal gets cure completely.

8). ***Clematis heynei*** Family – Ranunculaceae, Common name – Morwel, Ranjai.

Paste of fresh leaves of the plant is applied for curing eye cataract.

9). ***Clerodendrummultiflorum*****(Brum. F.)**

Family – Verbenaceae, Common name – Takli.

Take 200 grams of leaves of the plant and mixed with 500 ml of curd and give this mixture to animal three times in a day to treat three day sickness diseases. Leaves of the plant also use in broken bones and muscular pains.

10). ***Crotalaria verrucosa*****L.** Family – fabaceae, Common name – Ghatsarp.

Leaves of the plant crushed and the extract of crushed leaf with small quantity of water is given

to the animal for curing disease like Haemorgic septicemia.

11).*Enicostemaaxillare*(Lam.) Raynal.

Family – Gentianaceae, common name – Kadunai.

Whole plant is used as a medicine, Plant mixed with fodder is able to cure any type of fever, the whole plant also use to cure three day sickness.

12). *Launaeaprocumbens*(Roxb.) Ramayya and Rajgopal.

Family – Asteraceae, Common name – Pathri.

It is a very small plant only shows near about 8 to 10 leaves in numbers. Whole bunch of plants directly given as fodder to animals for removing worms.

13).*String gesneroudes*(Wild.)

Family –Scrophulariaceae.

Leaves of the plant finely crushed along with few quantity of water fill in the bottle and then given to cattle orally is very useful medicine for render pest.

Discussion

This study revealed that ethnoveterinary medicine has strong repute among community farmers, stock raiser. They believed that it provide a permanent and safe cure of commonly occurring diseases like Arthritis, tympani, weakness, indigestion, infertility. Availability of medicinal plants in the region influenced the treatment.

This investigation will further help in studies of ethno veterinary medicines throughout the region in further to add traditional knowledge base in the region.

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09

ADDITIONS TO ALGAL FLORA OF MEHEKARI DAM IN MAHARASHTRA-III

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Abstract

The present communication deals with the additions to algal flora at Mehekari Dam in Maharashtra. The collection of algal samples was done at monthly intervals from November 2016 to November 2017. Eleven species of different families *viz*. Hydrodictyaceae, Selenastraceae, Scenedesmaceae, Ulothrichaceae, Cladophoraceae, Oedogoniaceae and Zygnemataceae belonging to genera *Pediastrum, Dactylococcus, Crucigenia, Scenedesmus, Uronema, Geminella, Cladophora, Oedogonium, Zygnema, Mougeotia* and *Zygnemopsis* were recorded from the study area. A report of present study is described in the present communication.

Key words: Algae, Flora, Mehekari, Cladophoraceae

Introduction

Most beautiful microflora of the microscopic world is made up of algae. Collection and study of algae has a charm and fascination, which is better experienced than described (Randhawa, 1959). Algae are a group of organisms that have been generally described as photoautotrophic unicellular or multicellular, mainly water dwelling organisms lacking complex morphological organization. Algal samples from the different locations of water reservoir were collected in the convenient season and documented. In the present communication total 11 species of algae of have been taxonomically and morphologically described. Mehekari Lake is constructed on the Seena River in Ashti tehsil of Beed district of Maharashtra. The study was carried out to explore the presence of algal diversity of the water reservoir. Earlier the author reported the algal species of chlorophyaceae and cyanophyceae from the water reservoir.

Materials And Methods

Random sampling technique has been used for collection of algal samples. Sample collections from different locations were made during the period of November 2016 to December 2017 at monthly intervals. The algal samples were taken to laboratory. The Samples were preserved in 4% formalin for further taxonomic investigations. Temporary Mounts of algal specimen were prepared with suitable stains and observed under compound microscope. Identification of taxa was carried out by using Gonzalves, (1981), Prescott (1951), Philipose (1967), Prasad and Misra (1992), Ramnathan, (1964), Randhawa, (1959) and other relevant monographs and available literature.

Results And Discussion

During the present study following algal taxa belonging to were observed which are described as under

1) *Pediastum integrum*

Naegeli Prescott, 1951, p 225, pl 48, f 10 Colony entire; cells 5 sided; outer margin of peripheral cells smooth or with two short and much reduced processes, and granular walls, emarginate between the processes; cells 17.5 µ in diameter.

2) *Dactylococcus infusionum*

Naegeli var. ***fasciculatus*** G.M. Smith Philipose, 1967, p 211, f 119 (d)

Cells curved or sigmoid, twisted around one another and united in colonies of 50-200 cells with the median portion of the cells in contact and the apices free. Cells 5 µ broad, 55 µ long.

3) ***Crucigenia tetrapedia***

(Kirchner) W.et G.S. West Philipose, 1967, p 240, f 151 (a) Colony 4 celled, quadrate with a minute rectangular space at centre. Cells flattened and triangular with rounded ends. Outer side of cells always concave. Cells 10 µ.

4) ***Scenedesmus acutiformis***

Schroeder Philipose, 1967, p 260, f 169 (a) Colony 4 celled. Cells cylindrical fusiform and arranged in a single linear series. Cell wall smooth. Median cells with a lateral longitudinal ridge extending from pole to pole on each side. Terminal cells with two or four ridges. Poles of cells acute and without teeth or spines, but sometimes with a minute papilla. Cells 7.5 µ broad, 15 µ long.

5) ***Uronema confervicolum***

Lagerheim Prasad and Misra, 1992, p 46, pl 6, f 3 Filaments long, slightly curved, consisting of many cells, constricted at septa; cells cylindrical, slightly longer than broad, apical cells acuminating with pointed apex, basal cell narrow and long; each cell with one laminate chloroplast, accupying a part of the cell; pyrenoid not prominent. Long cell 10 µm, long basal cell 12.2 µm; lat cell 7.5 µm, basal cell 5 µm.

6) ***Geminella minor***

(Naegeli) Heering Ramnathan, 1964, p 65, pl 18, f (k) Cells cylindrical, 10 µ broad, 25 µ long, always united together and surrounded by a mucilaginous sheath 7.5 µ thick; chloroplast parietal, covering part of the cell, with one or more pyrenoid.

7) ***Cladophora fracta***

(Dillw.)Kuetzing Prescott, 1951, p 137, pl 20, f 3 Floating, forming coarse, light green masses of irregularly branched filaments, the branches often curving. Cells irregularly swollen or clavate; 65 µ in diameter in main axis, 1-3 times their diameter in length; 25 µ diameter in ultimate branches, 3-6 times their diameter in length.

8) ***Oedogonium cymatosporum***

Wittr. Nordst. Gonzalves, 1981, p 195, f 9.65 (A) Macrandrous, homothallic; vegetative cells cylindric, 7.5 µ in diameter, 32.5 µ long; oogonium single, subdepressed-globose, 22.5 µ in diameter, 25 µ long; spore wall three layered, outer and inner layer smooth, median layer scrobiculate.

9) ***Zygnema khannae***

Skuja Prasad and Misra, 1992, p 76, pl 12, f 9 Vegetative cells 22.5 µ broad, 65 µ long; conjugation not seen; reproduction by aplanospores; aplanospores ellipsoid to ovoid; outer spore wall punctate, median spore wall irregular, yellowish brown in colour.

10) ***Mougeotia scalaris***

Hassall. Prescott, 1951, p 304, pl 71, f 7 Vegetative cells 30 µ broad, 77.5 µ long, and chloroplast broad plate with 4 pyrenoids. Zygospores formed in the tube by scalariform conjugation.

11) ***Zygnemopsis jogensis***

Iyengar Randhawa, 1959, p 208, f 140 (a) Conjugation scalariform, vegetative cells 17.5 µ broad, 27.5 µ long; zygospores lying in the widened conjugation canal and extending far into each gametangium; zygospores irregularly cushion shaped, 40 µ broad, 45 µ long; chloroplast two, with one pyrenoid in each.

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10

Dietary importance of some unusual leafy vegetables

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Abstract

Unusual vegetables like *Oxalis corniculata, oxalis latifoliaand Bauhiniapurpurea* are the sources of nutrients for the body of human beings. Leafy vegetables are rich in dietary fibers, iron, calcium and vitamin C. Vitamin C of some of the unusual leafy vegetables was found to be considerably high. They can be included in the diet.

Introduction

Leafy vegetables are the source of major and minor elements. They are typically low in calories and fat. They have high protein content dietary fiber, vitamin C, Vitamin K, Vitamin A. Due to presence of phylloquinone content of leaves leaves contain high amount of vitamin K. They have chlorophyll content due to which they have their role in photosynthesis. Due to presence of chlorophyll they have occupied strategic position among the living organisms. The term vegetable includes all foods of vegetable origin, but the definitions now exclude cereals and dried seeds of pulses. Regular use of leafy vegetables supplies many of the most essential health building and protecting substances, such as vitamins and minerals. Food is a source of essential nutrients requires for health promotion and disease prevention.Increases in amount of natural dietary products are the basic necessity of human body for fighting again the diseases. These products produce antioxidants. (Barlow, 1990: Rice- Evans et. al. 1997). Among the various enzymatic and non-enzymatic parameters of antioxidants, vitamin C is one of the important, most powerful antioxidants (Smirnoff, 1996; Arrigoni and de Tullio, 2000; Horemans et. al. 2000b.). Vitamin C deficiency exacerbates atherogenesis in animal's models. In order to protect the body from degeneration of diseases, vegetables play an important role(Ogunlesi M., & et. al. (2010). Vitamin C is naturally synthesized in the body of human being, it is not synthesized endogenouslyand therefore it has to be consumed through leafy vegetables. (LIY and Schell horn H.E. (2007) Food and Nutrition Board at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences) recommended dietary intake for Ascorbic acid in the daily diet. (2000). There are many crops cultivated as vegetables suitable for different seasons and climate. But is appears that the people are not having a full choice for their tastes and requirements or they are not getting these according to their need in the season, and therefor people got diverted for the use of other plant parts of the crops that are found growing as wild plants and some as weeds. (Bhapkar and Bhore (1961). The weedslike*Boerhaaviadiffusa, Chenopodium album, Portulacaoleracea, Tamarindusindica, Oxalis corniculata, osalislatifolia, Bauhinia purpureaandAlternantheratriandrawere* selected for the experiments, are consumed as unusual vegetables (Chauhan, (1989).

Material and methods

Eight types of leafy vegetables were selected for analysis of Reducing sugar, polyphenols and Ascorbic acid. These were *Oxalis corniculata, osalislatifolia, Bauhinia purpureaand*The plant material neatly washed in tap water. The analysis of Vitamin C (Ascorbic acid) in all the leafy samples were carried out by Sadasivan and They moli Balasubramenan, 1987) Polyphenols (Farkas and Kiraly, 1962) Reducing sugar (Nelson, 1944)

Result and discussion

Sr. No.	Name of the plant	Reducing Sugars		Polyphenols		Vitamin C (Ascorbic Acid) mg/g	
		Tender	Mature	Tender	Mature	Tender	Mature
1	*Oxalis corniculata*	16.2162	88.2882	72.4454	532.6876	81.6314	122.4492
2	*Oxalis latifolia*	14.4144	153.153	95.8836	532.6876	122.4492	61.2246
3	*Bauhinia purpurea*	61.2612	309.9098	257.627	329.2978	102.0410	40.8164

Maximum amount of Ascorbic acid was found in tender leaves of *oxalislatifolia* and mature leaves of *Oxaliscorniculata*. The reducing sugar of these two ranges in between about 14 to 88 mg. The polyphenoliccontents of *Oxaliscorniculata*was maximum while that of *Oxalislatifolia* was moderate.These two plants were followed by Bauhinia tender leaves. Ascorbic acid, reducing sugars as well as polyphenolic contents is with moderate value. Among the unusual leafy vegetables, the highest level of vitamin C was recorded in the leaves of *Oxalis* Vitamin C contents of all these plants under study were found to be considerably high so it is advisory to include them in day to day diet. The person who suffers from deficiency of vitamin C. should consume the ***Boerhaaviadiffusa*** and oxalis leaves as a vegetable in their diet.

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11

Some medicinal plants and their Ethnobotanical Survey of Ajanta region District Aurangabad (MS) India

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Abstract

The survey region of study are Ajanta region in that Lenapur, Ajanta gaon, Savarkheda and Fardapur etc. in Aurangabad District (M.S.). Ajanta forests are situated between latitude 20__---_° 31" North and between 75°,44" east. In ethnobotanical study the selected plants are wild and endemic viz. *Catharanthus roseus* (L.), *Withania somnifera* (L.), *Cyperus rotundus* (L)., *Ocimum tenuiflorum* L. *Ficus benghalensis* (L. Hort.), *Terminalia ballirica* (Roxb), *Tinospora cardifolia* (Thunb). *Ricinnus communis* (L.) *etc.* We met tribal, old peoples, Hakims and Vaidu for collection of Ethnobotanical data. On the basis of data we concluded that these locally available medicinal plants having great importance in there different ailments.

Key Words: Ethnobotanical,Endemic plants, *Withania somnifera*, *Tinospora cardifolia, Terminalia ballirica,* Tribal.

Introduction

Traditional knowledge of medicinal plants and their use by indigenous healers and drug development in the present are not only useful for conservation of cultural tradition and biodiversity but also for community health care and drug development

in the local people. The indigenous knowledge on medicinal plants appears when humans started and learned how to use the traditional knowledge on medicinal plants **(**Emiru B. *et.al.***)**

Aurangabad district has very well preserved forest patch such as (Goutala) reserved as wild life sanctuary, Ajanta forest it has been observed that some tribal communities practicing tribal medicines live in jungles. This point laid me to study the matter seriously. I met with such persons and could find useful remedies for several diseases. Villagers from adjoining region of the forest take benefit in various treatments of these communities. In the present study the Ajanta, Lenapur and Fardapur comprise the tribal population and also some vaidhya of this region. Traditional practices of curing ailments using plants resources are practiced by all these people. In an enquiry to document their traditional knowledge of the medicinal plants. We conducted 2 months survey in this region from Sept. to Nov. 2012. The data was collected by interviewing local and traditional practioners and villagers. Our studies have shown that these people have accumulated a wide knowledge in the usage of plant wealth over the centuries. But due to the illiteracy this knowledge may be lossed. In this study an attempt has been made to give detailed information on plant species regarding their role in human welfare.

Material & Methods:

A combination of social survey and direct field observation was used in the study. The present work shows the little more than 02 moths of intensive exploration of the Ajanta region in Aurangabad district. This study was carried out mostly in hilly areas and some negligible area of Ajanta region. The study area is rural and somewhat backward. The entire area is divided in or 2 zones one is northern hilly region includes Ajanta, Savarkheda, Lenapur, Fardapur, Ajanta gaon, another zone is plane in nature not hilly. Ajanta, Ajanta caves, Lenapur, Fardapur, Savarkheda, were surveyed and the traditional medicines used for various diseases were gathered with the help of elderly and experienced individuals practicing indigenous medicines. During the study period from Oct. to Dec. 2012 several botanical tours have been conducted in various areas of the region. Emphasis has been given to visit the areas where more and diverse tribal belts and rural people in habiting different villages are studied. Some of the important places under study are listed below.

1) Sillod region – Ajantagaon
2) Soyegaon region – Lenapur, Savarkheda and Fardapur.

In these villages few are tadvi bhill mahadeokoli, and some shepherd. These tribal depend upon agriculture and forest products. In spite of the modern civilization the tribal and rural folks hold their traditional faith in the benefits of indigenous medicines. These people attribute most of the ills of the life to spirit and often seek help of the medico religious practices to get rid of them. Information was considered only after confirmation through or more informants. The interviews focused on plant species used in curing different or common disease.The botanical species were identified by the vendors; the part (s) of the species used and methods of application during utilization were identified and recorded. The abundance of the species identified was determined in the study area: for this purpose, rural and tribal communities which were far from the urban influence, were selected in each zone. Earlier workers like Faulks (1958) discussed the question relating to this field of inquiry in detail. (Rao and Hajra) 1967 emphasized the importance of special forms for ethno botanical work.

During investigation it is observed that the majority of the informants give most common uses of plants for food, agriculture for fish catching, fodder and particular ailments like headache, scorpion loiter, corn fracture. In tribal the elder person, in village vaidu or Hakeem were the resource person. The data collected on a particular ailment or species were verified by discussing about these aspects with tribal facilities. Information of species, their dosages days. During the dialogues care was taken not to contradicts or enforce them with the informants on any points.

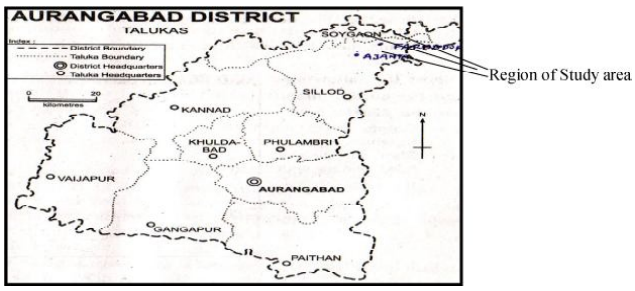
The discussion noted in the field dairy on

the spot and in the possible situation taken with the help of camera.

Proper information of the plant was filled in the herbarium sheet from the field dairy. The herbariums were arranged alphabetically. According to the Benthum and Hooker classification. While writing the test plants were mentioned according to Benthum and Hooker classification system followed by vernaculars name or local name and family. A brief information of habit, habitat and distinguishing characters of the plant is mentioned. After the locality and distribution is explained initially the general uses of the species and medicinal uses narrated.

The dosages are narrated is separate chapter by different men. The above information compared with the literature published by Naik V.N, 1998. Flora of Marathwada, Chopra (1956), and Jain S.K. (1987, 1991

Study area map



Result:

In the present investigation "An Ethno botanical survey of Ajanta region". Various field trips were arranged at the different places. Ajanta, Lenapur, Fardapur, Savarkheda and other places for getting information of the plants.

The discussion one arranged with the tribals, tadvi bhills, hakeems, rural people for the drag plants and the animal health care in different area. The discussion were also arranged with the people of different communities to know the use of plants. Knowledge is consolidated from the traditional practitioner. Their practice has come from their ancestor. During this investigation some people hesitate to disclose their knowledge to other person who belongs to other community.

In this research we met tribal and local old peoples who are worked as a traditional Hakims and Vaidu on different ailments. All results shown in tabulated manner. (Table no.1)

Table No. 1 : Medicinal plants name and medicinal used guided by tribal's of Ajanta region Dist. auranagabad.

Sr. No	Taxonomical Name of Plant	Common Name	Family	Locality and Distribution	Part used	Medicinal uses
1	*Catharanthus roseus* (L.)	Sadhaphuli	Apocynaceae	Ajant, Lenapur, Fardapur.	Leaves	Handful of fresh leaves mixed with water and boiled in 500 ml water is used for diabetes. one time in a day. It is long process.
2	*Tridax aprocumbens* L.	Ekdandi, tantani.	Asteraceae	Naturalized and very common on waste land.	Leaves	Fresh juice of leaves is antiseptic, insecticidal hence used in fresh wounds and to check length of wound. With caundel and gulwel it is used in jaundice. (10g of leaves)
3	*Cyperus rotundus* L.	Motha, Nagaramoth a	Cyperaceae	common weed of wet and agricultural fields, marshes along the bank of water.	Tuberou s roots.	General Use: The dried tuberous roots used in holly bath of dipawali festival. Also used for making perfumes. Medicinal use: Root powder 10gm mixed with buttermilk applied externally in piles. Decoction of rhizome is given on sunstroke.
4	*Ricinus communis* L.	Erand	Euphorbiaceae	All region of Ajanta	Leaves	It is helpful against Jaundice and skin diseases.
5	*Colocasia esculenta* L.	Alu, Pothinichepa n, Chamkura	Araceae	Ajanta hilly region and Lenapur	Rhizome and leaves	Rhizome in useful in tumour of tonsil, tumour on hand, leg. The Rhizome should be rubbed on infected part for 3-4 days. That tumour automatically vanished
6	*Tirchersanthes tricuspidata* Lour.	Kavandal	Cucurbitaceae	Ajanta and Lenapur	Seeds	It is used in jaundice with tantani and gulwel. (4-5 seeds only)
7	*Ocimum tenuiflorum* L.	Tulsi	Labiatae	: It is ethnic plant worshiped by women. Special worship in Ashadhi Ekadashi and in Dipawali	Leaves and roots	Decoction of leaves is given in malaria, gastric disease of children and liver disorder. Crushed leaves are used externally on skin disease. Also decoction of leaves used in asthma. The handful leaves and piper nigrum chewed in case of cough and cold. This one is blood purifier. The decoction of roots is given in malarial fevers. More than 100 uses are shown in ayurveda.
8	*Tinospora cordifolia* (Willd)	Gulvel	Menispermiaceae	Common in hedge and on trees in moist, shady places along streams.	Stem and Root	The stem is reputed ayurvedic medicine. 100gm of stem boiled in one liter water with tantani leaves (50g) + caundel seed (10g). It is useful remedy in Jaundice. This remedy given 3 days continuously. The juice of stem is useful in debility and anemia. The water extract of root is used in leprosy.
9	*Allium sativum* L.	Lasun	Liliaceae	Cultivated throughout the region.	Leaves, Capsule like Seed.	The juice of garlic is used in paralysis the process of making juice is as follows. 1) 100 to 150gm garlic bulb applied sun heat for 02 days. 2) That bulb is crushed in mortar and pestle 3) Juice is filtered through cotton cloth. 4) That juice kept. In bottle and applied sun light for 2 days. 5) On infected organ massage is important in paralysis. Fresh bulb eaten with chick pea in cold and cough. Also in heart ailment.
10	*Azadirachta indica* A. Juss.	Kadu limb, Neem	Meliaceae	Common in all over the region. Also planted along road side	Leaves, bark	50gms of leaf juice given orally in a day in case of malaria The decoction of bark (30 to 40gm) mixed with 200ml water and boiled. The half remaining part of the decoction given orally for chronic ulcer and acidity. Also it is useful in late menstrual cycle. The leaf and camphor mixed together and used in skin disease only at bath time.

Discussion

According to WHO report, several diseases of modern times are generally life style diseases. Medicinal plants have great importance in providing health care to about 80% of the population in India. Plants have been an important source of

precursors and products used in a variety of industries, including those of pharmaceuticals, food, cosmetics and agrochemicals. The continuing search for new drugs has seen researchers looking to the natural world for potential products. On the other hand the traditional medicines are enjoying an upsurge in popularity because of their low or no residual toxicity.

This investigation shows that various professions of these communities are plant based have the important place in the village economy and self employment. The health care problem on primary stage and save diseases such as fever, jaundice, skin diseases are cured by these people. In herbal drag more than 23 species being used. This is constant with the other general observation which has been reported earlier in relation to medicinal plant studies by the Indian Traditional System of Medicine like Siddha and Ayurvedha (Kirtikar and Basu, 2001; Gogte, 2000; Anonymous, 1992; Asolkar et al., 1992). Different types of preparation made from medicinally important plants included decoction, juice, powder, paste, oil and plant part extract. Some plants were even used in more than one form of preparations. The leaves and roots are the two major plant parts which are frequently used for the treatment of by traditional healers. Preparations from medicinal plants are applied externally to cure the disease like, skin, wound, rheumatism and poisons bites oral consumption was recommended against the disease like fever, cold, cough, diarrhea, indigestion etc. Drugs are prescribed either as a single or in a combination of more than one plant / parts of same or different plants to the people suffering from various diseases.

In this study or survey it is observed that deforestation and water problem one rapidly increased. It is necessary to secure and develop the forest area of Ajanta and Soygaon region. During this investigation we have visited different localities, libraries for the proper information.

Acknowledgment

I thankful to the traditional healers of the study area for their hospitality and kind response for sharing their accumulative indigenous knowledge to our inquire data. Next our truly grateful goes to Ajanta region tribals to support the Ethnoobotanical survey of medicinal plants and the Ajanta region agricultural experts.

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12

An Overview of Medicinal Uses of Custard Apple (*Sitaphal*)

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Abstract

Custard Apple (Sitaphal) is a yellowish green edible fruit. The botanical name of the custard apple is *Annona squamosa* and the family is Annonaceae. Custard apples contain anti-oxidants like Vitamin C, which helps to fight free radicals in our body. It is also high in potassium and magnesium that protects our heart from cardiac disease. Not only that, it also controls blood pressure and preventing cancer.

Keywords: Sitaphal, Custard Apple, Medicinal uses, Annonaceae

Introduction

The custard apple plant is a large shrub. It is native to the tropical rainforests of Central America and naturalized in many parts of the world, spreading along the tropical stretches, from South America to Africa and Asia. The genus name, '*Annona*' is derived from the Latin word 'anon', which means 'yearly produce'. *Annona squamosa*, *Annona cherimola* and *Annona reticulate* are the related species varieties. Botanically, custard is a "multiple-fruit" wherein the fruit is developed from the merger of several individual flowers (ovaries) into a large fruit mass (infructescence). Eating custard apple will help you to save from many diseases and disorders. The fruit is good for heart, skin, and bone and maintains blood pressure. Custard apple is also helpful in curing of boils, ulcers and gum related problems. The leaves of this fruits work against cancer and bark can be used in case of toothache and gum pain. Custard Apple is full of vitamin C anti-oxidants, which helps to combat many diseases and also enhances the immune system. Custard apple is abundant with potassium, magnesium and contains vitamin A, calcium, copper, fiber and phosphorous. It has high calorific value, able to provide sustained energy and delicious in nature. The luscious fruit is eaten throughout the world.

Fig.1 Custard Apple (Sitaphal)

Nutritional Value of Custard Apple

Custard apple is a delicious, pleasantly fragrant fruit in the Annona family. The fruit is popular for its sweet and slightly tangy, creamy textured flesh. It is also known as bullock's heart in the English speaking countries.

Custard apple contains many nutrients and minerals that are beneficial for healthy life. The different nutrients with its health benefits are as following:

- **Vitamin C**: Custard apple contains anti-oxidant vitamin C, which fights free radicals thereby preventing diseases.
- **Vitamin A**: Vitamin A in the fruits is beneficial in keeping skin and hair healthy. This vitamin is good for eyes too.
- **Potassium:** Potassium in the sweet fruits makes you active and removes the lethargies.

Potassium also helps to fight muscle weakness.

- **Magnesium**: Magnesium helps to maintain water balance in the body. It is good for arthritic patients by removing acids from joints.
- **Copper**: Copper in custard apple works against constipation.
- **Fiber:** It has plentiful of fiber, which is helpful in smooth digestion and eases constipation

Medicinal uses of Custard apple

There are many advantages of custard apple. The heath benefits of custard apple have been proven in various research studies, folklore remedies and Ayurvedic medicine. The roots, leaves, bark and flesh of custard apple fruit are all used for their medicinal benefits. The advantages of custard apple include, rectifying cardiac, hepatic or kidney problems. Traditionally it has been recommended for use in people with cardiac, hepatic, kidney conditions or osteoporosis. Custard apples can be used at any age.

Antioxidant Activity

Many studies showed that extracts of *Annona squamosa*, *Annona cherimola* and *Annona muricata* have high anti-oxidant activity (Noichinda S. *et al*., 2003; Kaur C and Kapoor H., 2005).

Effects on Cardio-Vascular Disease

Hole *et al*. tested the protective effect of aqueous extract of the fruits on isoproterenol induced myocardial infarction in rats. Pretreatment with pulp of custard apple decreased the myocardial damage (Hole R. et al, 2006). Consumption of one quarter of the normal sized custard apple daily for a 80 kg human exhibited cardioprotective effects similar to therapeutic doses of captopril (Kaleem M. *et al*.,2006).

Antidiabetic properties

The leaf extracts are also effective in lowering blood glucose levels and several reports indicates that *Annona squamosa* leaf extract can substitute effectively with decreased doses of externally administered insulin (Gupta *et al*., 2005).

Cancer Treatment

Research studies have revealed that the chemicals isolated from the plant are the main reason behind the great potential of custard apple in renal failure and cancer treatment. The chemical ingredients such as acetogenins and alkaloids impart anti neoplastic activities to custard apple. The custard apple seed extract can cause cell death in many cancer cell lines including bladder cancer cells and melanoma tumour cells. The root extracts have shown inhibitory activity against human melanoma cells. It qualifies as chemoprotective agent in cancer treatment (Dutta, 2016).

Anti-infective properties

The fruit of *Annona* spp. have been shown to have anti-microbial activities due to several diterpene, against *Staphylococcus aureus* and *Streptococcus pneumonia* is being established (Dutta, 2016). The compounds which include Ent-kauranes, Acetogenins, essential oils and Benzylisoquinolines alkaloids.

Anti Inflammatory Properties

Custard apple is a great source of magnesium, which helps in maintaining the water level and eliminating acids from the joints. Custard apple is used in the treatment of rheumatoid arthritis and gout. Custard apple fruit reduces the pain related with auto-inflammatory conditions. The leaves of custard apple also possess anti-inflammatory properties. Advantages of custard apple also include its use as an analgesic and a central nervous system (CNS) depressant agent (Dutta, 2016).

Remedy for Diarrhoea and Dysentery

The unripe custard apple fruit has antidysentric and antidiarrhoeic properties. The unripe fruit is dried and pulverized for the treatment of diarrhoea and dysentery. The seeds are also used in the treatment of diarrhoea and dysentery. Bark decoction is also used as a remedy for diarrhoea and dysentery.There are many health benefits of custard apple leaves including its use as a vermifuge and in the treatment of diarrhoea and dysentery. A decoction of the leaves, bark and green fruits of custard apple is prepared by boiling in one litre of water for 5 minutes. This decoction is efficacious as

a home remedy in the treatment of severe cases of diarrhoea and dysentery (Dutta, 2016).

Anti-aging Benefits

Custard apples are a rich source of Vitamin C. Custard apple has great antioxidant properties. The leaf extract has great benefit in quenching free radicals. It also aids in synthesis of collagen boosting immune system. It has excellent anti-aging benefits.

Custard Apple is good during Pregnancy

Custard apple has high fibre content which makes it helpful in fighting off the constipation commonly found in pregnant women. Custard apple is good for pregnancy because it is a great source of antioxidants and helps in alleviating morning sickness, nausea, numbness and food cravings associated with pregnancy.

An Excellent Antioxidant

The chemicals present in custard apple which are responsible for its antioxidant properties are mainly the phenolic compounds Vitamin C is also responsible for the antioxidant properties of custard apple. Hence, it is much better to have natural antioxidants through a balanced diet having adequate amount of custard apple fruit (Dutta, 2016).

Remedy for Wound Healing

The crushed leaves of custard apple or custard apple paste can be applied as a poultice on boils, abscesses and ulcers. The paste to be used as poultice can be prepared from the leaves and also the flesh of the fruits (Dutta, 2016).

Conclusion

Custard apple or the sugar apple is the fruit of *Annona squamosa*, which is one of the most widely grown species of *Annona.* The fruit pulp has shown numerous medicinal properties which include antioxidant, anti-diabetic, anti-infective and anti dyslipidemic properties.The government should come up with subsidies, advertisements with film personalities to make it more popular. There should be resurgence of intake of this fruit especially in the wake of increased percentage of diseases due to improper dietary habits. Custard Apple is full of vitamin C anti-oxidants, which helps to combat many diseases and also enhances the immune system. Custard apple is abundant with potassium, magnesium and contains vitamin A, calcium, copper, fiber and phosphorous. It has high calorific value, able to provide sustained energy and delicious in nature. The luscious fruit is eaten throughout the world. Eating custard apple will help you to save from many diseases and disorders. The fruit is good for heart, skin, and bone, and maintains blood pressure. Custard apple is also helpful in curing of boils, ulcers and gum related problems. However, the most important advantages of custard apple are healthy heart, beneficial in pregnancy, improve eye vision, cure arthritis, fighting fatigue, and protects against anemia.

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13

POTENTIAL MEDICINAL PLANT RESOURCES FROM LONAR CRATER

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Abstract

Since the ancient times, the plants have been used as a source of medicine. About 80% of the local individuals depend and regularly used traditional medicines, because only these medicines are safe and eco-friendly. Currently all the nations, realizing the value of these natural resources, has embarked on a mission of documenting the traditional knowledge. These plants act as a great economic source for the poor people, due to their enormous market value. While exploring the various forest pockets of Lonar crater and its surrounding, author collected the unexplored but potential bio-resources. The present paper highlights a rich and unique profile of potential medicinal plant resources of the area surveyed, with 15 families belongs to 19 species with correct botanical identification, vernacular names, parts used, used for, doses and mode of administration in respect to different diseases with their current market value.

Key words: Herbal drug plants, Lonar, Buldhana.

Introduction:

The Lonar crater is the third biggest meteoritic impact crater in the world. This Lake is mysterious because it is a saline and alkaline lake located at Lonar in Buldhana district in Maharashtra, India. Lonar Lake has a mean diameter of 1.2 kilometers (3,900 ft) and is about 137 meters (449 ft) below the crater rim. The Biologists, Geologists, ecologists, archaeologists and astronomers from the world have reported several studies on the various aspects of this Crater Lake. (Malu *et.al*., 2000; Tambekar *et. al*., 2010)

The rain water along the different minerals of surrounding area flow inside the lake and constant evaporation over the years has led to a high concentration of salt in the lake. Such alkaline water do not support life-forms. Yet, biologists have reported the presence of primitive life forms like both the chemotropic and phototrophic organisms (Varier, 1997; Rathod, 2014). The Lonar Crater is also famous as a cradle for Biodiversity. Therefore, many of foreigner as well as Indian biologist attract towards the lake. This area rich with economically and medicinally important plants, also many of animals and Birds has survived in this area. Therefore, forest department declare this area as a smallest 'Wild Life Sanctuary' to conserve this biodiversity. Lonar crater is the unique ecosystem with its own feature (Ahirrao & Patil, 2010; Tambekar et. al., 2010). These plants possess various types of pharmacological drugs and can be used as the medicine in various ayurvedic preparations.

The medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc.) extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans as well as for plants and animals. Besides the use of medicinal plants by local individuals as their raw material, the demand for medicinal plants has also increased day by day by the modern pharmaceutical industries (Patil *et. al*., 2010; Dabhadkar & Borul, 2013). From the thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs (Maheshwari, 2000; Tambekar *et. al*., 2012). However, the local peoples of the

surrounding area of Lonar Lake largely depend on plant resources in their vicinity for healthcare and other necessities of their life. Therefore, in present study, the authors find out some important herbal drug plants and communicate in present paper which has high potential as a medicinal resource and economic importance, with correct botanical identification, vernacular names, parts used, used for, doses and mode of administration in respect to different diseases with their current market value.

Methodology:

Lonar crater is situated between ý19°582 363 North latitude and 76°302 303 ÿþEast longitude in Southern region of Buldana district of Maharashtra state (India). During the year 2016-2017, the number of excursion tours was made to survey and collect various medicinal plants from Lonar crater in different seasons. The collected plants were firstly identifying by using different botanical floras (Singh *et. al*., 2000; 2001; Diwakar & Sharma, 2000; Patil *et. al*., 2007). The plant specimens were deposited as herbarium in college laboratory. The medicinal use of plants and their various data was also collected through interviewing local experienced informants, medicine men and women, and farmers. The information accrued was verified in different botanical literature (Agharakar, 1991; Maheshwari, 2000; Ahirrao *et. al*., 2009; Patil *et. al*., 2010). Photographs of these medicinal plants were taken in the field and from its record. The potential medicinal plant species have been arranged alphabetically in Table 1 with their uses.

Results and Discussion

The present investigation comprises 19 plants belonging to 15 different plant families showing medicinal potential and wildly used locally. For each species botanical name, family, vernacular name, part use, Doses and mode of administration and Current Market value of each plant species are provided in the table 1. Traditional healers and local individuals have been used these plants to cure various diseases. The specific plant parts like root, leaves, stem, bark, flowers, fruits, latex etc. in specific dosages is used for the treatment of ailments. The plant products are used as a raw material or in the form of decoction, infusion, juice, oral treatment or applied externally as paste or ointments. Each of the plant having some specific chemical constituents or secondary metabolites like alkaloids, steroids, volatile oils, etc those have economic value in Pharmaceuticals. These ingredients are extracted and the drugs have been prepared in Pharmaceuticals. These plants may be used separately or in mixture of several plants for better and quick result.

Now a day's these plants have great demand in ayurveda and some herbal product industries like, Dabar, Baidhyanath, Sharangdhar, Patanjali, etc. These medicinal plants also sold by traditional vendors and vaidus. Therefore, these plants have great economic value in local as well as in an international market. So, such a medicinal plants are great potential resource and can be full fill the basic demand of surrounding poor peoples. Besides, the conservation and multiplication of these potential plants is also needful.

Helicteres isora (Murudsheng)

Withania somnifera (Ashwagandha)

Sr. No	Botanical Name	Family	Vernacular Name	Part Use	Uses	Doses and mode of administration	Market Value
01	*Asparagus racemosus* Willd. Var. *javanica* Baker.	Liliaceae	Shatavari	Tuberous Roots	Blood purification, Tonic	A tea spoon of root powder in a glass of milk is given for 21 day's	25Rs/50 gm
02	*Balanites aegyptica* (L.) Del.	Zygophyllaceae	Hinganbet	Fruits	Headache and to improve lactation	A decoction of the bark are also used as an Abortifacient	15/kg
03	*Caesalpinia bonducella* Flemi.	Caesalpinaceae	Sagargota	Seeds	Tonic for children	The aquatic paste is given in milk	10Rs./10 Piece
04	*Calotropis procera* (Ait.) R.Br.	Asclepiadaceae	Rui	Latex, leaves, Root bark	Fast healing of wound, purgative and emetic agent, anticancer, anticoagulant and antifungal agent	The crushed leaves and milk can apply directly on wound, the powder of Root bark and crushed leaves used as antifungal agent	22,000/bag
05	*Cardiospermum halicacabum* L.	Sapindaceae	Kapalphodi	Leaves, whole plant	Against arthritis, inflammations, constipation and abdominal disorders	The decoction of leaves in castor oil is ground and applied over the affected areas or juice of leaves is directly taken	--
06	*Cassia fistula* L.	Caesalpiniaceae	Bahava	Fruit, Pod	Wound healing	The aquatic paste is externally applied on wound	5Rs./piece
07	*Eclipta erecta* Linn.	Compositae	Maka, brungraj	Whole plant, seed	Liver tonic, anti-inflammatory, jaundice, hair nourishment	The essential oil is used for various treatment	150/kg
08	*Evolvulus alsinoides* (L.) L	Convolvulaceae	Shankhapushpi, Vishnukrant	Whole plant	Brain tonic, sleeplessness, chronic bronchitis, asthma	Juice of Whole plant is give twice in a day or syrup is prepared from whole plant	1,200/kg
09	*Helicteres isora* L	Sterculiaceae.	Murud shengh	Fruit, Pod	Tonic for children	The fruit paste is given in milk	10Rs./10 Piece
10	*Hemidesmus indicus* R.Br.	Asclepiadaceae	Gavati Kavali	Whole plant	Rheumatism	Decoction of whole plant in tea is given for 7 day's	10Rs/pauch
11	*Justicia adhatoda* L.	Acanthaceae	Adulsa	Leaves	Cough, asthma, sore throat, diarrhea and dysentery	A cup of leaf decoction is given twice in a day	350/kg
12	*Mucuna pruriens* (L.) DC.	Fabaceae	Khaj-kuyili	Fruit/ pods, seeds	Anti-helminthic, to control the intestinal worms of childrens and calves	The pod trichomes mixed in curd or in rice soup, is given once in a day	300/kg
13	*Solanum xanthocarpum* Schr. & Wendl.	Solanaceae	Kantakari, kate ringani	Whole plant, dried roots, fruits	Stomach, respiratory disorder, snake bite, antiasthma tic, kidney stone	The decoction of plant part with a tea spoon of honey is given twice in a day	2600/bag
14	*Tectona grandis* L.f.	Verbenaceae		Flowers, wood	Piles, leucoderma, dysentery, urinary discharges, headache, burning sensation	The cup of decoction of dried flowers or wood are given twice in a day	1,200/ Cubic feet
15	*Tinospora cordifolia* (Willd.) Hook. F. & Thomson	Menispermaceae	Gulvel	Stem	Acidity, Fever, Abdominal Pain	Stem – powder or aquatic paste in a cup of water is given twice in a day	20Rs./piece
16	*Tylophora asthmatica* W. & A. (*T. indica* (Burm.f.) Merr.)	Asclepiadaceae	Kavali, anantmul	Leaves, root, whole plant	Asthma, Bronchites, constipation, Antitumer	The decoction of leaves or root is used twice in day	200/250 gm
17	*Withania somnifera* (L.)Dunal.	Solanaceae	Ashwgandha	Leaves, root	Weight loss or gain, tuberculosis, backache, menstrual problems, and chronic liver disease.	Decoction of leaves is used for weight loss, Powder of root with a glass of milk is taken for other benefits	300/kg
18	*Woodfordia fruticosa* (L.)Kurz.	Lythraceae	Dhayati	Flowers	Menstrual disorder	The fried flowers in butter is given once in a day for 7 days	20Rs./50gm
19	*Wrightia tinctoria* R. Br.	Apocynaceae.	Kala kuda	Stem bark	Skin disease, anti-dandruff and anti-inflammatory, Carminative	Paste, hair oils is used for hairs and on skin diseases	250/kg

Caesalpinia bonducella (Sagargota)

Woodfordia fruticosa (Dhayati)

Balanites aegyptica (Hinganbet)

Solanum xanthocarpum (Kateringani)

Wrightia tinctoria (Kalakuda)

Tinospora cordifolia (Gulvel)

Mucuna pruriens (Khajkuilu)

Cassia fistula (Bahava)

Asparagus racemosus (Shatawari)

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14

Effect of Integrated Fertilizer Management with and organic and inorganic fertilizers on growth and productivity of groundnut.

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Abstract

Indian agriculture has made significant progress in recent years. The concept & implementation of green revolution introduced during 1950 & 1960 and five year plan resulted in such a Situation that presently India is not only self sufficient in producing adequate food grains but supply it to under development & developing countries. In Spite of food, however, several people to living below poverty line suffer from hunger malnutrition & protein. In order increase productivity and efficiency of agriculture system in present investigation efforts are made to evaluate effect of integrated fertilizer management (IFM) on Productivity Some field Crop Plants of Beed district & Simultaneously efforts are made to avoid use of only chemical fertilizers, which is used by the farmers in adequate quantity, due to which there is depletion of soil fertility & over use of chemical fertilizer which causes pollution health problems.

Experimental :

In present Study to evaluate effect of integrated fertilizer management with inorganic & organic fertilizers on growth and productivity of groundnut.Pot experiments were made i.e. plants were sown in the pots having 34 cm diameter. The pots were filled with soil (PH. 7.9). The Seeds of groundnut were sown.After emergence, extra Seedlings were removed to maintain uniform plant population of 5 plants per pot. There were in all five treatments, each replicated for five times. The treatments were as under:

1)	Control	:	Untreated
2)	Rhizobium	:	2 g/pot
3)	NPK	:	2g/pot
4)	Rhizobium + urea	:	1: 1 g/pot
5)	Rhizobium + Nitrogen + compost	:	2g/pot

All fertilizers were added in the Soil of pots. The crops were raised in pots under irrigation. Fertilizer doses were given at the age of 15, 30, 45 day after Sowing. The Control or untreated pots were without treatment.

After 25 days of age at every 10 days interval a plants from each pot was removed randomly without damaging it's root system. The roots were wasted with water to remove adhering Soil particles & blotted.

The observations were made on height & root length per plant. The numbers of leaves per plant were measured, and total leaf area was determined by measuring the leaf area of each leaf using gravimetric method (Mungikar, 1986). The plants were then dried to a constant weight at 95 + 5 °c & dry weight per plant was recorded. Dry plant material was used to estimate nitrogen (N) Content which was determined by microkjeldahl method by digesting the material with H_2SO_4 in presence of catalyst (9 K_2SO_4 + 1 $CuSo_4$ + 0.02 Seo_2) and distillation followed by titration of liberated ammonia with boric acid. (Bailey, 1967).

Results & Discussion :

Groundnut is an important leguminous Crop of Beed district, cultivated during either rabi or summer Season. It is mainly cultivated for ponds & subsequently, Kernels which are used for the extraction of edible oil. Growing population mounting more pressure on natural resources to meet increased food demand. According to conservative estimate (Kanwar, 1998), the food grain demand in India for the year 2010 & 2020 is projected to be

246 & 294 mt. respectively, population grew at an annual growth rate of around 2% in 1970s, 1980s, 1980s & 1990s, to reach 1027 million in 2001 & estimated to increase further to 1282 & 1542 million by the year 2011 & 2021, respectively (Sekhon, 1997). As grains are rich in protein it also serves as a content, potential food material to 'Supplement proteins'. During present Study the effect of Chemical & organic fertilizers Separately & in Combination was observed.

Table 1 gives information of effect of integrated fertilizer management on growth & productivity of groundnut during 25 to 85 days after sowing.All pots received treatments except control. First group of pots received only water. They show very less development & production during period of 15 to 85 days. Plant height ranges from 4.7 cm to 35.8 cm & Number of pods 03 to 09. Pots received treatment of Rhizobium, or NPK Shows some more improvement as compared to control.

It was observed that there is an enhancement in growth & productivity in those plants who received treatment of both chemical & organic fertilizers. (i.e. Rhizobium + Urea + Compost).Plant height ranges from 9.5 cm to 65.5 cm & productivity & Productivity ranges from 13 pods to 18 pods. Table 2 Shows effect of integrated fertilizer treatment on productivity of groundnut.

Conclusion:

India accounts for 2.2% of the global land & 16% of the world's population. The country's population has crossed the one billion mark in 2001 while the population is likely to further increase at an alarming rate &, Side by Side as the land for cultivation of crops will decrease tremendously in the following decades, it will results in an increased demand for food, fodder, Shelter, energy, employment etc. The long term fertilizer experiments have shown that continuous application of suboptimal doses of chemical fertilizers alone to Soil has resulted in the deterioration of Soil health, thereby culminating in environmental pollution and Stagnation in crop productivity. Hence integrated use of organic manures with optimal levels of NPK fertilizer is the need of the hour, as it will not only improve the nutrient Status and Soil health, but also prove to be a boon in stabilizing the crop yields over a period of time.

Therefore INM System (Integrated Nutrient Management) is the only way to maintain and improve the nutrient Status of Indian Soil, In other words, INM Syst. is an ecologically, Socially & economically Viable approach, which on the whole is non-hazardous.

Overall results obtained during present Study indicate wide Scope to increase productivity of Crop plant in Beed District by integrated use of fertilizers or integrated fertilizer management author thought that there is need of further investigation on present Study on chemical composition of the final product residue effect & phyllosphere micro flora.

Table 1 : **Effect of Integrated Fertilizer Management with and organic and inorganic fertilizers on growth andproductivity of groundnut.**

Sr. No.	Treatment	Parameter	Days						
			25	35	45	55	65	75	85
1	Control	Height of plant	4.7	10.2	21.2	22.2	29.2	45.5	35.8
		No. of leaves	35	44	59	73	87	79	39
		Leaf Area	7.50	18.5	24.8	55.5	1135	1960	1345
		T.w.of pods	395	633	1315	503	1960	722	806
		D.W.	130	230	486	445	902	340	555
		%%	1.50	1.50	1.89	1.02	2.30	1.79	1.30
		Height of the plant	6.1	11.9	22.4	25.3	35.5	44.5	48.5
		No. of leaves	40	50	65	79	92	79	53
2	Rhizobium	Leaf Area	8.10	20.5	45.5	65.2	1850	2350	1108
		F.w.	397	662	1263	540	1742	722	1020
		D.W.	220	305	490	490	1602	468	750
		%%	1.85	1.85	2.50	2.20	2.30	1.79	1.32
		Height of the plant	7.5	12.3	23.2	24.4	36.6	47.7	60.2
3	NPK	No. of leaves	45	53	69	95	206	81	62
		Leaf Area	9.50	21.2	48.4	75.5	1950	3440	1489
		F.w.	420	688	1363	1648	17.60	761	1220
		D.W.	230	325	590	674	1702	380	850
		%%	1.85	1.88	2.92	2.28	2.35	2.09	
		Height of the plant	8.5	13.5	24.2	25.5	37.2	48.5	61.2
		No. of leaves	47	68	81	89	135	115	77
		Leaf Area	10.10	22.2	52.2	92.5	1980	3550	1.99
4	Rhizobium+Urea	F.w.	430	714	1543	1668	21.16	993	1430
		D.W.	235	420	630	760	1722	420	950
		%%	1.95	1.95	2.95	2.85	2.70	1.99	1.93
5	Rhizobium+Urea+Compost.	Height of the plant	9.5	14.5	26.9	27.5	39.8	53.2	65.5
		No.of leaves	57	86	107	125	158	125	100
		Leaf Area	12.90	30.6	70.4	95.5	2030	2750	2.20
		F.w.	670	853	1654	1686	28.90	1306	1710
		D.W.	290	530	850	950	1804	470	1040
		%%	2.20	2.20	2.99	2.95	2.90	2.22	1.90

Table 2:

Effect of Integrated Fertilizer Management with and organic and inorganic fertilizers on productivity of groundnut

Sr No.	TREATMENT	Height of The plant	No.of Pods/Plant	No.of seeds	Pods weight		Seeds Weight	
					Fresh (mg)	Dry (mg)	Fresh (mg)	Dry (mg)
1	Control	38.2	10	12	4950	2550	1720	1440
2	Rhizobium	49.5	11	14	5150	3240	1880	1480
3	NPK	50.5	13	17	5950	3550	1910	1850
4	Rhizobium+urea	55.2	15	18	7700	4400	2550	2320
5	Rhizobium+Urea+ compost	68.2	17	25	8250	6700	3550	3300

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STUDY OF PHYSICO-CHEMICAL PARAMETERS AND CORRELATIONSHIP AMONG DIFFERENT PARAMETERS IN WATER OF WAINGANGA RIVER FROM DIFFERENT AREA OF GADCHIROLI DISTRICT OF MAHARASHTRA

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Abstract:

The present investigation was conducted for the time periods of four months (1November 2016 to 28 February 2017) to evaluate the physicochemical parameters of water at different sites of Wainganga river, Gadchiroli. Maharashtra and also to study the influence of fortnightly, changes in physico-chemical parameters and to study Correlationship among different parameters. Three sites were selected for the investigation and the water sample were collected on fortnightly basis. The water samples were processed for analysis by adopting standard methods to investigate correlation of various physico-chemical parameters and to study Correlationship among different parameters. The study area experiences a fortnightly climate change and there by a fluctuation in physico-chemical parameters. Due to increase population, advanced agricultural practices, industrialization, man-made

activity, water is being highly polluted with different contaminants. Water is a vital resources for human survival. The availability of good quality of water is an indispensable feature for preventing diseases and improving quality of life. It is necessary to know details about different physico-chemical parameters such as Water temperature, p^H, EC, TDS, TA, TH, DO, BOD, Turbidity, Acidity.

Key words: Physico-chemical parameters, Fortnightly variation, Correlationship , Wainganga river, Gadchiroli

Introduction

Water is a transparent and nearly colourless chemical substance that is the main constituent of earth streams, lake and oceans and the fluids of most living organisms. Water also known as "Blue gold 'one of the most precious natural resources. Water is a wonder of the nature. "No life without water" is a common saying depending upon the fact that water is the one of the naturally occurring essential requirement of all life supporting activities.Pollution of a river first affects its chemical quality and then systematically destroys the community disrupting the delicate food web. Diverse uses of the rivers are seriously impaired due to pollution and even the polluters like industry suffer due to increased pollution of the river. Gadchiroli is district headquarter in Maharashtra, India. Gadchiroli district is situated in the south-eastern corner of Maharashtra state, and is bounded by Chandrapur district to the west, Gondia district to the north, Chhattisgarh state to the east, and Telangana state to the south and southwest.The main river basin of the district is the Godavari, which flows west to east and forms the southern boundary of the district. The major tributaries of the Godavari are the Indravati and the Pranhita, which is in turn formed by the confluence of the Wainganga and the Wardha near Chaprala village of Chamorshi Taluka. Due to use of contaminated water, human population suffers from water borne diseases. It is therefore, necessary to check the water quality at regular interval of time. Parameters that may be tested include temperature p^H, turbidity, EC (Electrical conductivity), TDS (Total dissolve solids), TA(Total alkalinity), TH(Total hardness), DO (Dissolve Oxygen), BOD (Biochemical Oxygen Demand) and Acidity. Thus, from the above study it is suggested to prevent human contravention and restrict excess of iron from the reservoir to procure good quality water from consumption.

Study Area: Gadchiroli town, a district headquarter belonging to eastern Vidarbha of Maharashtra state is located at 20.1884 North and 80.003 East longitude. The district stretches over an area of Maharashtra State. The Waingana (Bormala ghat, Kotagal, Pardi) river selected for the present study is situated on the Chandrapur path around 15 km from the Gadchiroli. This project is highly benefited by several villages along with entire city of Gadchiroli. The three study sites will be merges from the Wainganga river of the Gadchiroli town.

Fig.(A)Map shows location of Wainganga Gadchiroli River .Gadchiroli

Fig.(B) Map shows study locations highlighted in red colour of Wainganga River (three sites viz. B1, B2 and B3) Gadchiroli.

Material And Methods:

Water samples are collected from the different sites of river Wainganga viz, site-B1, B2 & B3 respectively, at fortnightly intervals from November 2016 to February 2017. Water sample were taken fortnightly for analysis and after determining field parameters(Temperature) they were kept in umber colour bottle. Before being transported to a laboratory for quantification of the other parameter (p^H ,turbidity, EC, TDS, TA, TH, DO, BOD, Acidity).

Following different physico-chemical parameters are tested regularly for monitoring quality of water.

Sr. no	Studied Parameters	Unit	Methods use
1.	Water Temperature	^{0}C	Recorded by thermometer
2.	P^H	-	Recorded by Digital pH meter
3.	EC	Cus/cm	Digital conductivity meter
4.	TDS	mg/L	Digital TDS meter
5.	TA	mg/L	Titrometry with Std.Hcl.
6.	TH	mg/L	Titrometry with EDTA
7.	DO	mg/L	Titrometry/Winkler method
8.	BOD	mg/L	Titrometry/Winkler method
9.	Turbidity	NTU	Turbidity meter
10.	Acidity	mg/L	Titrometry with NaoH

Table1:Data processing for statistical analysis

Month	Area	Temperature	P.H	E.C	T.D.S	T.A	T.H	D.O	B.O.D	Turbidity	Acidity
November- 1	B1	23°C	7.66	0.35	021	500	91.10	0.64	8.96	000NTU	500
	B2	23°C	7.65	0.32	020	250	65.00	0.62	9.36	000NTU	500
	B3	22°C	7.72	0.37	029	700	68.62	0.74	10.11	000NTU	250
November-16	B1	24°C	7.96	0.36	031	250	99.35	0.74	12.60	000NTU	250
	B2	23°C	7.84	0.42	034	250	95.20	0.68	9.17	000NTU	250
	B3	23°C	7.90	0.41	039	500	124.12	0.67	14.36	000NTU	500
December-1	B1	24°C	7.70	0.43	020	750	100.00	0.80	9.36	000NTU	700
	B2	23°C	7.75	0.38	027	750	97.36	0.89	9.87	000NTU	500
	B3	22°C	7.68	0.34	035	500	120.32	0.78	8.66	000NTU	250
December-16	B1	23°C	7.74	0.39	032	250	122.32	0.99	9.87	000NTU	500
	B2	24°C	7.80	0.42	029	250	120.00	0.75	12.36	000NTU	250
	B3	21°C	7.91	0.48	025	500	98.35	0.68	11.98	000NTU	250
December-31	B1	24°C	7.72	0.36	026	700	102.35	0.96	14.00	000NTU	500
	B2	23°C	7.74	0.40	030	750	84.14	0.68	15.63	000NTU	750
	B3	23°C	7.91	0.36	031	250	75.33	0.78	13.52	000NTU	700
January-16	B1	19°C	7.68	0.38	027	750	78.69	0.68	14.36	000NTU	500
	B2	21°C	7.86	0.48	025	500	123.32	0.88	13.25	000NTU	750
	B3	21°C	7.87	0.45	027	500	147.32	0.77	12.32	000NTU	250
January-30	B1	23°C	7.89	0.35	029	500	98.00	0.79	11.36	000NTU	500
	B2	23°C	7.68	0.37	024	750	63.25	0.66	12.35	000NTU	500
	B3	22°C	7.65	0.34	026	700	76.66	0.87	14.35	000NTU	750
February-14	B1	22°C	7.90	0.36	028	750	67.38	0.68	12.36	000NTU	700
	B2	24°C	7.90	0.42	039	500	99.24	0.78	12.35	000NTU	250
	B3	24°C	7.95	0.35	032	250	106.35	0.91	11.98	000NTU	250
February-28	B1	23°C	7.98	0.32	038	250	127.36	0.76	13.35	000NTU	500
	B2	22°C	8.16	0.42	035	500	99.30	0.84	14.65	000NTU	750
	B3	23°C	7.95	0.47	033	500	124.248	0.85	13.45	000NTU	750

OBSERVATION TABLE

Table 2: Fortnightly intervals mean values of physicochemical parameters of Wainganga River from three different area ;(B1), (B_2) & (B_3), Gadchiroli during (1 November 2016) to (28February 2017)

Results:

The result obtained from analysis of water sample of river Wainganga are shown table -1.

The reported value referred to be mean value of water sample collected in different month at different sites (B_1, B_2, and B_3) along the stretch of Wainganga River. The results indicate that the quality of water various considerably from location to location. The present study showed that the permissible limits of p^H, Water Temperature, Turbidity, Total Dissolved solids (TDS), Alkalinity, Total Hardness (TH), Acidity, and Dissolved Oxygen are mostly narrative and the water from the present water body is acceptable for the drinking purpose.

Temperature- The minimum water Temperature was observed 21^{0}C and maximum 24^{0}C.

P^H- The minimum P^H was observed in (1 Nov.2016) at site B_2 & (30 Jan.2017) at site as 7.65, while maximum P^H was observed in (28 Feb, 2017) at site B_2 is 8.16.

Electrical conductivity- The minimum EC was Observed in (1 Nov 2016) at site B_2 & (28 Feb 2017) as B_1 as 0.32 mhos, while maximum E.C was observed in (1 Dec 2016) at site B_3& (16 Jan 2017) B_2 as 0.48 mhos.

Total Dissolved Solid- Minimum E.C. was observed in (1 Nov 2016) at site B_2& (1 Dec 2016) B_1 as 020 mg/L, while maximum TDS was observed in (16 Jan 2017) at sites B_3 (16 Jan 2017) at B_2 as 039.

Total Alkalinity-The minimum T.A was observed in (1Nov 2016) at B_2; (16 Nov 2016) at B_1, B2; (1 Dec 2016) B 1&B_2 (14 Feb2017) at B_3 & (28 Feb 2017) at B_1 as 250 mg/L $CaCo_3$. Maximum T.A was observed in (1 Dec 2016) at B_1 & B_2; (31 Dec2016) B2, (16 Jan 2017) B1& (14 Feb 2017) at B_1 as 750mg/ L $CoCo_3$.

Total Hardness – The minimum TH was observed in (1 Nov 2016) at site B_2 as 65.00 mg/L, and maximum TH was observed in month (16 Jan 2017) at sites B_3 as 147.32 mg/L.

Dissolve Oxygen- The minimum DO was observed in (29 Jan 2017), at site B_2 0.62 mg/L, while maximum DO was observed in (1 Dec 2016) at B_2 as 0.99 mg/L.

Biological Oxygen Demand- The minimum value of BOD was observed in (1 Dec 2016) at B_3 8.66 mg/L, while maximum value of DO was observed in (31 Dec 2016) at B_2 site as 15.63 mg/L.

Turbidity-The turbidity is present study area

was found to be range of 000 NTU.

Acidity- Minimum value of Acidity was observed in (1 Nov 2016) at $B_{3,}$ (16 Nov 2016) $B_{1,}$ $B_{2,}$ and (1 Dec 2016) $B_{3,}$ (1 Dec 2016)$B_{2,}$ $B_{3,}$(16 Jan 2017)B_3 ;(14 Feb 2017) B_2B_3 as 250 mg/L CaCo3 while maximum value is (1 Dec 2016) at B_2 ,(30 Jan 2017) at $B_{3,}$(28 Feb 2017) at B_2,B_3 as 750 mg/L CaCo3 was observed in various months.

Discussion:

Temperature –Water Temperature is important physical parameter for aquatic ecosystem, water temperature in quality of the water, it deports as catalyst activities, stimulants, controller or as killer of life for some aquatic organisms. Also temperature has Nov 21 ^{0}C to 24^{0}C. and Dec.23^{0}C-24 ^{0}C , Jan 23 ^{0}C-25 ^{0}C was observed Feb.22^0 C-24^0 C. the water temperature varied from 21 ^{0}C – 24 ^{0}C. Wainganga River at different site.

P^H -P^H is important parameter in evaluating acid- base balanced of water. The p^H value of water at sewage at discharge points are usually higher than that of the river water. The graphical representation of changes in pH value are shown in fortnightly interval. The BIS (Bureau of Indian Standard)limits for drinking water is in the range of 6.5 – 8.5 it is found that pH of river water slightly various in studied location. This variation is due to change in Alkalinity of water sample. The pH value ranges 7.67- 8.27 units. The pH is graphically represented in graph no (1).

EC - Conductivity is measurement of ability of an aqueous solution to carry an electrical current (Manora online, 2012) Electrical Conductivity (EC) is a measure of water capacity to convey electric current. It signifies the amount of total dissolved salts EC values were in the range of 0.45 µohms/cm-0.28 µohms/cm. high value were observed Dec 31 at site w1 as 0.45 µohms/cm and minimum value observed January and February month.

TDS - The value are also within permissible limit drinking water standards (500- 1500mg/L) suggested by the WHO. The minimum value 018 mg/L and maximum value found as 028 mg/L.

TH -Total Hardness which is very important parameter determining useful of water in different is sector is also very much below the permissible limit that is, 132- 260 mg/L. This good for drinking purpose.

TA -Total Alkalinity of water is capacity neutralised to strong acid and it is normally due to the presence of bicarbonate, carbonate and hydroxides compound of calcium, sodium and potassium. Total Alkalinity values for all investigated sample were found to be greater in sample w1 site in the present study. Total Alkalinity range between 44-56 ppm. That is also within permissible limits suggested by WHO in (1984).

DO - Dissolved Oxygen content in natural and waste water depends on physical, chemical and biological activities in the water bodies. The WHO suggested that standard of DO is 7.5-00 mg/L. The graphical representation of DO presented in studied water sample are shown in graph no. (6) the concentration of the DO in the Wainganga river water sample in Nov(2016), Dec(2016) ,Jan (2017) and Feb (2017) found to range of maximum 14 Feb at site w2 as 0.98 and minimum in Dec at site w1 as 0.63 mg/L. DO is suitable for survival of aquatic life. The percentage DO is graphically represented in graph no (6).

BOD - In the present research BOD is ranged between 11.42mg/L in Feb 2017 and 7.65 mg/L in Nov 2016. The BOD values increased or decreased due to bacterial activities and other organisms as they feed upon and bring about the decomposition of organic matter. The plant life in the water will have an effect on the test BOD.

Turbidity - Turbidity is comparatively lower than the limit. Therefore there is no turbidity found in the water samples.

Acidity -Carbon dioxide level can fluctuate daily due to photosynthesis and respiration in the water. The degree of change depends on the alkalinity of the water CO_2 is the most common cause of acidity in water. Usually dissolves CO_2 is the measure acidic component present in the unpolluted surface water. These method are applicable to the determination of acidity in water and waste water

the applicable range is 0.52-500 mg/L acidity as $CaCo_3$. The recorded values are also compared with quality standard as proposed by BIS in February 1987.

Conclusion:

During study,water samples from three sampling sites of Wainganga river, Gadchiroli (M.S) were collected from the duration of fortnightly interval of 4 months from (1Nov.2016 to 28 Feb.2017) and analysis of various physico-chemical parameters had been carried out, correlation matrix was also prepared to show relationship among various hydrological parameters. Due to heavy discharge of effluent waste and domestic sewage in the river basin induction deterioration of Wainganga river water quality. Inside the crater, some farmers doing farming and hence the use of inorganic fertilizers, insecticides and pesticides like toxic compounds enters in river. Simultaneously, Hygienic activates are carried out by the local people in the fresh water springs and used waste water indulged in river at last. Therefore some steps must be taken towards human awareness to prevent human infringement to protect water from contamination and the divinely gifted river of Gadchiroli Maharashtra. It diminishes the aesthetic quality of rivers. The present work done undertaken to account to bring an acute awareness among the people about the quality of water. The individual and the community can help minimize water pollution by simple housekeeping and management practices the amount of waste generated can be minimized.

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Aeromycological survey of vegetable market at Sironcha, Dist. Gadchiroli

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Abstract

Aeromycological survey of vegetable market at sironcha was carried out for one year. During investigation period total 12 types of fungal spores were recorded. Along with fungal spores fungal hyphae were also recorded. Among fungal spores *Aspergillus* species (21.63%) were dominant followed by *Helminthosporium* (19.25%) & *Alternaria* (17.55%). Quantitatively fungal spores were maximum in the month of January and December. While minimum in the month of May. *Alternaria* species occurred throughout the year.

This information will be significant to the people who visit the market and are allergenic to fungal spores.

Introduction

Sironch is a town in Gadchiroli district of Maharashtra state. It is at the border of Maharashtra state and Telangana state. For selling the vegetable people from Telangana state come tosironcha. So it was decided to study aeromycofloraof vegetable market Various workers studied aeromymycoflor of vegetable market, Kumar *et.al*. (2013), Medhi and Sharma (2010), Sharma and Bhattachrjee(2001), Tilak and Kulkarni (1970).

Material And Method

Vertical cylinder spore trap was used to study aeromycoflora of vegetable market of sironcha. The study was carried out for one year from Junuary2016 to December 2016. The sampler was kept in the market for 24 hours. Total 360 slides were prepared. Along with this daily meteorological data was collected. Fungal sores were identified by using standard literature.

Observation

During investigation period total 12 types of fungal spores were recorded.viz. *Alternaria, Aspergillus, Cercospora, Chetonium, Cladosporium, Curvularia, Helminthosporium, Mucor, Nigrospora, Penicilium, Pringshemia* and*Rhizopus.* Among these *Aspergillus* species (21.63 %) were dominant followed by *Helminthosporium*(19.25 %) and*Alternaria* (17.55 %).Quantitatively fungal spores were maximum in the month of January and December. While minimum in the month of May. *Alternaria*species occurred throughout the year.

Table .1 – month wise occurrence of fungal spores with the percentage.

Sn	Name of fungal spores	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	%
1	*Alternaria*	31	17	14	09	02	05	08	04	21	20	25	29	185	17.55
2	*Aspergilus*	34	18	17	17	08	20	09	---	24	22	28	31	228	21.63
3	*Cercospora*	08	02	02	02	---	---	03	04	09	08	07	09	54	5.12
4	*Chetonium*	03	---	---	---	---	---	---	01	---	---	---	02	06	0.56
5	*Cladosporium*	04	03	---	---	---	---	---	---	04	02	05	05	23	2.18
6	*Curvularia*	28	22	08	---	01	---	---	---	11	13	22	29	134	12.71
7	*Helminthosporium*	27	20	14	04	---	---	14	21	24	29	22	28	203	19.25
8	*Mucor*	17	08	03	03	01	04	---	---	09	08	11	13	77	7.30
9	*Ngrospora*	09	03	01	---	---	---	---	---	04	---	05	04	26	2.46
10	*Penicilium*	12	17	08	02	---	01	---	---	06	09	07	11	73	6.92
11	*Pringshemia*	04	---	---	---	---	---	---	---	01	---	03	01	09	0.85
12	*Rhizopus*	06	07	03	01	---	---	---	03	03	02	05	06	36	3.41
13	Fungal hyphae	22	19	05	06	01	---	03	04	09	12	21	24	126	
	Total	183	117	70	38	12	30	34	33	116	113	140	168	1054	

Fig. 1.
Map of Gadchiroli District showing Sironcha.

Fig. 2. Vegetable Market of Sironcha

Result And Discussion

Aeromycoflora collected from air of vegetable market of sirocha show 12 types of fungal spores. During investigation period total 1054 fungal spores were recorded. The most dominant spore recoded was *Aspergillus* (21.63 %). *Alternaria*species occurred throughout the year. Maximum spores occurred during month of January followed by December and November. Similar observation was made by Kumar *et.al* (2013) while studying the seasonal variation of fungal spores of vegetable market of Haripur (U.P.).

Fungal spores were dominant in winter season. This may be due to low temperature and high humidity which is a favourable condition for fungal growth. Minimum fungal spores were recorded in the month of April and May which is a summer season. This may be due to high temperature and less humidity. Jadhav (1996), Tiwari (1996), Tiwari And Saluja(2009) shende and Kalkar(2013) observed maximum fungal spores in winter season.

This information will be significant to the people who visit the market and are allergenic to fungal spores.

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17

Effect of weed green manure and compost manure on Productivity of Spinach

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Abstract

Organic manures were prepared from common weeds like *Parthenium hysterophorous*, *Cassiatora* and *Ipomoeacarnea*. Spinach seeds was sown at the seed rate 30 kg/ha, frequent irrigation was given as per requirement. In a course of time 3 regrowths were studied after 41, 76 and 111 days of sowing. Productivity of spinach shows maximum amount of yield produced by weed compost and green manure as compared to chemical fertilizers and control.

Key words: Weed organic manure, Green Manure, Compost, Spinach productivity.

Introduction

Weed biomass is one of the easily available source of organic matter and plant nutrients. Economic utilization of this weed biomass for the production of various compost will open a new horizon. Generally weeds like *Cassia, Crotalaria,* and *Achyranthus* are used as green manures (Chamle, 2007). The function of green manure is to add organic matter to the soil (Kipps, 1970). Many weeds can be used for composting and vermicomposting as nutrient source for many crops. (Naikwade*et al.*, 2011a, Naikwade*et al*., 2011b, Ghadge*et al*., 2013).

Material and Methods

The fresh green leafy vegetation of *Parthenium hysterophorous*, *Cassiatora* and *Ipomoeacarnea* collected from nearby wasteland during the early hours of the day at 10-20 %flowering stage, chopped into small bits (2-3 cm) by the traditional iron cutter. The weed plant material was incorporated into the pots at the rate of 13333 kg/ha about 5-10 cm in the soil as green manure (GM). The same amount of weed vegetation was used for the preparation of compost (CM), and vermicomposting (VM).

Chemical analysis

The leaf chlorophyll content (a,b and total) were estimated following Arnon (1961), using 80% acetone as a solvent for extraction of pigments. Nitrogen (N) content was determined by Micro Kjeldahl method (Bailey, 1967).

Statistical Analysis

All the results were statistically analyzed using analysis of variance (ANOVA) test and treatments means were compared using the least significant difference (C. D. p = 0.05) which allowed determination of significance applications (Mungikar, 1997). In present investigation, the effect of weed green manure and Compost manure on different parameters of spinach were studied. Spinach is a short duration vegetable crop, it requires proper and sufficient N and K for regular growth (Premshekhar and Rajshree, 2009). Pot experiment was conducted to evaluate the role of weed manures for improving biochemical content in spinach.

Abbreviations:

CON = Control; CFU = Chemical fertilizer urea; GMP = Green manure *Parthenium hysterophorous;* GMC = Green Manure *Cassiatora*; GMI = Green manure *Ipomoeacarnea*; COP = Compost *Parthenium hysterophorous;* COC = Compost *Cassiatora*; COI = Compos *Ipomoeacarnea*;S. E. = Standard error; C. D. = Critical difference.

Table 1.
Effect of weed manures on Spinach.
(Age of the plant: 41 DAS).

Treatments	FW (gm.)	%			Kg / ha			
		DW	N	CP	FW	DW	N	CP
GMP	55.30	5.40	1.38	8.63	8975	876	224	1400
GMC	65.60	6.40	1.00	6.25	10647	1039	162	1014
GMI	56.30	5.80	1.12	7.00	9137	941	182	1136
COP	73.70	7.40	1.16	7.25	11962	1201	188	1176
COC	72.30	6.80	1.84	11.50	11734	1104	299	1866
COI	71.00	7.40	1.84	11.50	11523	1201	299	1866
FER	49.00	4.70	1.00	6.25	7953	763	162	1014
CON	41.70	4.20	0.91	5.68	6768	682	148	922
S. E.					679	69	22	134
C.D. (0.05)					1609	164	52	318

Table 2. Effect of weed manures on Spinach. (Age of the plant: 76 DAS).

Treatments	FW (gm.)	%			Kg / ha			
		DW	N	CP	FW	DW	N	CP
GMP	114.50	12.00	1.49	9.31	18550	1948	241.8	1511
GMC	138.60	12.90	1.12	7.00	22490	2094	181.7	1136
GMI	118.60	11.20	1.33	8.31	19240	1482	215.8	1348
COP	144.60	14.70	1.99	12.43	23469	2386	322.9	2018
COC	113.30	10.50	1.12	7.00	18388	1704	181.7	1136
COI	126.60	14.70	1.99	12.43	20547	2386	322.9	2018
FER	103.30	9.60	1.16	7.25	16814	1558	188.2	1176
CON	97	8.70	0.83	5.18	15743	1412	134.7	842
S. E.					938	138	25	156
C.D.(0.05)					2223	327	59	370

Table 3. Effect of weed manures on Spinach. (Age of the plant: 111 DAS).

Treatments	FW (gm.)	%			Kg / ha			
		DW	N	CP	FW	DW	N	CP
GMP	136.60	12.90	1.99	12.43	22170	2094	323	2017
GMC	127.70	12.20	2.21	13.81	20726	1980	359	2241
GMI	133.70	12.60	2.12	13.25	21700	2044	344	2150
COP	151.60	14.90	2.65	16.56	24605	2418	430	2687
COC	151.00	13.60	2.40	15.00	24507	2207	389	2434
COI	132.30	14.40	2.30	14.38	21472	2337	373	2333
FER	119.30	12.20	1.33	8.31	19362	2109	216	1348
CON	118.30	11.40	1.16	7.25	19200	1850	188	1176
S. E.					723	66	46	186
C.D.(0.05)					1714	156	109	441

Results and discussion-

Chemical analysis

Table 1, 2, 3 illustrates the analysis of fresh weight, dry weight, nitrogen and crude protein at the age of 41, 76, 111 days. The fresh weight and dry weight of leaves was found higher in COP 11962 kg/ha, followed by the treatments COC 11734, COI 11523, GMC 10647, and GMI 9137, GMP 8975 and fertilizer 7953 kg/ha. It was minimum in control. During second harvest it was also maximum in COP 23469 kg/ha, followed by GMC 22490, COI 20547, GMI 19240, GMP 18550, COC 18388 and fertilizers 16814 kg/ha. It was minimum in control 15743. During third harvest fresh weight and dry weight was also highest in COP 24605kg/ha, followed by COC 24507, GMP 22170, GMI 21700, GMC 20726, COI 21472, and in fertilizer 19362 kg/ha. It was minimum in control 19200.

The content of nitrogen and crude protein was found maximum in the treatment of COC and COI followed by GMP, COP, and GMI. It was equal in fertilizer and GMC while lower in control during first harvest. During second it was observed highest in treatment of COP and COI followed by GMP, GMI, and fertilizer. It was equal in GMC and COC. It was observed minimum in control. During third harvest nitrogen and crude protein was also highest in COP followed by COC, COI, COP, GMI, GMP, Fertilizer and control.

On the basis of statistical analysis it has been observed that all the values of fresh weight, dry weight, nitrogen and crude protein was statistically significant in all the treatments. All the values of GMP, GMC, GMI, COP, COC, and COI were statistically significant over the control, while the value of FER was non-significant over the control. In all three harvests compost of *Parthenium* showed maximum yield, nitrogen content and crude protein content as compared to other treatments.

Chlorophyll content-

During first harvest period Chlorophyll a, Chlorophyll b and total chlorophyll content ranged from 1.71-2.71, 3-4.76, and 4.60-7.50 mg/g. The chlorophyll content were more in COI, and GMP. It was equal in GMP and COC and lower in fertilizer and control. (Fig 1.). Chlorophyll a, Chlorophyll b, and total chlorophyll content ranged from 1.49-2.71, 2.63-4.61, and 4.13-7.60. It was minimum in COC and control during second harvest season (fig-2). While during the third harvest Chlorophyll a, Chlorophyll b, and total chlorophyll content ranged from 2.39-2.79, 4.02-4.68, and 6.3-7.4. It was equal in GMP & GMI and minimum in control (fig-3).

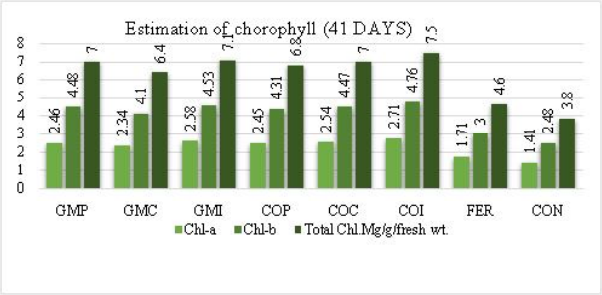

Fig-1 Estimation of chl-a, chl-b and total chlorophyll (age of the plant 41 days)

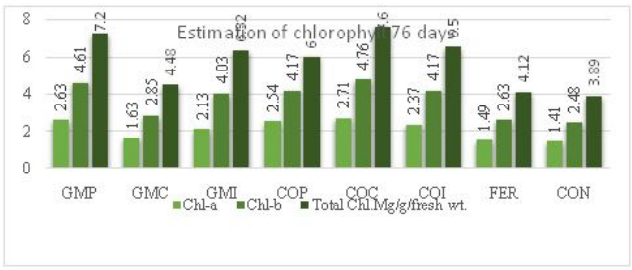

Fig-2 Estimation of chl-a, chl-b and total chl (age of the plant 76 days)

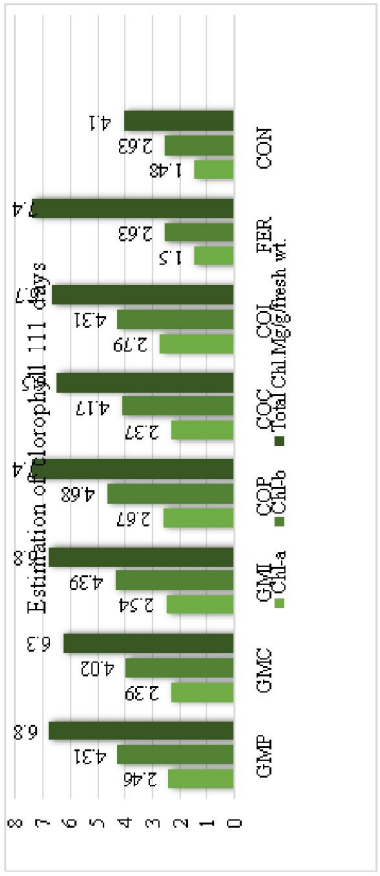

Fig-3 Estimation of chl-a, chl-b and total chl (age of the plant 111 days)

Use of organic weed manure amplify fresh wt., dry wt., nitrogen, Crude protein and chlorophyll content of vegetables which will be helpful to solve the problem caused by vitamin deficiencies (Mogle, 2013).

Conclusion- Green manure and compost manure of *Parthenium, Cassia* and *Ipomoea* served as very good manures for the crop.But the compost of *Parthenium* was best as compared to green manure.

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STUDIES ON BIOCHEMICAL CHANGES IN SOYBEAN SEED INFECTED WITH *FUSARIUM OXYSPORUM*

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Abstract

Studies on Biochemical changes were observed from healthy and artificially inoculated Dithane M-45 resistant F_{10} and sensitive F_{11} isolates of soybean seed (variety MACS-13) caused by *Fusarium oxysporum*. There was a significant variation between healthy seed and infected seed which showed significant changes with respect to estimation of crude protein, total sugars, oil, amino acids (methionine , tryptophan), minerals (calcium, iron). Among them, crude protein (41.50 %) it was increased in healthy seed. But in Total sugars (26.40%) it was decreased in healthy seed as compared with infected seed followed by oil (13.80%) and others. Infected seed of soybean seed by both resistant and sensitive isolates reduced the contents of all parameters. This was more pronounced due to utilization of nutritious compounds of the soybean seed (variety MACS-13) by fungal pathogen for their growth and metabolism which causes deterioration of the nutritious compounds of the seed.

KEYWORDS:*Fusarium oxysporum*, biochemical changes, soybean seed (variety MACS-13), Dithane M-45

Introduction

Soybean (*Glycine max* (L.) *merill*) is native of eastern Asia. The name of Soybean might be derived from the Chinese names "Shu" or "Sau" for Soybean. Soybean belongs to the family *Leguminosae*, subfamily *Papilinoidae* and tribe *Phaseoleace*. Soybean is considered the most important cultivated oil crop, comprising about 51% of the world production of vegetable oil. Also, because of its high protein content it has many commercial applications and soybean processing formed a large agro-industrial complex. It is most common species recorded on oil seed cum leguminous soybean crop (Glycine max L. Merrill.) fungi causing more than 30 per cent yield losses (Khan and Sinclair, 1992 and Mittal et al., 1993). Soybean is an ancient crop domesticated around the eleventh century in the North East China, after that it was spreaded towards South. Later on, it was known in Europe in 1721, in USA by 1804, in Brazil by 1903, in East Africa by 1907 (Mali and Thottappilly, 1990). Soybean was known to India some where between 1870-1880 (Andolle, 1884). Cultivation of soybean was initiated on large scale after the Second World War due to its nutritional value & multifarious uses (Synder & Kwon, 1987).

The common biochemical constituents like chlorophyll, sugars and phenols are important in imparting resistance to the crop plants. But almost most all living animals and plant show biochemical changes after infected by infectious agent (in Fishes by Mahananda et al., 2010 and in trees by Bora and Joshi, 2013). The present investigation was made to evaluate the biochemical changes observed in soybean seed (variety MACS-13) due to infected soybean seed (variety MACS-13).

Materials And Methods

Total 13 isolates of *Fusarium oxysporum* were isolated from infected part of soybean seed and maintained on Czapek Dox agar medium (CZA). *Fusarium oxysporum* isolates were tested against Dithane M-45 fungicide by food poisoning test

(Dekker and Gielink., 1979) Dithane M-45 resistant F_{10} and sensitive F_{11}; isolates were tested for biochemical analysis. This was studied by inject soybean seed with spore suspension of resistant and sensitive isolates. A deep well 1mm was prepared for spore suspension with the help of injection. After inoculation for 4 days, Seed were dried at 40°C in hot air oven and powder was obtained after crushing in grinder. Altogether 5 parameters were considered for analysis viz, Total sugars (Chenge and Bray 1951), crude protein (A.O.A.C. 1975), oil and minerals (calcium, iron) (Bangal and Gupta 1998).

Results And Discussion

Thirteen isolates of *Fusarium oxysporum* were tested against Dithane M-45 fungicide. The sensitivity (MIC) of Dithane M-45 resistant F_{10} showed 100ìg/ml while sensitive F_{11} showed 85ìg/ml. The sensitivity ranged from 80 to100 ìg/ml (**Table 1**). Biochemical analysis determined from soybean seed are shown in (**Table 2**), (**Fig.2.).** It was noted that the content of all parameters in the pathogen varied in sensitive and resistant strains. It was seen that crude protein were reduced in infected soybean seed when compared with healthy ones.

Table 1:

Sensitivity (MIC) of Dithane M-45 against *Fusarium oxysporum* isolates. Soybean seed (variety MACS-13)

Isolates	Locations	*Invitro* (MIC) µg/ml
F_1	Udgri	90
F_2	Parbhani	95
F_3	Deoni	98
F_4	Hingoli	95
F_5	Latur	99
F_6	Jalna	88
F_7	Beed	92
F_8	Amdapur	91
F_9	Aurangabad	87
F_{10}	Nilanga	100*
F_{11}	Dighol	85^+
F_{12}	Ashta	95
F_{13}	Chakur	89

❖ Minimum Inhibitory Concentration (MIC)

* - sensitive +- Resistant

Table 2: Estimation of biochemical analysis of healthy and infected Soybean Seed (variety MACS-13)

Sr.No.	Estimation	Healthy	Sensitive F_{11}	Resistant F_{10}
1.	Crude protein (%)	41.50	26.82	29.90
2.	Total Sugars (%)	26.40	17.2	19.50
3.	Oil (%)	20.50	11.10	13.80
4.	**Amino acids(g/60gm N)**			
	Methionine	1.27	0.85	0.95
	Tryptophan	0.58	0.10	0.16
5.	**Minerals (mg/100gm)**			
	Calcium	300	260	286
	Iron	9.70	5.40	6.80
	S. E. ±	40.849	35.859	39.367
	C.D. 05	96.813	84.985	93.3
	C.D. 01	142.97	125.5048	137.7842

Crude protein in the Seed infected with sensitive and resistant isolates were variable. Among them crude protein (41.50 %) was increased in healthy fruits, but reduced to sensitive (26.82 %) and resistant (29.90%). But in case of Total Sugars it was decreased (26.40 %) in healthy however, increased to sensitive (17.2%) and resistant (19.50%) followed by Oil. In case of Amino acids (Methionine and Tryptophan g/60gm N) and Minerals (Calcium and Iron mg/100gm) were decreased due to infection of both isolates. There was slight increase in total Sugars in seed inoculated with resistant and sensitive isolates in the healthy seed.

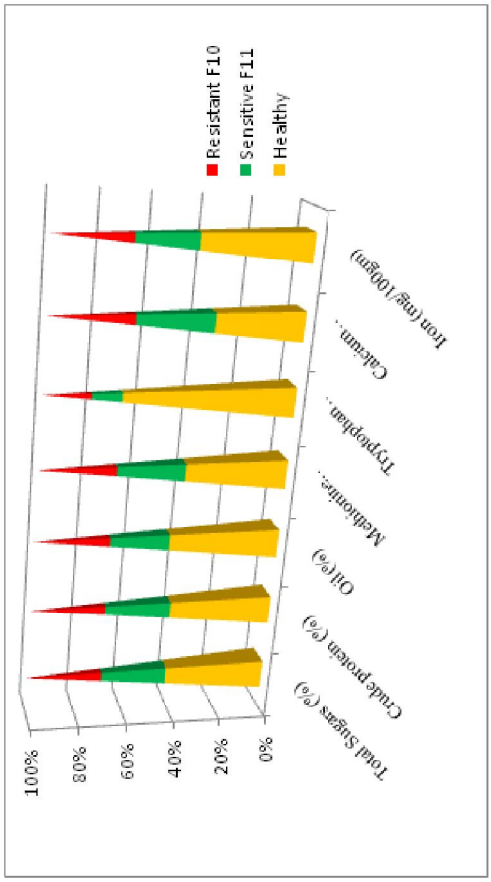

Fig. 2. Estimation of biochemical analysis of healthy and infected Soybean Seed

The comparisons of bio chemical contents of healthy and infected seed was made by estimating protein, oil, sugars (carbohyalrate) amino acids and minerals. It was observed that there was decrease in protein, oil, sugars, amino acids and minerals in infected seeds. These observations can be supported by the work of various scientists in case of soybean (Bangal and Gupta, 1998). The other workers also analysed the bio chemical containts of healthy and fungal infected seed of different crop plants. They are also in agreement with the descease in protein, oil, sugars, amino acids and minerals, (Singh, 1982., Mary Ragina and Tulsi Raman 1992., Kumar and Prasad, 1993., Gour and Singh 1995, Sinha and Sinha 1995)

These findings are in conformity with those reported earlier by many workers. Waghmare, et al. (2012) reported that the rose plants infected with leaf spot of rose showed significant increase in phenol content as compared to the healthy plant. Similarly, higher amounts of phenols were recorded for leaf spot resistant in groundnut cultivars by Gupta et al., (1985), Sindhan and Jaglan (1987) and Sindhan et al. (1987). This may be explained by the fact that as values of polyunsaturated fatty acids (linoleic and linolenic) decrease due to the lipid oxidation process, there will be an increase in the relative proportion of saturated fatty acids (Hildebrand, 1992). Soybean oil is one of the most preferred vegetable oils used for food and other applications. Oil content ranges from 8.3% to 27.9%, with an average of 18.1% on a 13% moisture basis in soybean seed (Wilson 2004). The concentration of the total amino acids varies across different soybean genotypes and during the days of sprouting (Song et al. 2000).

CONCLUSION

Exposure on consumption of these spoiled Seed may be responsible for serious health hazards. The nutritional value of Seed chiefly depends on the quality and quantity of nutritive substances. Fungi cause Seed of soybean. Post-harvest and per-harvest losses of Seed are very high and diverse post infection; biochemical changes reduce their food and market value considerably. Results of study showed that fungal infection brought about nutritional changes in Seed. This was more pronounced due to utilization by fungal pathogen for their growth and metabolism and causes deterioration of the nutritious of the Seed.

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Phytochemical Study of the medicinal plant Pudina (Peppermint)

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Abstract:

Pudina is herbsplants. The pudina is the aromatic properties. In most part of the herbs are grown mainly as field crops or on small scale crop among vegetable. The pudina is rich in volatile oil which gives pleasurable aroma. Essential oils have been extensively investigated for their activity against a number of storage fungi, plant and human pathogen.the menthol is covalent organic compound made synthetically or obtained from peppermint or other mint oil. It is a waxy crystalline substance clear. The menthol occurring in nature (1R,2R,5R) configuration. Chemical composition are present inpudina such as Fibre, Protein, Fat, Carbohydrate. Peppermint is a hybrid species of spearmint & water mint

Keywords: Phytochemical screening, analysis, Pudina.

Introduction:

Pudina also known as mint is one of the most common herbs & extremely popular in alternative medical treatment.Mint has several medicinal use in treating mint to skin problem.It is specially useful in treating inflammable.It solve the gastrointestinal problems.Pudina are used as the antisparmodic, peppermint stimulative and carminative.Peppermintoil a acts as an expectorantand decongestant they help clear respiratory tract and relieve tensionheadache pain .Pudina good for cold fever, pudina help as a digestion, rheumatism ear aches.

Material An Method- Tests for carbohydrate are Benedict's test , Barfoed's test, Non reducing sugars, Tyrosine, Cysteine test. Alkaloids, Protein and Amino acid test are determined. Bertrand' reagent, Shinodatest Flavonoids, Lead acelateand ninhydrin tests are analysed.

General Test for preliminary phytochemical screening medicinal plant peppermint (pudina)

Test name	Inferences
Carbohydrates	
Benedict's test	+ ve
Barfoed's test	+ ve
Non reducing sugars	- ve
Tyrosine test	+ ve
Cysteine test	+ ve
Alkaloids	
Bertrand's reagent	- ve
Hager test	+ve
Marme's test	+ve
Flavonoids	
Shinoda test	+ ve
Sulphuric acid test	-ve
Lead acelate	-ve
Protein	
Millon's test	+ ve
Xanthoprotein test	- ve
Precipitation test	+ ve
Lead acetate test	+ ve
Amino Acids	
Ninhydrin test	+ ve

Result And Discussion- Benedict's test, Barfoed's test, Barfoed's test, Tyrosine , Cysteine test.. Shinodatest ,Million test, Precipitation test, Lead acelateand ninhydrin tests are positive.

Conculsion-

In the pudina contain carbohydrate, protein , alkaloids, flavonoids ,amino acid and mint are present. The mint oil is used as as environmentally friendly insecticide for its ability to kill some common pests such as wasps, hornets, ants and cockroaches. It also has antimicrobial properties it help freshen bad breath & soothe digestive issues.

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Assessment of Antagonistic Activity of *Trichoderma viride* on Seed Borne Pathogens of Pigeon pea (*Cajanus cajan* L.) Millsp.

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Abstract

The present study reveals that the antagonistic potential of some fungi isolated from the pigeon pea seeds which were screened against *Trichoderma viride. T. viride* isolated from soil and fruit west which collected from local market. It showed inhibitory effect on some common post harvest pathogen of pigeon pea. They are namely *Alternaria tenuissima* (70%); *Curvularia* sp. (66.66%), and *Drechslera* sp. (66.66%), *Fusarium* sp. (72.22%), *Aspergillus terrus* (76.66%) and *Aspergillus niger* (63.33%) were found. It means *Trichoderma* was found more inhibitory activity on *Aspergillus terrus* and *A. niger* shows the minimum inhibitory effect. All over result shows *Trichoderma viride* the maximum inhibitory effect on pathogens.

Key words: *Trichoderma viride*, Seed borne pathogens, Biological control

Introduction

Pigeon pea (*Cajanus cajan* L.) Millsp. is a annual crop. After harvesting it also affects by many endophytic or pathogenic fungal pathogens. The present study focused on seed borne pathogen of pigeon pea i.e. *Alternaria tenuissima, Curvularia* sp., *Drechslera* sp., *Aspergillus terrus*, *Aspergillus niger* and *Fusarium* sp. etc. these pathogens are affects on quality content of seed and reduce the productivity of crops. To control these pathogen use chemical and biological method everywhere. In biological method *Trichoderma* has giving the best results on controlling this pathogen. A number of microorganisms, which effectively control postharvest pathogens, have been identified for post harvest control (Wilson and Wisniewski, 1989). Biological control of plant pathogens by micro-organisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). *Trichoderma* sp. has proved to be useful in the control of phyto-pathogens affecting different crops (Benitez T. *et al* 2004), (Soytong K.*et al*.2005) *Trichoderma* spp. are now the most common fungal biological control agents that have been comprehensively researched and deployed throughout the world. Several fungal cell wall degrading enzymes, amongst them chitinase and glucanase, which seem to play an important role in the antagonistic action of *Trichoderma* against a wide range of fungal plant pathogens (Kucuk and Kivanc, 2008). *Trichoderma spp*. have been identified as most common fungal antagonistic. Several strains of *Trichoderma* have been found to be effective as bio-control agent of various soils and seed borne plant pathogenic fungi

Material and methods:

Isolation of pathogenic fungi:

The pathogenic fungi namely *Alternaria tenuissima, Curvularia* sp., *Drechslera* sp., *Fusarium* sp., *Aspergillus terrus,* and *Aspergillus niger* are isolated from seeds of pigeon pea and it collect on different locality. Seeds are sterilized by the using 1N HCL for one minute and cleaned with help of distilled water. Seeds are put on Potato Dextrose Agar plate and fourth day after that separates the each fungal colony and making pure culture and observed under microscope, on the basis

of their colony color, shape, size, conidia and mycelium and identified by using manual of The Illustration of Fungi (Mukadam D.S. *et.al.*2006)

Duel Culture Method

Antagonism of *Trichoderma viride* on post harvest pathogen was studied by duel culture technique (Rama Bhadra Raju et al., 2000). On potato dextrose agar plate seven days old culture of pathogen and *Trichoderma viride* were placed simultaneously on the periphery of petridish. The petridishes containing PDA medium inoculated with the tested pathogen alone served as control. All the plates were inoculated at room temperature and measurement taken after five day. At the end of incubation period radial growth was measured. The percentage inhibition growth of tested pathogen in presence of *Trichoderma viride* was calculated over control. The growth inhibition was calculated by using the formula 100×C-T/C, where C= growth in control, and T= growth in treatment. (Edington et al, 1971)

Result and Discussion

The result showed that *Trichoderma viride* could restrict growth of seed borne pathogen on potato dextrose agar medium in the dual culture technique (Table 1). The percent inhibition of radial growth of tested fungi viz. *Alternaria tenuissima* (70%), *Drechslera* sp. (66%), *Curvularia lunata* (66%), *Fusarium* sp. 72%), *Aspergillus terrus* (76%), *A niger* (63%) were reduced by *T. viride*. which grown over the pathogens colonies. The best result showed on *Aspergillus terrus A. niger* and *Fusarium* sp. shows a minimum inhibitory effect. The growth inhibition of seed born fungi by dual culture in this study could be due to its fast growing nature. Secretion of harmful extra cellular compounds like antibiotics cell wall degrading enzymes such as glucanase, endo-chitinase and chitinase and mycoparasitism in dual culture as found with other fungi. This supports earlier investigations. Ramesh Sunder et al., (1995), Thirumala Rao and Sitaramaiah (2000), Nakkeeram et al., (2002).

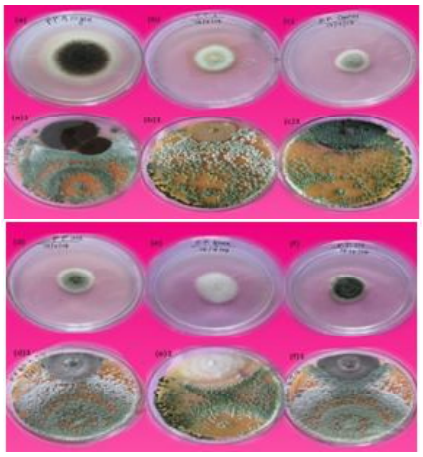

Photo *In-vitro* assessment of *Trichoderma viride* against seed borne pathogens of pigeon pea. (a) *A. niger* (a) 1- *A. niger* against *T. viride*, (b) *A. terrus* (b) 1- *A. terrus* against *T. viride*, (c) *Curvularia sp*. (c) 1- *Curvularia sp*. against *T. viride*, (d) *A. tenuissima* (d) 1- *A. tenuissima* against *T. viride*, (e) *Fusarium sp*. (e) 1- *Fusarium sp*. against *T. viride*, (f) *Drechslera sp*. (f) 1- *Drechslera sp*. against *T. viride*.

Observation table 1. - Antagonistic effect of *Trichoderma viride* on seed borne pathogens of Pigeon pea.

Seed borne fungi	Control without *T. viride*	Radial growth of tested pathogen (in cm)	Radial Growth of *Trichoderma viride* (in cm)	Growth inhibition due to *T. viride* (%)
Alternaria tenuissima	2.5	2.7	6.3	**70.00%**
Drechslera sp.	3.1	3.0	6.0	**66.66%**
Cuvrularia sp.	2.8	3.0	6.0	**66.66%**
Fusarium sp.	2.6	2.5	6.5	**72.22%**
Aspergillus terrus	2.1	2.1	6.9	**76.66%***
Aspergillus niger	4.0	3.3	5.7	**63.33%**

Highest inhibition of *T. viride

Acknowledgement

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Determination of Effect of Phytohormone on the Seed germination of *Sesbania grandiflora* of Maharashtra.(India)

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Abstract

Hormones may be defined as biologically active substances which regulate and stimulate plant growth and development .Most of the physiological activities and growth in plants is regulated by the action and interaction of chemical substances in them called hormone.(Rekha Balodi 2013) An experiment was carried out to study the effect of plant growth hormone on seed germination of *Sesbania grandiflora* belongs to family Fabaceae in the Marathwada region of Maharashtra.

Key Words: Marathwada, Seeds, germination, growth hormone, Sesbania

Material and Methods

The Seed samples were collected from Marathwada region of Maharashtra. The investigation was conducted in the laboratory during 2016-2017. Ten Seeds for each treatment of *Sesbania grandiflora,* were soaked in various plant growth regulators viz. NAA, KI and 2,4-D at 10, 20, & 30PPM for the duration of four days. Untreated seeds served as the control.

Result and Discussion

It can be observed from the data presented in Table-1 that all the treatments significantly influenced the germination percentage when compared to the control(water Soaking)Three

growth hormones NAA, KI and 2,4-D of different concentration i.e. 10, 20 and 30 ppm were afforded to study their effects on the germination of seeds of *Sesbania grandiflora* .In NAA 30%, 50% and 80% are germinated in 4days at 10, 20 and 30 ppm seeds. While the percentage of seed germination was % ($SGP^4_{30,}$ SGP^4_{50} and$SGP^4_{80).}$ While in KI at 10 ppm 6 seeds are germinate 04 days at 20ppm 7 seeds germinated and 30 ppm 10 seeds are germinated respectively. The percentage of seed germination was 50 % at 10 ppm and 70 at 20 and 100% at 30 ppm (SGP^4_{60} , SGP^4_{70} and SGP^4_{100}). In 2,4-D 2 seeds germinated in 4 days at 10 ppm and Zero seeds germinated at 20 and 30 ppm. The percentage of seed germination was 20 % at 10ppm and zero % at 20 and 30 ppm respectively (SGP^4_{20} $SGP^4_{00)}$

In KI (30ppm) treated seedling induced in enlargement and increased in Plumule, with increasing the concentration.while in NAA treated seedling reduced in length but increase in size of cotyledons as compared to control.Similar results have been reported byArumugam et.al. (2006); Dhanker & Sing (1996) In 2,4-D 10, 20 and 30 ppm, the seed germination is retarded**. (Table 01, plate 01)**

Conclusion

The result indicate that NAA,KI and 2,4-D affect the germination of seeds. Improvement in germination may be obtained with NAA and KI in Sesbania grandiflora.The responses of these growth hormone was specific and largely dependent on the nature of seeds.

Table.01.

Effect of Phytohormone on Seed Germinaton of Sesbania grandiflora

Sr. No.	Growth hormones in ppm	Total seeds	Days for Germination	Total Seeds Germinated	% of Seed germination %
1	Auxin :- (NAA) 10	10	04	03	30
	20	10	04	05	60
	30	10	04	08	80
2	KI 10	10	04	06	60
	20	10	04	07	70
	30	10	04	10	100
3	2,4-D 10	10	04	02	20
	20	10	04	00	00
	30	10	04	00	00
4	Control with distil Water	10	04	10	100

Plate :1

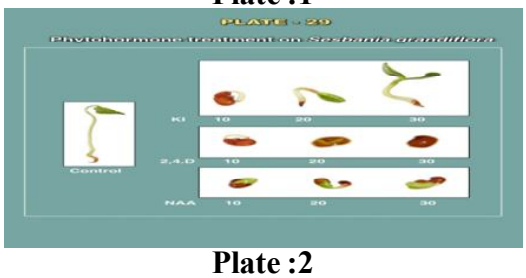

Plate :2

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TRICHODERMA STRAINS AGAINST *MACROPHOMINA PHASEOLINA* CAUSING FRUIT ROT OF IVY GOURD

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Abstract

Fruit rot of *Coccinia indica* Wight and Arn is caused by *Macrophomina phaseolina* (Tassi) Goid. Integrated management practice based on biocontrol is considered to be safe and sustainable measure for management of plant diseases. This paper describe the efficacy of different strains of *Trichoderma* species against *M.phaseolina* by dual culture method under *invitro* conditions. *Trichoderma viride* (12), *T. harzianum* (12), *T. virens* (10), T. *koningii* (07), and *T. pseudokoningii* (05) strains were isolated and used for antagonistic study. Among 12 isolates of *T. viride,* Tv_1, Tv_2 and Tv_9 isolates showed maximum whereas Tv_7 and Tv_{11} isolates showed minimum antagonism. In *T. harzianum* isolates, Th_1 and Th_5 showed indicative results as compared to others but only 50% inhibition was found in Th_2. In case of *T. virens,* significant inhibitions were found in Tvr_3, Tvr_4 and Tvr_8 isolates but Tvr_1 isolate was found less inhibitory. In *T. koningii,* only Tk_4 isolate was found eloquent to other strains. Out of five isolates of *T. pseudokoningii* Tp1, Tp_2 and Tp_3 were found maximal and Tp_5 minimal in inhibition.

Key words: *Macrophomina phaseolina,* Strains of *Trichoderma* species, dual culture

Introduction

Plants have always been an exemplary source of drugs and many drugs currently available have been derived directly or indirectly from them. A vast majority of population particularly those living in villages depends largely on medicinal plants for treating and curing diseases. One such medicinal plant ivy gourd (*Coccina indica* Wight and Arn.) of the family *Cucurbitaceae* is most important vegetable and medicinal plant, distributed in Tropical Asia, Africa, Pakistan, India and Sri Lanka (Cooke, 1903; Sastri; 1950). It is a climber and trailer (Nasir and Ali, 1973). Different names of ivy gourd like the parwal, kundru, tondli are in market. It is native to Africa and has been growing in the Indomalayan region of Asia for many centuries (Singh, 1990). It has white flowers and small cucumber like fruits which turn bright scarlet red when ripened. Ivy gourd has vitamin A, â - carotene and is a good source of protein. The fruit of *Coccinia* is used as vegetable when green and eaten fresh when ripened into bright scarlet color. Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for skin diseases bronchial catarrh, bronchitis and unani systems of medicine (Behl et al. 1993). It shows also hypoglycemic activities (Mukerjee et al., 1972 and Nahar et al., 1998). The juice of the roots and leaves are considered to be a useful in treatment of diabetes (Chopra et al., 1925). A post and pre-harvest food loss constitutes a vast complex of physical and biological changes due to microorganisms like fungi and bacteria. Diseases are very important in reducing market quality of ivy gourd fruit and are primarily responsible for the post and pre harvest losses of 10-35%.

However, ivy gourd fruits during field and storage are attacked by *Macrophomina phaseolina* which is severe in Marathwada region of Maharashtra. Since, biocontrol agents for protection of seeds and control of seed borne diseases offers

farmers an alternative source for chemical fungicides which is highly effective (Callan et al., 1997). It is therefore necessary to develop alternative ways of control. One such alternative is biological control, in which microorganisms are selected for their ability to antagonize pathogens. Various disease management methods have been implemented to combat and eradicate pathogenic fungi which include cultural, regulatory, physical, chemical and biological methods. All these methods are effective only when employed well in advance as precautionary measure (Kata , 2000). Therefore an investigation was made to evaluate the different isolates of *Trichoderma* species against *M. phaseolina* inciting fruit rot of ivy gourd.

Material and Methods

Isolation and identification of test pathogen

Fruits showing symptoms of fungal infection were collected and symptomatology of the disease was studied under natural and artificial conditions. Isolation of the pathogen was done from each distinct type of symptoms produced on fruits. Infected fruit parts were cut into small pieces by sterilized blade then surface sterilized with mercuric chloride (0.1%) for 1 min. The pieces were then washed thrice with sterilized distilled water and dried by sterilized blotting paper. These pieces were placed on potato dextrose agar (PDA) medium and incubated at 28±2°C. The fungus was isolated and identified as *M. phaseolina* (Ellis, 1971). The culture was deposited at Dept of Botany, Arts, Science and Commerce College, Naldurg. The pathogencity of test fungus was confirmed by inoculating ivy gourd fruits properly (Thompson, 1996).

Isolation of *Trichoderma* spp

Rhizosphere soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra. From the rhizosphere soil samples, desired strain of *Trichoderma* species were isolated by using potato dextrose agar (PDA) and *Trichoderma* selective medium (TSM) by dilution plate technique. The isolated strains were identified by reculturing on another petriplates containing sterilized TSM. The isolated strains were identified up to species level based on colony characters, growth of fungus and structure of mycelium, conidiophores and conidia (Kubicek and Harman, 2002). All *Trichoderma* spp. were purified by hyphal tip technique. The isolated strains of *Trichoderma* species were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

Dual culture experiment

Antagonistic efficacy of different isolates of *T. viride* (12), *T. harzianum* (12), *T. virens* (10), *T. koningii* (7), and *T. pseudokoningii* (5) were tested against the isolated pathogenic fungus by dual culture experiment (Morton and Stroube,1955). *Trichoderma* species and test fungus was inoculated at 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2° C for 9 days. Monoculture plates of both served as control. Nine days after incubation (DAI), radial growth of test fungus and *Trichoderma* isolates were measured. Colony diameter of test fungus in dual culture plate was observed and compared with control. The growth inhibition was calculated by using the formula: 100 X C - T / C, Where C = growth in control and T = growth in treatment (Vincent, 1947).

Statistical Analysis

Arcsine transformation of biological control (*Trichoderma* species) percentage was done by using following formula -

$$y = \arcsin e\sqrt{p} = \sin^{-1}\sqrt{p}$$

Where, p is the percentage and Y is the result of transformation

Statistical analyses of the experiments were performed using the Handbook of Biological Statistics (McDonald, 2008) and Mungikar (1997).

Results and Discussion

Isolation and identification of test pathogen

Fruits showing blackish, gray, black, grayish white, blackish gray containing symptoms were collected from different locations of Marathwada region of Maharashtra and ten isolates of *M. phaseolina* were isolated.

Isolation of *Trichoderma* spp

Isolates of five species of *Trichoderma*, T. *viride* Pers. (12), *T. harzianum* Rifai, (12*), T. virens* J. Miller, Giddens and Foster (10), *T. koningii* Oud.(07) and *T.pseudokoningii* Rifai. (05) were isolated from irrigated and non-irrigated rhizosphere soil of Marathwada region of Maharashtra. Isolates are deposited at Dept. of Botany, Arts, Science and Commerce College, Naldurg.

Dual culture experiment

Among 12 isolates of *T. viride*, Tv_1 (78%), Tv_2 (74.11%) and Tv_9 (75.12%) showed maximum antagonism as compared to others whereas, Tv_7 (47.03%) and Tv_{11} (49.26%) were reduced the antagonism (Table 1). *T. harzianum,* isolates Th_1 (83.3%) and Th_5 (85.68%) showed indicative results as compared to other isolates. But only 50% percent inhibition was found in Th_2 (Table 2). Table 3 illustrated that, *T. virens* isolates i.e. Tvr_3 (83.57%), Tvr4 (80.24%) and Tvr_9 (83.46%) were found better antagonistic and followed by other isolates. In case of *T. koningii* isolates, only Tk_4 (74.56%) was found eloquent followed by Tk_6 (69.02%) and Tk_5 (66.97%) (Table 4). Out of five isolates of *T. pseudokoningii,* Tp_1 (81.01%), Tp_2 (83.34%) and Tp_3 (85.84%) showed maximal percent inhibition (Table 5). Among all isolates of *Trichoderma* species, $Tv_{1,2 \text{ and } 9}$, $Th_{1,5 \text{ and } 12}$, $Tvr_{3,4 \text{ and } 9}$ and $Tp_{1,2 \text{ and } 3}$ isolates were found better but *T.koningii* isolates were failed to showed more antagonism (Fig.1).

The findings of workers have been reported use of *Trichoderma* species as biological control against number of plant pathogenic fungi. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum* (Hesamedin Ramezani, 2008). Interaction implies that a single isolate of antagonist can be highly effective against an isolate of *M. phaseolina*, but may have only minimal effects on other isolates of *M. phaseolina* (Aly et al., 2007). In *in vitro,* control of *R. solani, F. oxysporum* and *M. phaseolina* were achieved with *T. koningii, T. hamatum* and *T. harzianum* (Arora, 1990). Krishnaveni (1991) reported that seed treatment with *T.viride* was very effective in controlling charcoal rot of soybean and similarly, seed treatment with *T.viride* and *T.harzianum* also reported to be reducing incidence of charcoal rot of cowpea (Ushamalini et al., 1997). The use of bioagents and oil cakes do not harm the environment but improve sustainably of field soils (Jaiman et al., 2009). Deshmukh and Rout (1992) reported *T.harzianum* and *T.viride* were effective in inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence in pot culture. Indra and Subbiah (2003) reported the least incidence of root rot in black gram (*M. phaseolina)* treated with *T.viride* and *Rhizobium*. In dual cultures, *T.viride, T.harzianum* and *Aspergillus vesicolor* were effective in inhibiting the growth of *M.phaseolina* (Choudhary et al., 2010). Recently, Waghmare and Kurundkar (2011) reported efficacy of *Trichoderma* species against *Fusarium oxysporum* f. sp. *carthami* causing wilt of safflower and isolates no. 29 and 33 were found to minimum growth of the pathogen as compared to others. The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda et al., 2011).

According to Papavizas and Lumsden (1980) the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably due to antibiosis, lysis, competition and mycoparasitism. Ayers and Adams (1981) indicated that interactions observed *in vitro* do not necessarily confirm their operation for decrease in pathogen populations and reduction in diseases observed in natural conditions.

The *in vitro* screening with our arbitrary system of bio-antagonists effective against soil borne pathogens is a simplistic approach to understand a small sector of biological system in disease control. Therefore, it may be more prudent to search for biological antagonists against specific pathogen and evaluate blends of antagonists for wider applications (Baker and Cook, 1974). Our results showed that although considerable success in biocontrol is achieved under laboratory conditions the outcome

is also proportionate under field conditions. Hence, work is needed towards a better understanding and development of technologies that allow the biocontrol agent to spread and proliferate in soil. Papavizas (1985) suggested that the research should be directed towards the improvement of strains of biological agents that are more capable of becoming established and surviving under adverse field conditions. Thus, it is obvious that biological control offers durable, environmentally safe and cost effective alternative to chemicals for the efficient management of plant disease.

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Table 1: Influence of *Trichoderma viride* isolates on radial growth of *M.phaseolina.*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tv_1	Naldurg	12.00	86.60 (98.84)
Tv_2	Osmanabad	16.02	82.34 (95.80)
Tv_3	Latur	29.77	67.91 (80.03)
Tv_4	Nanded	31.12	65.55 (79.95)
Tv_5	Jalna	24.13	73.50 (87.67)
Tv_6	Aurangabad	34.00	60.17 (70.75)
Tv_7	Beed	43.00	52.25 (59.99)
Tv_8	Paranda	38.12	57.09 (70.31)
Tv_9	Ashti	15.19	83.70 (96.89)
Tv_{10}	Omerga	32.99	63.46 (74.58)
Tv_{11}	Ahmedpur	41.29	54.73 (63.85)
Tv_{12}	Hingoli	30.00	66.66 (79.44)
Control		89.46	--
SEm ±		10.34	7.71
CD (p=0.05).			

Radial growth and percent inhibition values are means of three replications

Figures in parentheses are arcsine transformed values of % inhibition.

Table 2: Influence of *Trichoderma harzianum* isolates on radial growth of *M.phaseolina*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Th_1	Tuljapur	14.77	83.30(96.95)
Th_2	Kallam	45.11	50.01(55.68)
Th_3	Ausa	41.00	54.6863.86)
Th_4	Nanded	22.00	75.8190.21)
Th_5	Badnapur	13.11	85.68(98.32)
Th_6	Beed	38.17	57.96(67.72)
Th_7	Paithan	31.00	66.10(79.44)
Th_8	Paranda	34.77	62.41(72.90)
Th_9	Patoda	28.12	69.62(82.49)
Th_{10}	Nilanga	39.11	54.88(63.85)
Th_{11}	Udgir	33.12	63.45(74.59)
Th_{12}	Parbhani	19.00	79.13(94.24)
Control		90.00	--
SEm ±		3.43	
CD (p=0.05).		7.54	

Radial growth and percent inhibition values are means of three replications.

Figures in parentheses are arcsine transformed values of % inhibition.

Table 3: Influence of *Trichoderma virens* isolates on radial growth of *M.phaseolina.*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tvr_1	Naldurg	40.00	55.55 (65.15)
Tvr_2	Murum	23.00	74.44 (88.12)
Tvr_3	Beed	15.00	83.57 (97.12)
Tvr_4	Badnapur	18.00	80.24 (94.95)
Tvr_5	Kannad	20.11	77.90 (91.67)
Tvr_6	Parbhani	34.77	61.11 (72.06)
Tvr_7	Nilanga	30.99	67.91 (80.03)
Tvr_8	Udgir	36.02	71.11 (83.77)
Tvr_9	Nanded	15.11	83.46 (97.19)
Tvr_{10}	Hingoli	20.00	77.77 (92.23)
Control		89.00	--
SEm ±			2.98
CD(p = 0.05)			6.73

Radial growth and percent inhibition values are means of three replications.

Figures in parentheses are arcsine transformed values of % inhibition.

Table 4: Influence of *Trichoderma koningii* isolates on radial growth of *M.phaseolina.*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tk_1	Naldurg	40.00	55.55(64.29)
Tk_2	Osmanabad	40.00	55.55(64.29)
Tk_3	Latur	45.00	61.11(71.16)
Tk_4	Aurangabad	23.11	74.56(88.05)
Tk_5	Jalna	30.00	66.97(79.05)
Tk_6	Parbhani	28.14	69.02(78.60)
Tk_7	Nanded	47.01	48.04(56.13)
Control		87.88	--
SEm ±			3.40
CD(p=0.05)			8.26

Radial growth and percent inhibition values are means of three replications.

Figures in parentheses are arcsine transformed values of % inhibition.

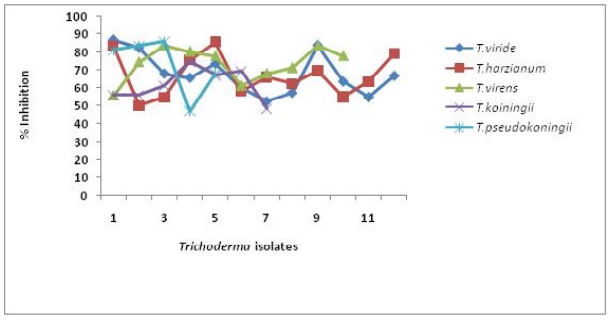
Table 5: Influence of *Trichoderma pseudokoningii* isolates on radial growth of *M.phaseolina.*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tp_1	Ausa	17.11	81.10 (95.42)
Tp_2	Tuljapur	15.00	83.34 (96.89)
Tp_3	Jalna	13.11	85.84 (98.32)
Tp_4	Sillod	47.66	46.81(53.14)
Tp_5	Ardhapur	29.12	67.91 (80.42)
Control		90.00	--
SEm ±			1.68
CD(p=0.05)			4.66

Radial growth and percent inhibition values are means of three replications.

Figures in parentheses are arcsine transformed values of % inhibition.

Fig.1: Influence of different isolates of *Trichoderma* species against *Macrophomina phaseolina.*



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Effect of aqueous and methanolic leaves extract of *Cassia fistula* L. against *Fusarium solani* causing rhizome rot of ginger

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Abstract

The *in vitro* aqueous and methanol leaves extract of *Cassia fistula* L. plant at different concentrations from 10 to 40% each was tested by following poisoned food technique. The different concentrations of leaves extract used were as 0.0 (control), 10, 20, 30 and 40%. The *Cassia fistula* L. aqueous leaves extract at 40% (41.66 mm) and methanolic leaves extract at 30% (17.66 mm) concentration was found to be most effective in reducing the mycelial growth of the *Fusarium solani*. Similarly the methanolic leaves extract at 30% and 40% concentration was found to be most effective in reducing the mycelial growth of the pathogen.

Keywords: *Cassia fistula* L.*, Fusarium solani,* rhizome rot, ginger

Introduction

Ginger (*Zingiber officinale* Rosc.) is an important commercial crop cultivated throughout India for its rhizome as spice and has high medicinal value. Among the major constraints for growing ginger is the rhizome rot. Even though important foliar diseases do exist, rhizome rot is very important in view of severe crop losses. It occurs in several parts of India wherever these crops are grown (Spices Board, 2005). The term rhizome rot is loosely used for all the diseases affecting the rhizome irrespective of pathogens involved, since the ultimate result is the partial or total loss of rhizome. Ginger is affected by several fungal pathogens during storage (Dohroo, 1993). Among which, rhizome rot caused by *Fusarium solani* is most common (Kumar, 1977). *Cassia fistula* L. is medium-sized tree up to 24 m in height as well as 1.8 m in girth and it is cultivated in almost all over India (Rajagopal et. al., 2013). *C. fistula* is a deciduous tree with greenish gray bark, leaves are compound, leaflets are each 5-2 cm long pairs (Danish et. al., 2011) and it contains around three to eight pairs of opposite leaflets. It produced fowlers in golden yellow color and shows showering bunch of up to 40 cm long earning its popular names golden shower tree (Neelam et. al., 2011).

Materials and Methods

The *in vitro* aqueous and methanol leaves extract of *Cassia fistula* L. plant at different concentrations from 10 to 40% each was tested by following poisoned food technique as given by (Mishra and Tiwari, 1992). Fresh and healthy leaves of *Cassia fistula* L. were collected locally and the leaves were washed under tap water followed by sterilized water, shade-dried and pulverized to obtain dry powder. The fine powder, and the precisely weighed amount of the powder was extracted with aqueous and 80% methanol solvents and was vacuum dried to obtain the dried aqueous and methanol extracts. One liter of 80% methanol extraction solvent was mixed with 200 g of powdered plant material. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. This mixture was filtered through muslin cloth. Further extraction of the residue was repeated 3 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible.

The extracted liquid was subjected to water bath evaporation at 400 C to remove the solvent. The same procedure was used for the aqueous extract. The semi-solid extract produced was kept

under a ceiling fan to dry. The extract was weighed and portion of it used for phytochemical screening (Thakare, 2004). To study the efficacy of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1973). The required amount of stock solution was mixed with sterilized molten PDA medium, respectively so as to get 10, 20, 30, and 40 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. 20 ml of medium was poured into 90 mm sterilized Petri plates and all plates were inoculated with actively growing 5 mm mycelial disc in the centre of media and incubated at room temperature for 7 days. Control was maintained without adding any plant extract to the medium. Three replications were maintained for each.

Results & Discussion

The aqueous and methanolic leaves extract of *Cassia fistula* L. plant was used to study its effect on growth of *Fusarium solani* causing rhizome rot of ginger. The different conc. of leaves extract used were as 0.0 (control), 10, 20, 30 and 40 %.

The *Cassia fistula* L. aqueous leaves extract at 10 % shows 81.66mm growth, at 20% shows 59.00 mm growth, at 30% shows 55.00 mm growth, and at 40% shows 41.66 mm growth on 7th day of incubation period. 40 % concentration was found to be most effective in reducing the mycelial growth of the pathogen.

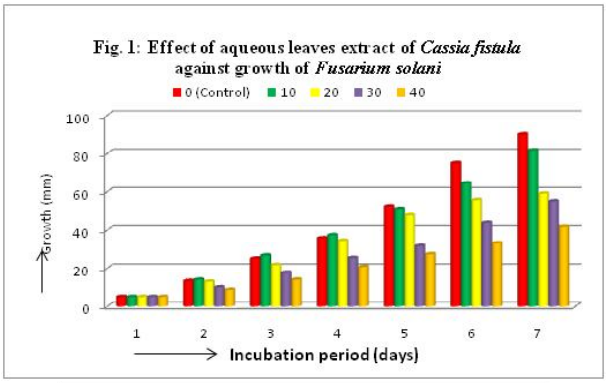
Similarly the methanolic leaves extract at 10 % shows 64.00 mm growth, at 20% shows 37.00 mm growth, at 30% shows 17.66 mm growth, and at 40% shows 5 mm growth on 7th day of incubation period. 40 % concentration was found to be most effective in reducing the mycelial growth of the pathogen. The observations indicated that, aqueous and methanolic leaves extract of *Cassia fistula* L. reduces the growth over control. The above data was shown in Table 1, Fig. 1& 2.

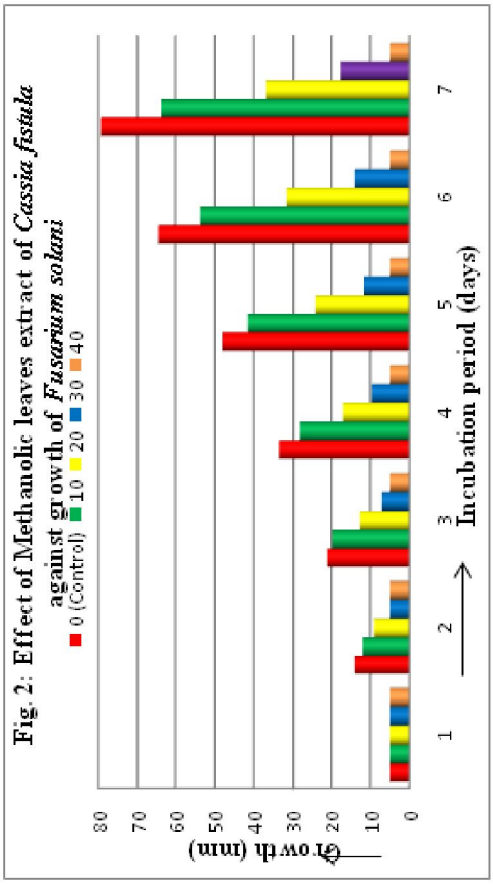
In the present study, among the aqueous and methanol solvent extracts tested, only methanol extracts of *Cassia fistula* L. was found to be more effective to inhibit fungal growth than aqueous extract, which may be due to the wide range of solubility of various polar compounds present within plant in methanol. Several other studies also showed that methanol extract of various plant sample are rich in antimicrobial agents (Babu et.al; 2010 and Nam et. al; 2008) which is having some degree of similarity with our study.

Table 1:

Effect of *Cassia fistula L.* leaves extract against growth of *Fusarium solani*

Incubation period (Days)	Growth (mm)						Methanol				
	Conc. of plant extract (%)										
	Aqueous										
	0 (Control)	10	20	30	40		0 (Control)	10	20	30	40
1	5.00	5.00	5.00	5.00	5.00		5.00	5.00	5.00	5.00	5.00
2	13.33	14.00	13.00	10.00	8.66		14.00	12.00	9.00	5.00	5.00
3	25.00	26.66	21.66	17.66	14.00		21.33	19.66	12.66	7.00	5.00
4	35.66	37.33	34.33	25.33	20.66		33.66	28.33	17.00	9.66	5.00
5	52.33	51.00	47.66	31.66	27.33		48.00	41.66	24.33	11.66	5.00
6	75.00	64.33	55.66	43.66	33.00		64.66	53.66	31.66	14.00	5.00
7	90.00	81.66	59.00	55.00	41.66		79.33	64.00	37.00	17.66	5.00
SE ±	1.257	1.382	1.363	1.355	1.037		1.942	1.227	1.142	1.012	0
CD @ 5%	3.869	4.227	4.198	4.192	3.192		3.913	3.861	3.513	3.076	0





Conclusion

The present investigation showed that the active bioactive compounds from *Cassia fistula* L. can inhibit the growth of *Fusarium solani* for the control of rhizome disease of ginger. It is economical and easily available and could be used as a biocontrol agent control of rhizome rot disease of ginger. It is suggested the farmers can use plant extracts along with minimum fungicides to increase yield of rhizome plants and reduce the environmental concerns regarding negative impact of fungicides.

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Fluoride content of Man Reservoir, Shirla Nemane, District. Buldana, (MH), India.

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Abstract:

The study was carried out monthly over a period of one year from Jan – 2016 to Dec. 2016 to examine the variations of fluoride content, pH and temperature of Man reservoir water at ShirlaNemane for analyzing the suitability of water for drinking, irrigation, industrial and aquatic biota purposes.

An average fluorideconcentration of water is 0.25 mgll having range of 0.19 mgll in summer to 0.54 mgll in winter, while in Dec. 2016 was 0.20 mgll having range of 0.18 mgll in summer the prescribed standards for drinking.The pH was 7.5 in the range of 7.6 to 8.4 during 2016 is within the prescribed lineits. Maximum pH was in January, while minimum in August.Monthly temperature variations of water was in the range of 24.2°cto 34°c.

Keywords: Fluoride, pH, Temperature, Man Reservoir.

Introduction:

Water is the most abundant ant most useful compound in the world and hence it is called "Jeevan" in Sanskrit. It is one of the most difficult substances to obtain in pure state. It has the ability to dissolve different materials which include physical, chemical, biological and radiological impurities. It is an essential component for survival of life on the earth, which contain minerals important for humans as well as for earth and aquatic life. Fluoride is one of the most important parameters in water quality management. Fluoride is an element of high biological activity. Fluoride should have in the proper amount in plants, humans and animals. In adequate intake of fluoride causes various physiological disorders in humans stated by Choubisa*etal.,*(2001); Valithan. (2001); sargaonkar and Deshpande (2002); and fluorosis in various Livestock by Sahoo*et al.,* (2003); Water, soil, air and most foods are the sources of fluoridestated by Gupta *et al.,* (2002). Hence, it is important to monitor the fluoride status, pH and temperature of major sources of water in Buldana and to analyse the quality of water for drinking, irrigation, industries and aquatic biota. Thus, this study reports the fluoride con castration of Man Project, Buldana district.

Materials And Methods :

Man Dam is an earth fill dam on Munriver near Khamgaon in the Buldana district in the state of Maharashtra in India. The height of the dam above lowest foundation is 30.2 m (99 ft), while length is 1.466 m (4.810 fit) and the gross storage capacity of Man Dam is 42.480.00. It is Major reservoir supplying the water for agriculture irrigation, drinking and domestric use, industries and aquatic biota in Buldana District. Every month during Jan, 2016 to Dec, 2016, one liter of water samples were collected from Man project reservoir at ShirlaNemane in clean plastic bottle with cork. The bottle were sealed, labeled and bought to the laboratory before noon of the same day and analyzed for fluoride by digital fluoride meter. The pH and temperature of water samples were measured at the same spot, time and period by using standard methods of APHA (1998).

Observation :

Mean fluoride concentration (mgll), pH and temperature of water for every month from Jan-2016 to Dec, 2016 is given in table No.1.

Sr. No.	Months	Fluoride (mgll)		pH		Temperatures	
		2015	2016	2015	2016	2015	2016
1	Jan.	0.18	0.16	8.4	8.1	22.2	23.0
2	Feb.	0.17	0.17	7.6	7.7	25.4	26.0
3	March	0.19	0.18	7.6	7.6	29.2	29.0
4	April	0.28	0.22	7.5	7.6	34.0	31.6
5	May	0.34	0.28	7.6	7.5	31.8	31.9
6	June	0.31	0.45	7.7	7.6	31.4	31.0
7	July	0.28	0.29	7.9	7.4	30.1	29.2
8	August	0.26	0.27	7.3	7.2	28.2	25.6
9	Sept.	0.28	0.21	7.5	7.6	26.1	27.6
10	Oct.	0.29	0.28	7.3	7.4	24.6	24.2
11	Nov.	0.27	0.20	7.7	7.8	24.2	22.2
12	Des.	0.19	0.21	7.9	7.8	32.0	20.2
Average		0.25	0.25	7.5	7.6	29.9	26.7

Result And Discussion:

The mean fluoride was in the range of 0.16 to 0.45 mgll having average 0.25 mgll from Jan, 2016 to Dec, 2016 and similar values of fluoride content of various rivers and lakes in India were reported by Lakshmanan*et al.,* (1986); Dasgupta*et al.,* (2001); Madhavan and Subramanian (2001); Shingh (2002).Maximum valus of fluoride concentraction of water were recorded in the months of summer, while minimum in the months of winter earlier report of Sreeniwasrao*et al*., (2001).The observed concentration of fluoride limits set by WHO. Thus, monitoring of water samples for fluoride is very important. Monthly recorded pH was in the rang of 7.6 to 8.4 and is within (WHO and ISI). Same range of pH for various reservoirs in India were reported by Sakhare and Joshi (2002); Shaikh and Yeragi (2004) and Pawar and Pulle (2005).The lowest pH values were recorded in monsoon months because of probably mixing of rainwater, while highest values in winter month may be due to the consumption of $CO_2$by the algal population during the process of photosynthesis studied by Jameel (1998). Monthly temperature of water was recorded in the range of 20.2°c in Dec. Dec, 2016 to 34°c in April 2016 having difference 12.2°c during the study period and was supported with the earlier reports of Walecha et al 1993 and Pawar and Pulle 2005 for various reservoirs. This study concludes the water of Man reservoir is suitable for drinking and industries as per the fluoride content pH and temperature.

Table: 1) Monthly values of fluoride concentration and pH and temperature at Man reservoir during 2015-2016.

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Bacteriological analysis of public place drinking water from Buldana district (M.S.)

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Abstract:

On the basic of the result differences in quality and quantity of the microbiological parameters between the different places of collection of water sample.Indeed,salmonella typhi was more frequently detected in bus stand water sample also Escherichia coli and staphylococcus aureus were detected in higher number especially in the water from dispensers.The contamination of the water dispenser may be derived from the poor sanitation low level of hygiene the regular refilling of the bottles and uncontrolled parameters.Therefore,a periodic adequate disinfection of water dispensers had to be indicated in order to keep the level of microbiological contamination under control.To avoid problems with cross-contamination of machines or devices,it is extremely important to ensure that the staff responsible for the cleaning and sanitizing of water dispensers are correctly trained and are awere of the potential for contamination during the cleaning process.

Keyword- Public places, drinking water, Bacteriological study.

Introduction

Water is the most important natural resource in the world,since life cannot exist without water. The health and well being of a population is directly

affected by the coverage of water supply and sanitation.the impact of poor environmental condition on the transmission of communicable disease is well established(Mengesha et al.,2004). Water is the elixier of life, a precious gift of nature to mankind and millions of other species living on the earth.it is fast becoming a scare commodity in most part of the world. Water resources comprising of surface water,ground water,marine and coastal water support all living thing including human being through water is available in the universe in huge quality in the order of 1400×106 km,only 3% of the water in the universe are fresh water. Among the fresh water, only about 5% of them or 0.15% of the total world waters are readily available for beneficial use.the total water resource available in india is 1850 km.which is roughly 4% of the world fresh water resources(EPA-PWD,2001). Drinking water quality has always been major issue in many countries, especially in developing countries(assembly of life sciences,1977).the world health organization in its guidelines for drinking water quality publication highlighted at least seventeen different and major genuses of bacteria that may be found in tap water which are capable of seriously affecting human health(WHO,2006).The proportion of waterborne disease outbreaks association with the distribution system failures has been increasing over the years(Moe & Rheingans etal.,2006). About 1.3 billion people still lack safe drinking water and more than 6 million children die from diarrhea in developing countries every year (Lithierland et al.,1995).only 61% of people in developing countries are estimated to have access to a potable water supply,greater in urban than rural areas and 36% to sanitation greater in urban than in rural areas(WHO,1998) The safety of drinking water can be monitored in a number of ways because the constituents of drinking water quality is to determine wheater the water supply system is being operated correctly,implying that the water is safe for drinking or not.indicator micro-organisms survive better and longer than the pathogen with a uniform and stable properties and may easily be detected by standard laboratory techniques.the present study was designed to detect the coliform and assess the quality of drinking water and also the quality of water supplied by mobile vendors,protected walls and municipality water(FAO,1997). The evaluation of potable water supplies for coliform bacteria is important in determining the sanitary quality of drinking water. High levels of coliform counts indicate contaminated source,inadequate treatment or post-treatment deficiencies.Many developing regions suffer from either chronic shortage of freshwaters or the readily accessible water resources are heavily polluted (Lehloesa and Muyima et al.,2000).

Materials and Methods:

Study site: study site is different location of public places of buldana.

Sample collection:

Take total 5 sample of drinking water collected from state bank of india,bus stand,government hospital.jaysthamb chaupati and court office from buldana.the source of the drinking water was tap water from the water supplies were carried out using the procedure given by APHA.water collected using sterile sample bottles.the samples were labeled immediately after collection and were transported to the laboratory for analysis.

Table no.1s: collection of water samples for bacteriological analysis.

Sr. no.	Sample collected	Sample collection place	Total samples
1	BSW	Bus stand of buldana	4
2	SBW	State bank of india	4
3	GHW	Government hospital	4
4	COW	Court office of buldana	4
5	JCW	Jaysthumb chaupati	4

Bacteriological analysis of water samples:

The bacteriological analysis of water samples are analyzed for total coliform this was carried out using the most probable number technique as described by American public health association.a combination of positive and negative tubes and the MPN index each of water sample were determined using most probable number standard table.

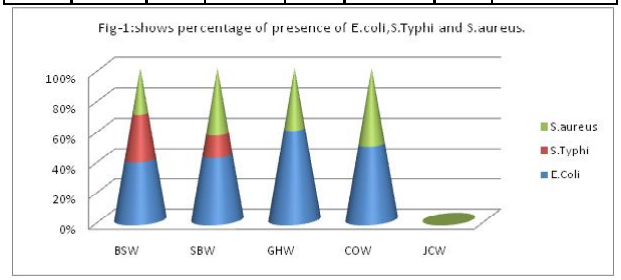
Result and Discussion:

In present study,a total 20 water samples from buldana were analyzed for water quality.Isolation and detection of coliform from all water sample were analyzed by MTFT.After isolation on selective media well grow colony are again subculture on nutrient agar for observing their morphological characteristics.After isolation of Escherichia coli,salmonella typhi and staphylococcus aureus they further used for morphological and biochemical characterization.After all test Escherichia coli,Salmonella typhi and Staphylococcus aures found.The isolates screeed were identified by using the cultural, morphological and biochemical characteristics.So identification of screened isolated was confirmed as Escherichia coli,Salmonella typhi and Staphilococcus aureus. Table 2 shows that in bus stand water sample Escherichia coli present 100%,Salmonella typhi present 75% and Staphylococcus aureus present 75% state bank water sample Escherichia coli present 75%,salmonella typhi present 25% and staphylococcus aureus present 75%,in government hospital water sample Escherichia coli present 75%,staphylococcus aureus present 50% and salmonella typhi is absent in court office water sample Escherichia coli present 25%,staphylococcus aureus present 25% and salmonella typhi is absent and in jaysthamb chaupati water sample Escherichia coli,staphylococcus aureus and salmonella typhi are absent.from this table observe that the water sample from bus stand is more hygienic and jaysthamb chaupati water is safe for drinking purpose. The effect of drinking water contaminated water result in thousand of deaths every day,mostly in children under five year,in developing countries(who,2004).in addition diseases caused through 264 consumption of contaminated water and poor hygiene practices are the leading cause of death among children worldwide after respiratory diseases (WHO, 2003). Thus lack of safe drinking water supply basic sanitation and hygienic practices is associated with high morbidity and mortality from excreta related diseases.

Table 2 :

Percentage of presence of Escherichia coli, Salmonella typhi and Staphylococcus aures

Places	No. of samples	Escherichia coli.		Salmonella Typhi.		Staphylococcus aureus	
		+ve	%	+ve	%	+ve	%
BSW	4	4	100%	3	75%	3	75%
SBW	4	3	75%	1	25%	3	75%
GHW	4	3	75%	0	0%	2	50%
COW	4	1	25%	0	0%	1	25%
JCW	4	0	0%	0	0%	0	0%



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OCCURRENCE AND CONTROL OF SEEDBORNE PATHOGENIC FUNGI OF SOYABEAN *(Glycine max* (L.) Merril)

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Abstract

Seed-borne pathogens causes enormous losses to crops in the world as well as in India. The presence of pathogenic propagules in a seed lot is pivotal because infected seed may fail to germinate, causes infection to seedlings and growing plants. In psesent study fungi associated with seed of three cultivars comprising three samples of soybean were investigated and four species were isolated by two different methods i.e. Blotter method and agar plate method. Pathogenic fungi frequently isolated were *Macrophomina phaseolina, Fusarium, Apergillus niger* and *Aspergillus flavus.* 70% to 80% of fungi isolated was of *Fusarium* and hence control of this fungi was performed by using tulsi plant extracts in different concentration. It was observed that 20% concentration of extract shows 100% inhibition of fusarium.

Keywords: Soyabean, Blotter method, Agar plate method, Pathogenic fungi, plant extract

Introduction

Grain legumes, in particular soybean, are attacked by a wide range of diseases many of which are seed-borne. Sinclair*et al.,* (1977) reported that there were at least 66 fungi, 6 bacteria and 8 viruses found to be associated with soybean seeds. These seed-borne micro-organisms have adverse effects on soybean seeds. They can reduce seed germination or seedling emergence or cause blights, leaf spots and other diseases on mature plants.Seed borne fungi causes losses in terms of seed quality and quantity in all oil seed crops. These fungi also reduce the germination and storability of the oil seed. They are responsible for seed rot, seedling blight, root/shoot rot, foliar infection as well as pod blight diseases (Agrawal *et al*, 1972; Agrawal *et al*, 1974).Soybean the "golden bean" is one of the fore most important oil seed crop known for its excellent protein (42-45%), oil (22%) and starch content (21%). It is good source of vitamin - B complex, thiamine and riboflavin. Soybean protein is rich in valuable amino acids like lysine (5%) in which, most of the cereals are deficient. Low yield and productivity of soybean in India is mainly due to various diseases and pests occurring in the field and causing yield losses. Seed health testing methods like blotter paper method, deep freeze blotter, 2, 4 - D blotter paper method and agar plate methods have been employed for detection of internal and external seed borne mycoflora of soybean (Solanke RB *et al.,* 1997; Paul YS, 1989; Rajeswari B *et al.,* 2009). The main objective of this study was to isolate and control seed borne fungi of soybean.

Materials and Methods

Collection of soybean seed samples

Samples were collected from three different industries i.e. Mahamandal seeds, Nirmal seeds and Ankur seeds. These three different samples were collected and stored in laboratory for further studies.

Isolation of Fungi

Two generalized isolation procedures were employed for the isolation of pathogenic fungi (Neergaard, 1977; Wan Zamun*et al.*, 1978). The two methods were the moist blotter and the potato dextrose agar (PDA) method.

Standard blotter method

The standard blotter method was developed by Doyer in 1938, which was later included in the International Seed TestingAssociation Rules of1966. Four hundred seed of each variety weretested by employing standard blotter method in 3 replications. Threepieces of blotting paper of 90 mm size were moistened with distilledwater and placed in 90 mm sterilized Petri plates after drainingexcess water. Untreated seeds were placed at the rate of 25 seedsper Petri plate at equal distance. The plates were incubated at roomtemperature (20± 2º C) under alternate cycles of 12 hours NUV lightand darkness. After eight day of incubation the seeds wereexamined under stereoscopic –binocular microscope for theassociated fungi and they were identified based on "habit and colonycharacters (Anonymous,1996)

Agar plate method.

In this method, pre sterilized petri plates were pouredwith 15 ml of autoclave potato dextrose agar (PDA). On cooling themedium, the seeds per plate of the sample to be studied wereequidistantly placed aseptically. The plates were incubated at roomtemperature (20± 2º C) under alternate cycles of 12 hours NUV lightand darkness. After eight day of incubation the seeds wereexamined under stereoscopic –binocular microscope for theassociated fungi and they were identified based on "habit and colonycharacters

Identification of fungi

Pure cultures of individual fungal isolates were critically examined and identified. Fungi were identified were based on gross colony morphology and microscopic characters. Colony identification was based on colony characteristics such as color and the texture of mycelia and type of pigmentation. Microscopic characteristics of spores such as shape and color also used to identify the pathogens associated with the seeds.

Efficacy of selected tulsi plant extract against*Fusarium.*

Preparation of plant extract

Tulsi plant extract was prepared in acetone. Fresh leaves of tulsi were collected and washed with distilled water to remove surface dust. 10gm of leaves were chopped into fine pieces. These chopped leaves were added to 100ml of acetone and placed for overnight. Next day filtered and filtrate was used as plant extract and stored at 4^0c for further use.

Antifungal activityof plant extract

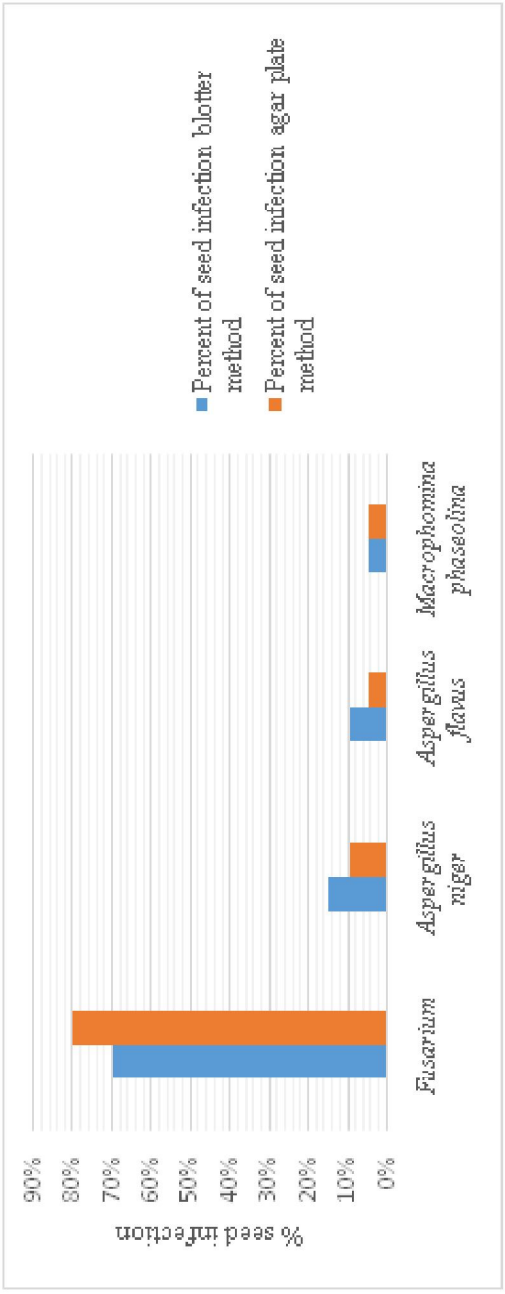
Antifungalactivity of leaf extract was tested by poisoned food technique.Control of *Fusarium* was performed using different concentration agar plate method. Concentration used were 5%, 10%, 15%, 20% of extract. To this plate Loopful of *Fusarium* was placed and growth was observed after 5 days. Diameter of *Fusarium*colony was measured and compared to the control. Using this data The percent inhibition of mycelial growth was calculated using the formula: %I = [(C-T)/C] x100).

I = percentage inhibition.
C = radial growth in control.
T = radial growth in the treatment.

Resultand Discussion

Significant differences in occurrence of seed mycoflora were observed and the results indicated that irrespective of the locations and sources, a total of 4 fungal species viz., *Macrophomina phaseolina, Fusarium sp., Aspergillus Niger*and *Aspergillus flavus* belonging towere detected. Total per cent incidence of seed mycoflora in Blotter method is 5%, 10%, 15%, 70% of*Macrophomina phaseolina*, *Aspergillus flavus*, *Aspergillus Niger, and Fusarium sp.* respectively.Total per cent incidence of seed mycoflora in agar plate method is 5%, 5%, 10%, 80% of Macrophomina*phaseolina*, *Aspergillus flavus*, *Aspergillus Niger, and Fusarium sp.*respectively. (Graph.1)

Graph 1. Percent of seed infection by blotter method and agar plate method



Efficacy of selected tulsi plant extract against *Fusarium.*

Tulsi plant acetone extract was used for control of the *Fusarium sp*. as this fungal genera observed high prevalence. Concentration used were 5%, 10%, 15%, 20% of Tulsi extract. The antifungal activity of the extract was enhanced by increase in the concentration of the extract. Extract concentration 20% was showing 100% inhibition of *Fusarium sp.* which was calculated by measuring mycelial growth after 5 days of incubation. (Table 2. Figure 1.)

Table 2. Percent inhibition of *Fusarium sp.*

Extract concentration	Fungal colony diameter after 5 days	% Inhibition
Control	50mm	--
5%	40mm	20
10%	25mm	50
15%	15mm	70
20%	00mm	100

Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. The detection of seed-borne pathogenic fungi and seeddiseases is an important aspect of disease management. Determining the presence of seed-borne pathogens allows managers to apply the appropriate controls or modify management practices to avoid the problem in the future. All the fungal isolates observed in this study were listed among the eight species isolated from ten soybean cultivars with agar (PDA) plate and blotter methods in India (Khayum *et al*., 2006) and the thirty-nine species isolated fromone cultivar of soybean using four methods of isolation in Pakistan (Nasreen, 2003). Both studies also found the agar plate method of isolation to perform better than the blotter method in isolating the fungi.

Conclusion

Out of total fungal species recorded, *Fusarium sp.*were found predominant in the samples. The seed can be used by treating with the 15-20% extract concentration to prevent seed infection with plant pathogens. The present investigation is an important step in developing plant based pesticides which are ecofriendly for the management of the seed borne fungi and development of commercial formulation of botanicals. Further investigation will be done for developing commercial formulation based on field trail and toxicological experiment.

Figure 1. Efficacy of selected tulsi plant extract against *Fusarium sp.*

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PRODUCTION, PURIFICATION AND CHARECTRIZATION OF BENZONITRILE PRODUCED BY *Aspergillus fumigates F11*

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Abstract

Dihalogenate benzonitrile are active compounds in a number of herbicides and poses to have a deleterious health effect. The biocatalyst was applied to the biotransformation of benzonitrile,3-cynopyridine,(R,S)-3-hydroxy -2- ethylene butanenitrile The enzyme involved in the degradation of benzonitrile by fungal identified as *Aspergillus fumigates F11*.a strain which utilized benzonitrile as sole source of carbon and nitrogen were partially purified by ammonium sulphate precipitation of (80%). The GC results revealed that benzonitrile in single step pathway was converted to ammonia with the formation of benzoic acid as an intermediate by enzyme benzonitrilase.In the present study nitrilase enzyme was produced and purified using *Aspergillus fumigatus*. The purified enzyme was showing maximum activity at a pH 4 (68.22 µmole/min) and maximum benzonitrile degradation with immobilized enzyme was observed at temperature 60^0c (110.51µmole/min).The metal source $MgSo_4$ (30.85 µmole/min) and enzyme stable up to 30 min (88.14 µmole/min) The Vmax is 0.188µg/ml and km is 0.00180µm.The molecular weight of benzonitrilase were found to be 97400 Dalton

Keywords: Biodegradation, benzonitrilase, *Aspergillus*, Purification.

Introduction

Nitrilase, which are generally highly toxic due their cyno functional group, can be used by some microorganisms as carbon and nitrogen sources. Nitrilase catalyzes the direct cleavages nitrilase to the corresponding acid and ammonia (kobayshi 1994), whereas nitrile hydratase catalyze the hydration of nitriles to amides (Asano1980, Kobayshi 1992). Both the enzymes are involved in biosynthesis of the plant hormone indol 3 –acetic acid in plants(Barteling 1992, Barteling 1994, Bartel 1994). Nitrilase are widely manufactured and extensively used by the chemical industry, and nitrile herbicides are also widely applied in agriculture.

Thiemann and Mahadevan demonstrated that nitrilase (EC 3,5,5,1) purified the hydrolysis of indolacetonitrile to indolacetic acid and ammonia ,several nitriles found and characterized.(Asano 1982) reported that the formation of nitrile hydretase and amides, and purification and enzymological properties of the former enzyme from Artrobacter Sp.J-1.Only two fungal nitrilase –from Fusarium solani and F.Oxysporum were purified and characterized (Gold lust and Bohak 1989).We improved the production of nitrilase in several species of filamentous fungi by using picolinitrile (Kaplan et al 2006). Layhe et al. (1992) isolated several bacterial strains with nitrilase activities from the environment. They were isolated from enrichment cultures using different arylacetonitriles such as 2-methyl- or 2-ethylbenzylcyanide as sole sources of nitrogen. One of these strains, *Pseudomonas fluorescens* EBC191, was able to use different arylacetonitriles (e.g. 2-phenylpropionitrile) as nitrogen sources and converted the nitriles to the corresponding -substituted carboxylic acids. It was demonstrated that strain EBC191 synthesized a nitrilase, which converted *O*-acetoxy mandelo nitrile preferentially to *R*-acetoxy mandelic acid (Layh et al.1992). The enzyme was subsequently partially purified, biochemically characterized, and the N-

terminal and some internal amino acid sequences were determined (Moser, 1996; Layh et al. 1998). This enzyme seems to possess some potential for the enantio selective production of carboxylic acids from racemic nitriles. In the present work, the nitrilase gene was identified in a genomic library of *P. fluorescens* EBC191, expressed in *Escherichia coli* and the recombinant protein biochemically characterized.

The enzymes involved are different from the nitrile –degrading nitrile hydretase and nitrilase,and organic nitriles cannot be degrade by these becteria. Benzonitrile was chosen as a substract,because it is the simplest organic nitrile ,widely used as solvent and an imported environmental polluntant.The results indicates the presence of a specialized group of previously unknown haloalkophilic bacteria capable of growing with acetonitrile as sole substract (Dimitry 2007)

Materials And Methods

Elective Enrichment and Isolation

One gm of soil sample was suspended in Basal salt medium containing (KH_2PO_4 1.5 gm; K_2HPO_4 3.5 gm; $Mgso_4.7H_2O$ 0.19 gm; Yeast extract 50 mg; Trace element ; P^H 7.5; Distilled water 1000 ml.) Benzonitrile 0.05% was added aseptically to sterilized and cooled medium. The suspension (100ml) in 250 ml Erlenmeyer flask was incubated at 30^0 C on rotary shaker. After 7 days 2ml of this culture was transferred to 100ml of fresh medium with little rise in benzonitrile concentration. The process was repeated for a total four transfers by step by step raising the concentration of benzonitrile (0.05 to 0.2%). After one month of acclimatization, the last enrichment culture flask was used to isolate microorganisms on basal salt agar containing 0.2% benzonitrile. The colony characterization of fungal cultures was carried out. The pure cultures were maintained on basal salt agar for further studies.

Optimization of cultural condition

All isolates were capable of growing on mineral medium containing benzonitrile as sole source of carbon and nitrogen. Out of these 30 isolates 11 fungal strains were screened based on maximum production of ammonia. Secondary screening was carried out based on benzonitrile biodegradation at various pH. Three strains were selected showing maximum biodegradation in terms of ammonia production at basic, acidic and alkaline pH. In These strains the enzyme activity was found to be maximum in cell supernatant as compair to cell lyzate.The strain F19 was used further for optimization of growth parameters. The intact cells of F19 was showing maximum benzonitrile biodegradation at pH 7and temperature 30^0c incubation for 72 hrs.The presence of malt extrat as a nitrogen source and maltose as carbon source were found to enhance the benzonitrile hydrolysis

Development of inoculam

Basal salt broth containing 0.2% benzonitrile was prepared and it is inoculated with selected bacterial strain S 15 This flask was incubated on rotary shaker at 100 rpm for 72 hrs. After 72 hrs cells were harvested by centrifuging the culture flask at 10,000 rpm for 10 min. washing of cell pallet was carried out using saline. These intact cells were suspended in saline and used further to study growth parameters.

Enzyme production

The crude enzyme was submerged fermentation .The Basal salt medium was prepared (pH 7) inoculated the culture of bacterial and incubated at 30^0c for 72 hrs. After incubation, centrifugation the sample at 10000 rpm for 10 min.The cell free supernatant was taken the phosphate buffer and sonicated and crude enzyme was prepared

Enzyme Assay

The benzonitrilase assay was performed using both cell supernatant as well as cell free extract The standard reaction mixture consisted of 50, µmol of potassium phosphate buffer (pH 8.0), 3 ,µmol of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml. The reaction was started by adding the substrate and was carried out at 30°Cfor various times. The activity was estimated in terms of ammonia production. Protein was determined by the method of Lowry. One enzyme

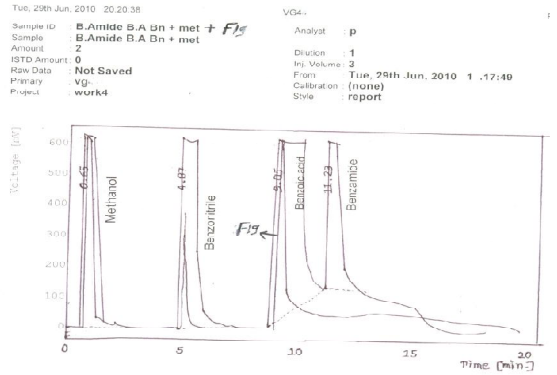
(Benzonitrilase) unit was defined as the amount of enzyme which catalyzed the formation of 1 micromole of ammonia per min.

GC analysis method

The isolated strains were cultured aerobically at 28^{0}c for the 3 days on the isolation medium. The cells were centrifuged, washed with physiological saline and suspended in 0.1M potassium phosphate buffer, pH 7.0.The reaction mixture for the screening of benzonitrile producing strains contained 100 µmole of potassium phosphate buffer, 300 µmole of benzonitrile as substract, washed cells from 3 ml of culture broth in a total volume of 1.0 ml. The reaction was carried out at 30^{0}c for 1 hr. with moderate shaking and terminated by addition of 0.2 ml of 1 N HCL.

The mixture was determined with a Chemito Gas chromatograph, Model GC -7610 equipped with flame ionized detector. The column used was stainless steel silicon 30, packed with porapack Q (80 to 100 mesh) operational conditions were: column temperature 200^{0}c; injection and detector temperature 151^{0}c and 201^{0}c.The carrier gas was N_2 at 40 cm^3/min.

Fig 5. Detection of metabolite of benzonitrile biotransformation by gas chromatography analysis for *Aspergillus fumigatus* F19



Peak No.	RT	Area (mv.S)	Height (mv)	W05 (min)	Area (%)	Height (%)
1	0.767	19830.8966	623.5239	0.4700	57.2997	47.5322
2	2.117	65.4515	10.4240	0.1067	0.18910	0.7946
3	5.070	2373.3678	189.1799	0.1367	6.8576	151.1076
4	9.110	12339.3700	479.6649	0.1667	35.6536	36.5656
-	Total	34609.0859	1311.7927	-	-	-

Purification of enzyme from bacteria

Step 1 –Ammonium sulphate precipitation

Ammonium sulphate was added to 90% saturation. The crude enzyme prepared was brought to 80% saturation with ammonium sulphate at pH of 6 and kept overnight in cold room. After equilibration, the supernatant was brought to 90% saturation with ammonium sulphate and centrifuged at 8000 rpm, at 4^{0}C for 10 min. Then precipitates were collected separately and dissolved in a 0.05 M phosphate buffer at pH 7 stored at 4^{0}c for the purification.

Step 2- Dialysis

The precipitation dissolved in 0.05M potassium phosphate buffer and dialysis .after, dialysis the samples were used for protein estimation and enzyme activity

SDS page analysis

Molecular weight and puriety determinations on the nitrilase were performed by electrophoresis on polyacrylamide gel in the presence of SDS by thin layer techanique that enabled up to 1samples to be analysed simultaneously on the same gel slab. This methods used was based on that described by (weber et al 1972) for disc gel electrophorasis. Thin layer gels of 0.2%(w/v) SDS dissolved in 100mM-sodium phosphate buffer, pH7.2 were suitable, and were polymerized by using ammonium persulphate as catalyst and NNN'N-tetra methylenendiamine s accelerator in the usual manner. Sample of standards proteins and of nutrias were prepared for application to the gel in 10mM-sodium potassium buffer, pH 7.0containing 1%(w/v)SDS and 1%(v/v) mercaptoethanol at 100^{0}c.pieces (1.5mm×6mm) of Whatmann 3MM chromatography paper were soaked in the sample solution dried to remove superficial moisture and placed vertically in slides along the length of the gel (25cm×12.5cm) on the side adjacent to the cathode. The slite was filled to the surface of the gel with 10mM-sodium phosphate buffer pH7.2containing 0.1% micropipette. Bromophenol blue was used as the tracking dye. The gels were subjected to transverse electrophoresis on the instrument, the reservoir buffer, pH 7.2

containing 0.1%(w/v) SDS and 0.1% micro-pipette. Bromophenol blue was used as the tracking dye. The gels were subjected to transverse electrophoresis. The reservoir buffer in the cathode compartments being 50mM-sodium phosphate buffer pH 7.2, containing 0.1% (w/v) SDS with voltage of 80-100V and a current of 40-50mA at a temperature of 17.5^{0}C,a running time of about 16 hours was required to complete electrophoresis, i.e. for the tracking dye approach the anodic side of the gel. The position of the dye was then marked with water proof black ink and the gel stained with coomassie brillent blue. The distance migrated by the stained protein bands and the dye was measured and the relative mobilities of the sample proteins with respect to the tracking dye were calculated From a plot of the weights of the polypeptide chains of standard proteins against their electophoretic mobility the molecular weight of the subunits of the isolated nutrias enzyme was determined from their relative mobility in addition to certain of the standard proteins used in proteins used in gel filtration experiment, the following proteins were also used for callibration

Characterization of purified nitrilase parameter

The partially purified enzyme was used for the characterization of nitrilase and optimization of its activity

Optimization of pH

The pH activity profile of the partially purified enzyme (pH range 3 to 10) was studied citrate buffer (pH range 3.0-4.0), Citrate phosphate buffer (pH range 5.0-6.0), Phosphate buffer (pH range 7.0-8.0), glacial sodium hydroxide (pH range 9.0-10.0). The standard reaction mixture consisted of 50, umol of potassium phosphate buffer (pH 8.0), 3 ìm of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml. The reaction was started by adding the substrate and was carried out at 30°C for 20 times, the activity was estimated in terms of ammonia production. Protein was determined by the method of Lowry.

Optimization of temperature

The activity of enzyme was measured at different temperature ranging from10^{0}c to 80^{0}C. The standard reaction mixture consisted of 50, ìm of potassium phosphate buffer (pH 8.0), 3 ìm of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml. The reaction was started by adding the substrate and was carried out at various temperatures for 20 min., the activity was estimated in terms of ammonia production. Protein was determined by the method of Lowry.

Optimization of metal ion concentration

The standard reaction mixture consisted of 50, umol of potassium phosphate buffer (pH 8.0), 3, umol of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml as supplementary as metal source like Cr^{2+}, Mg^{2+}, Fe^{2+},Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}. The reaction was started by adding the substrate and was carried out at various temperatures for 20 mintues; the activity was estimated in terms of ammonia production. Protein was determined by the method of Lowry.

Optimization of enzyme stability

The benzonitrilase assay was performed using both cell supernatant as well as cell free extract The standard reaction mixture consisted of 50, umol of potassium phosphate buffer (pH 8.0), 3 ,umol of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml. The reaction was started by adding the substrate and was carried out at 30°C for various times; like 10 min, 20 min. The activity was estimated in terms of ammonia production. Protein was determined by the method of Lowry.

Results And Discussion

By elective enrichment 30 different fungal cultures were isolated from soil capable of utilizing benzonitrile as a sole source of carbon and nitrogen (Heper 1977) show that cultures actively growing on benzonitrile as carbon and nitrogen source were tested for their ability to oxidized possible intermediate in the degradation of benzonitrile. The production of ammonia was estimated from culture filtrate of all thirty isolates. At primary level 11 isolates were screened out on the basis of maximum production of ammonia as one of the metabolite of degradation. To study the impact of pH on

benzonitrile degradation all 11 isolates were grown at three distinct pH. The results has shown that the strain F12, F18 and F19 were degrading benzonitrile at high rate in respective pH viz. 7, 9 and 4.Harper (1977) has shown that in *Arthobacter* sp.J-1 benzonitrile was directly hydrolyzed to benzoic acid and ammonia by nitrilase. In These strains the enzyme activity was found to be maximum in cell supernatant as compair to cell lyzate.The strain F19 was used further for optimization of growth parameters. The intact cells of F19 was showing maximum benzonitrile biodegradation at pH 4 and temperature 30°C incubation for 72 hrs.

In course of time benzonitrile was degreaded by accumulating benzoic acid and ammonia but benzamide was not detected throughout the cultivation of all three selected strains. Tentatively all three strains are showing the direct degredation of benzonitrile to benzoic acid and ammonia by enzyme nitrilase which was confirmed by Gas chromatography. (Asano 1982) reported that a new enzyme aliphatic nit rile hydretase catalysed the hydration of nitriles to amides in *Arthrobacter* sp. J -1. The expression of nitrilase was studied by observing enzyme activity in cell supernatant as well as in cell lysates. All these three strains the enzyme activity was more in cell supernatant as compare to cell lysates. Further the strain F19 was showing highest activity which was selected for study on purification and characterization of enzyme.

Fig 1.
Effect of pH on nitirlase activity

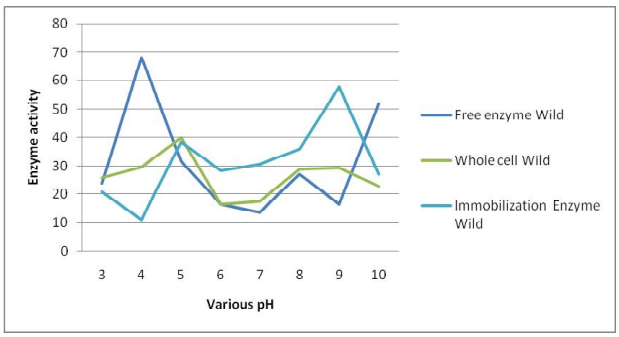


Fig 2.
Effect of temeprature on nitirlase activity

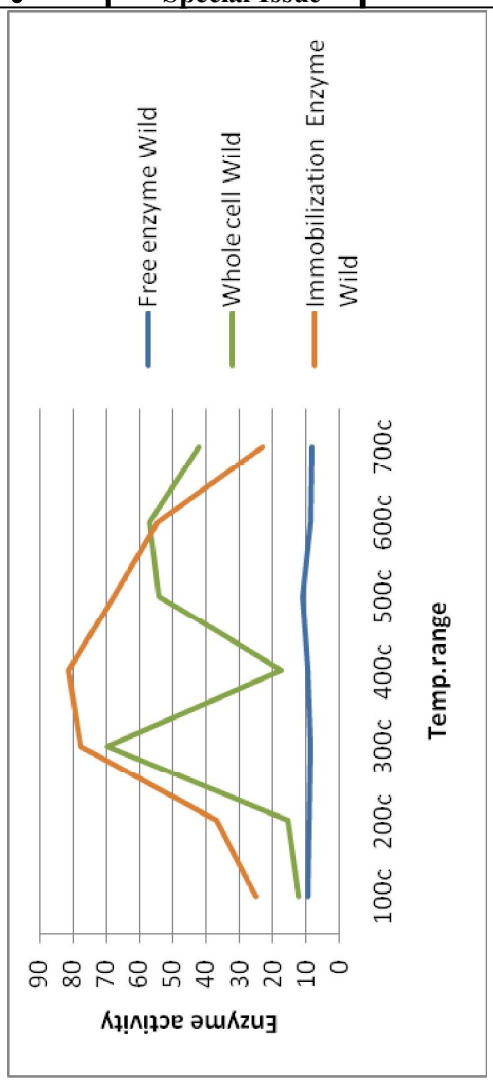
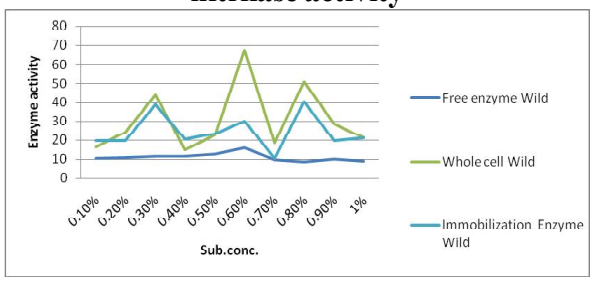


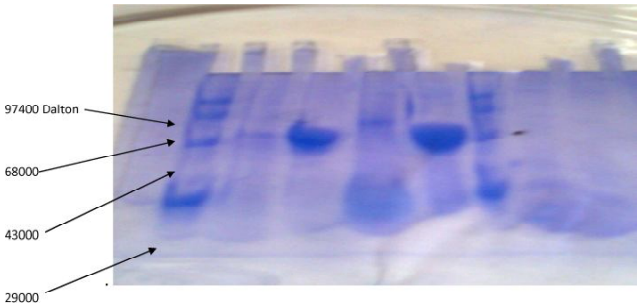
Fig 3. Effect of substrate concentration on nitrilase activity



The strain F19 has shown maximum partially Purified enzyme at 80% by ammonium sulphate method (Dias 2000) reported that different encapsulated matrices were tested for purified cells of *Candida guilliermondii* UFMG- Y65 used for acetonitrile degradation. Acetonitrile degradation by free cells and cells immobilized in Ba-alginate,k-

carrageenan. The partially purified enzyme was used for the characterization of nitrilase and optimization of its activity. The enzyme activity found at pH 4of phosphate buffer (68.22µm/min). Similarly the enzyme activity was maximum at mesophilic temperature 60°C (110.51µmole/min). The enzyme is found to be stable up 30 min (88.14 µm/min).The metal source $MgSo_4$ (30.85 µm/min) and the Vmax is 0.188 µg/ml and km is 0.00180

Photo no.1 SDS PAGE



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28

Microbial degradation of Reactive Red 195 by *Bacillus cereus* PCS 8 JN234858

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Abstract

Many dyes and pigments are hazardous and toxic for human as well as for aquatic life in the concentration at which they are being discharged to receiving water bodies. Synthetic dyes released into the environment in the form of effluents by textile, leather, food, paper and printing industries cause severe ecological damages. Dyes used in the textile industry are difficult to remove by conventional methods that are recalcitrant against light, oxidizing agents and biodegradation processes. Azo dyes are the main constituents of such pollution because of their wide applicability and usages, and therefore, these are present majorly in textile industrial effluents.

Reactive Red 195 was selected for decolourization and detoxification studies by *Bacillu cereus* PCS 8 JN234858. Decolourization studies were carried out under aerobic condition. Under optimized condition, decolourization of Reactive Red 195 by *Bacillus cereus* was found to be 97.943% in 24 hours. Degradation of the dye was confirmed by UV- Visible spectrophotometric studies.

Keywords: Azo dyes, Reactive Red 195, *Bacillus cereus*, decolourization, degradation

Introduction:

Synthetic dyes released into the environment in the form of effluents by textile, leather, food, paper and printing industries cause severe ecological damages. Wastewater resulting from dyeing and finishing processes has an adverse impact in terms of total organic carbon, biological oxygen demand and chemical oxygen demand. Azo dyes are the main constituents of such pollution because of their wide applicability and usages, and therefore, these are present majorly in textile industrial effluents. Moreover their toxicity and resistance to degradation offer great challenge for removal technologies. In many cases the products formed after the degradation of the parent azo dye molecule are more toxic. These products are mainly in aromatic amine form. Azo dyes have been shown to be mutagenic to the human hepatoma cell line where frame shift mutation was observed [Shah et al 2013].

Azo dyes are the largest and most versatile class of dyes but have such a structural properties owing to which they are not easily degradable under natural conditions (Gomare *et al.*, 2009).

Various physical and chemical methods are used for textile effluent treatment but these methods have some demerits like production of large amounts of sludge which require safe disposal and are very expensive. Hence the need for effective treatment method becomes very imperative. Biological process as an alternative method involves the use of microorganisms is cost effective, eco-friendly nature and produce less sludge are now being used as an alternative method [Adebajo et al, 2017].

Dyes, however, are more difficult to treat because of their synthetic origin and mainly complex aromatic molecular structure. Such structures are often constructed to resist fading on exposure to sweat, soap, water, light or oxidizing agents and this renders them more stable and less amenable to biodegradation [Banat *et al.,* 1996]. Still these dyes can be removed from environment by microbial methods of decolourization and degradation which are cost effective methods [Verma *et al.*, 2003; Asgher *et al.*, 2007].

Reactive Red 195 is one of the reactive azo dyes that are commercially used for textile dyeing that contains reactive group which is often a heterocyclic aromatic ring substituted with chloride or fluoride, e.g. dichlorotriazine. In this study, a

bacterial strain *Bacillus cereus* PC8 JN234858 capable of decolorizing azo dyes was isolated and, in addition, the effects of various parameters such as pH, temperature, dye concentration, effect of carbon and nitrogen sources on dye decolourization by the bacterial strain were investigated

Material And Methods:

Microorganism and culture conditions:

Microorganisms were isolated by acclimatization to Reactive Red 195, an azo dye in the basal nutrient medium, nutrient broth. Dye industry effluent contaminated soil, sewage, dung and dye waste were the sources of organisms. The most promising bacterial isolate was used for further dye degradation studies. The culture was identified as *Bacillus cereus* PC8 JN234858. Pure culture was maintained on the nutrient agar slants.

Dyestuff and chemicals: Reactive Red 195 (RR 195) was obtained from Spectrum dyes, Surat, India. All chemicals used were of the highest purity and of analytical grade. Nutrient broth dehydrated was purchased from Hi-Media, Mumbai, India. .

Identification of the culture: The isolate was identified preliminary by morphological, cultural, biochemical characters. 16sr-RNA sequencing of the isolated organism was done in GeneOmbio Technologies Pvt. Ltd., Pune, India.

Decolourization experiments: Ten percent inoculum of $O.D_{600}$ 1.0 of *Bacillus cereus* PC8 grown for 24 hrs at 37°C on nutrient agar was used throughout the study. The isolate was inoculated in nutrient broth to study the decolourization performance of the culture. The dye was filter sterilized by using 0.2 ìm cellulose acetate filter and added after sterilization of medium throughout the study. The dye (50mg/L) was added immediately and incubated at static condition at 37°C. The aliquot (3ml) of culture media was withdrawn at different time intervals and centrifuged at 6000g for 20 min. Decolourization was monitored by measuring the absorbance of the culture at ëmax of the dye at 542 nm and change in pH was also recorded. Growth of microorganism in the dye containing medium was determined by wet weight method [Dawkar et al 2009].

Decolourization at different dye concentration:

In order to examine the effect of initial dye concentration on the decolourization in static condition 50-250mg/L of RR 195 was added to the nutrient broth inoculated with 10% *Bacillus cereus* of O.D600 1.0 and incubated at 37°C under static condition. The % decolourization was measured after every hour up to 72 hours. All decolourization experiments were performed in three sets. Abiotic controls (without microorganism) were always included. The % decolourization and average decolourization rate was measured.

% Decolourisation =

Average decolourization rate =

where C = initial concentration of dye mg/L and % D = Dye decolourization % after time t [Sartale et al 2009].

Effect of pH on dye decolourization:

Sterile nutrient broth of different pH 3, 4, 5, 6, 7 and 8 was inoculated with 10% inoculum and incubated at 37°C under static condition. The dye concentration was 50mg/L. All decolourization experiments were performed in three sets. Abiotic controls (without microorganism) were always included. The % decolourization was measured [Sahasrabudhe et al 2012].

Effect of temperature on dye decolourization:

Sterile nutrient broth of pH 7.5 was inoculated with 10 % inoculum and filter sterilized dye at 50 mg/L was added after sterilization. The broth was incubated at 28°C, 37°C, 40°C, 45°C and 50°C. The experiment was carried out in triplicate. Abiotic controls (without microorganism) were always included. The % decolourization was measured

Effect of carbon and nitrogen sources:

To study the effect of carbon and nitrogen sources on decolourization of RR195, semi synthetic medium having following composition was used (g/L)- $(NH4)_2SO_4$ 0.28, NH_4Cl 0.23, KH_2PO_4 0.067, $MgSO_4.7\ H_2O$ 0.04, $CaCl_2.2H_2O$ 0.022, $FeCl_3.6H_2O$ 0.005, yeast extract 0.2, NaCl 0.15, $NaHCO_3$ 1.0 and 1 ml/ L of trace element solution containing (g/L) $ZnSO_4.7H_2O$ 0.01, $MnCl_2.4\ H_2O$

0.1, $CuSO_4.5H_2O$ 0.392, $COCl_2.6H_2O$ 0.248, $NaB_4O_7.7\ H_2O$ 0.177 and $NiCl_2\ .6\ H_2O$ 0.02 with different carbon and nitrogen sources (1% each) such as glucose, sucrose, lactose and starch, yeast extract, peptone, malt extract, meat extract and urea respectively[Saratal et.al 2009]. 50 mg/L of the dye concentration was used. Filter sterilized dye was added after sterilization of the medium and after inoculation of the isolate.

Analytical procedure:

The metabolites produced during the biodegradation of RR195 hrs i.e. after decolourization of the medium were extracted twice with equal volume of dichloromethane (DCM) [Moutaouakkil A. et al.2003] The DCM extracts were pooled and evaporated at 40°C in rotary evaporator and then transferred to test tube. The extracted residue was dissolved in methanol and the same sample was used for analysis. Uv-Visible analysis of the extracted product was done by recording changes in absorption spectrum of the decolourized medium [200-800nm].

Results And Discussion:

Isolation and identification of dye decolourizing bacteria: Isolation of bacteria from mixed sample was carried out by the enrichment technique using nutrient broth and RR 195 as source of carbon and nitrogen that has rapid decolourization capacity. Decolourization occurred only when carbon and nitrogen sources were available for growth. The isolate was Gram positive facultative anaerobic nonmotile rod. An identification of the culture was based on 16sr-RNA analysis done by geneOmbio Technologies, Pune.

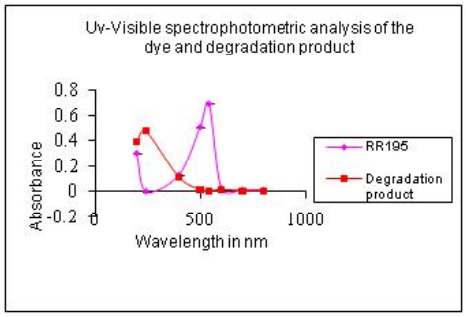
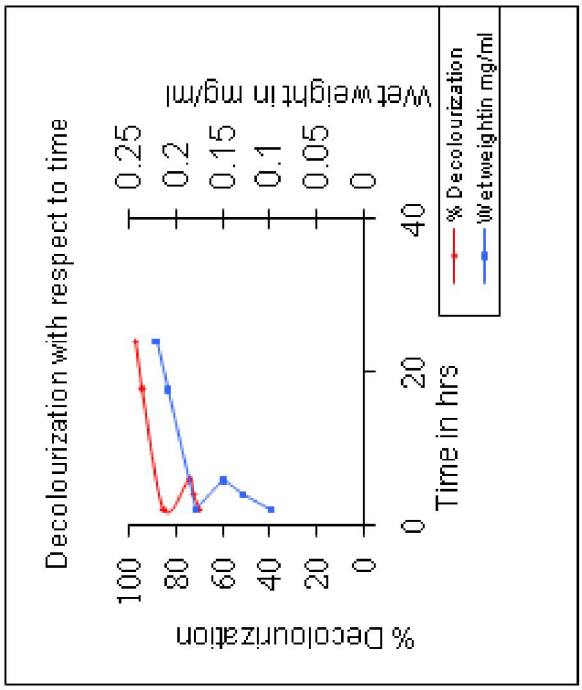


Fig 1 Uv-Visible spectrophotometric analysis of the dye and degradation products

The absorbance peaks in the visible region disappeared indicating complete decolourization. In the UV spectra the peak at 542 nm was replaced by new peak at 244 nm (Fig.1).

Fig 2 Decolourization with respect to time



Decolourization with respect to time showed within 24 hours 97.94 % decolourization along with simultaneous increase in wet weight of the cell. This indicated the ability of the isolate to grow in presence of the dye. (Fig.2)

There was no abiotic loss of RR195 within 24 hrs incubation indicating that the decolourization of RR195 was due to biological mechanism rather than adsorption. To confirm whether this decolourization was due to the bacterial action or variation in pH, change in pH was recorded in the range of 7.4±0.2.

Effect of physiochemical conditions on the decolourization performance:

The effect of various physiochemical conditions such as pH, temperature, dye concentration, effect of carbon and nitrogen sources on decolourization of RR 195 by the isolate were studied in detail. All parameters were studied at 37°C under static condition. 10% inoculum with O.D600 1.0 was used at a dye concentration 50 mg/L.

Effect of pH:

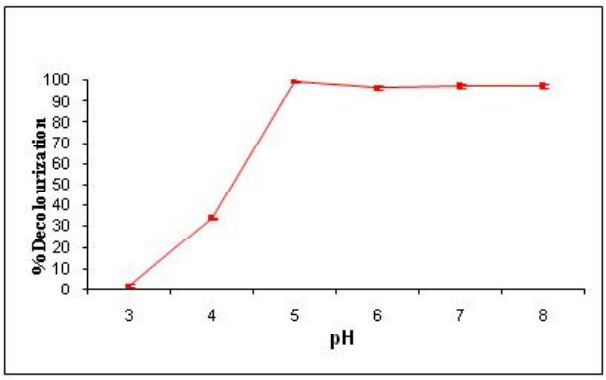


Fig 3 Effect of pH on decolourization

Bacterial cultures generally exhibit maximum decolourization at pH values near 7.0, our culture exhibited decolourization activity in the range of pH 5-8. At pH 3 and 4 decolourization observed was 2.16% and 33.68%% respectively. The isolate showed more or less constant decolourization from pH 5 to 8, maximum 88.41% at pH 7 (Fig. 3). *Enterococcus faecalis* grows very well in the pH range of 5-9. *E.coli* and *Pseudomonas luteola* both exhibited best decolourization at pH 7.0 with constant decolourization rate up to pH 9.5. *Klebsiella pneumoniae* RS-13 completely degraded methyl red in the pH range from 6.0 to 8.0.

Effect of Temperature:

Various microorganisms showed their survival at various temperatures ranging from 25°C to 50°C. The isolate showed decolourization at all selected temperatures but maximum decolourization was observed at 40°C (Fig. 4). This may be owing to a greater production of enzymes and optimal growth conditions of the isolate for its dye decolourizing ability.

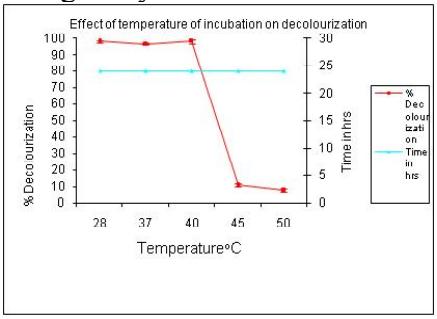


Fig 4 Effect of temperature on decolourization

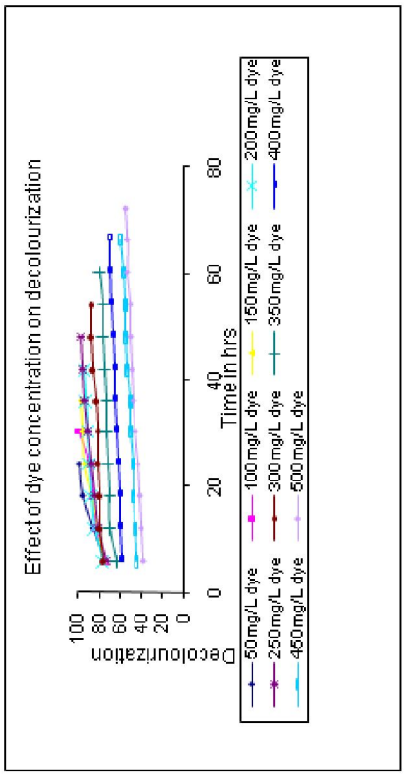
The decolourization at this optimum temperature may be owing to higher respiration and substrate metabolism. This also demonstrates that decolourization of the dye was through microbial reaction which relies on optimal temperature and not by adsorption. Hence pH and temperature optimum for RR 195 were found to be pH 7 and 40°C respectively.

Effect of initial dye concentration:

Actual concentrations of reactive dyes in dye house effluent have been reported to range from 60-250mg/L.28 Fig. 5 showed decolourizing ability of culture with increase in dye concentration from 50-250mg/L. It has been proposed that dye concentration can influence the efficiency of microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration. Thus, the isolate which could decolourize the dye at the reported dye concentration in wastewater, can be successfully employed for treatment of dye bearing industrial wastewater.

Fig 5

Effect of dye concentration on decolourization



Effect of carbon and Nitrogen sources:

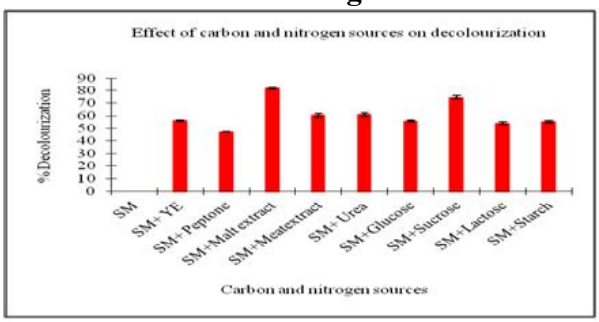


Fig 6 Effect of Carbon and nitrogen sources on decolourization

B.cereus showed 81.38% of decolourization of the dye in presence of malt extract while in presence of remaining C and N sources it showed moderate decolourization within 24 hours.

Analysis of metabolites resulting from decolourization and biodegradation of RR 195:

To understand the possible mechanism of the dye decolourization, we also analyzed the products of biotransformation of RR 195 by UV visible spectral analysis,

UV visible scan (400-800nm) of the culture supernatant withdrawn at different time intervals indicated the decolourization and decrease in dye concentration from batch culture. Peak obtained at 542 nm decreased at complete decolourization. The absorbance peak in the visible region disappeared indicating complete decolourization. In the UV spectra, the peak at 542 nm was replaced by new peak at 244 nm. TLC and HPLC analysis confirmed degradation of the dye (Data not shown). Thus such an isolate can be successfully employed for treatment of dye bearing industrial wastewater.

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Avian Diversity of Pandharkawaa Taluka, Maharashtra, India

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Abstract

The present study was made to estimate the avian fauna in terms of species richness and diversity and guild structure of Pandharkawada Taluka in Yavatmal District (M.S.), India. The present investigation was carried out to study avian diversity with aspect to ecological condition. The study was carried out near Pandharkawada,district Yavatmal situated far southern corner of Maharashtra State, adjoining the Adilabad district of Telangana pradesh. The study was undertaken during January-2015 to December 2016. A checklist of total 149 birds species belonging to 46 families was observed of which 122 were resident, 12 were local migrant and 15 were migrant .The favorable ecological conditions like availability of food, wetlands and roosting places were attracting the various birds.

Key words- Aves, Checklist, Pandharkawada.

Introduction:

India has a numerous diversity of plants and animals both domesticated and wild in variety of habitats and ecosystems. Food, water, space and cover are four essential components required of a habitat (U.S.Fish and Wildlife Service,2002).The diversity and richness of avian species in a community shows the diversity and richness of that habitat. Investigations on the bird communities of Western Ghats to plan for biodiversity friendly development are gaining significance (Pramod, 1995). Population studies have been traditionally used to monitor long term changes in avian to assess both habitat quality and the responses of birds to both natural and human caused environmental changes (Wiens,1989). Understanding the needs and requirement of different species leads us to make conservation strategy.

Study area:

Pandharkawada tahsil of Yavatmal district (Maharashtra) is located between 19.9594527°N 78.7261256°E. and is known as white diamond as there is maximum yield of cotton. The study was carried out for one year from January-2015 to December-2016 at different Villages and areas of Pandharkawada taluka.

Fig:-Map of Study area

Materials and Methods:

The bird survey was conducted according to a standard point count method. The data collected from the surveys can be used to estimate populations of birds, to identify the habitats that are used by specific species and, with repeated surveys over the years, to identify trends in bird population. I used a 25-m fixed-radius point count method to census the avifauna at each count station (Hutto et al. 1986). Surveys were conducted two days in a week, either from sunrise to 4 hours after sunrise or from 4 hours before sunset until sunset. Morning and evening counts was altered between sites. Each of the sites was survey daily depending on weather conditions. Birds seen were identified and recorded along the habitat type and status and checklist were prepared.

For each census, a map and aerial photographs was use to identify sites. So that camera Nikon was used of 45x zoom and 12.6 megapixel. A Tape recorder was also utilized during each survey to record the particular calls, which were later analyzed and used to identify the species.

For watching, counting and identifying birds, Binocular (10x50), telescope (25-40x), Notebook, Pen, pencil, Compass, Observation sheet, metal or wooden stakes, permanent Marker, Flagging Tape, Handheld GPS, Guide book of birds of Indian continents, Birds of south India etc were used.

Identification and Classification:

For identification and field diagnosis of birds, colored plates of Ali and Ripley (1974), Ali (1996), Grimmett et.al.,(1998), Grewal et.al.,(2002) were used. Classification of birds was made in accordance with Inskipp et. al., (1996).

Result and Discussion:

A total of 149 species of birds were observed and recorded during the course of study while sampling (Table-1). Out of them, Number of birds species recorded in each habitat were different as resident birds species were 122, local migratory were 12 , migratory were 15 species . It was observed that out of 151 species, 90 found were common, 31 were occasionally seen and 28 were uncommon. 44 Families represent the 149 species with corvidae comprising more birds species following Passeridae and accipitridae. Birds such as lapwings and larks were found using wetland habitat extensively for nesting in their breeding season (Narwade et.al.in press). The presence of birds in large quantity is seen by species like black drongo, parakeet, crested serpent eagle, sparrow hawk and dove. Green bee eater and spotted dove are two common species of these areas.

The record of 149 species of birds during the dry and wet seasons shows the diversity is very high. The occurrence of winter birds in the area indicates that the area is important for migratory birds. The rich and high vegetation might be providing heterogeneous and suitable site for nesting, roosting and foraging of bird. The most important threats to the birds are the spread of agriculture which is main reason behind degradation and conversion of bird population affecting globally of 86% of threatened bird species. Some birds have become locally extinct. The possible reason for this decline is the loss of habitat due to conversion to plantation.

Table 1: Order wise status and feeding guilds of birds in TWS.

Sr.No	Scientific Name	Common Name	Occurrence	Status
1.	**Order-Ciciniiormes Family:Podicipedidaea** Podiceps ruficolis	Little Grabe	C	R
2.	**Family:Phalacrocoracidae** Phalacrocorax niger	Little Cormorant	C	R
3.	Phalacrocorax carbo	Great Cormorant	O	LM
4.	**Family:Ardeida** Ardea purpurea	Purple Heron	O	R
5.	Ardeola grayii	Pond Heron	C	R
6.	Bubulcus ibis	Cattle Egret	C	R
7.	Ardea alba	Larger Egret	O	LM
8.	Egrettaa intermedia	Medium Egret	C	R
9.	Egretta garzetta	Little Egret	C	R
10.	**Family:Anhingidae** Anhinga melanogaster	Darter	O	R
11.	**Family:Ciconiidae** Anastomus oscitans	Asian Openbill Stork	O	LM
12.	Anastomus leucocephala	Painted Stork	O	LM
13.	Ciconia episcopus	Wooly-necked Stork	C	R
14	**Family:Phoenicopteridae** Pseudibis papillosa	Black Ibis	Un	R
	Family: Pteroclidae Pterocles indicus indicus	Painted Sandgrouse	Un	R
15.	**Family-Scolopacidae** Tringa ocropus	Wood Sandpiper	C	M
16.	Tringa hypoleucos	Common Sandpiper	C	M
17.	Tringa tetanus	Common Red Shank	O	M
18.	**Family:Jacanidae** Metopidius indicus	Bronzewinged Jacana	C	R
19.	**Family:Charadriidae** Himantopus himantopus	Blackwinged Stilt	O	LM
20.	Charadrius dubius	Little Ringed Plover	C	R
21.	Vanellus indicus	Yellow-watted Lapwing	C	R
22	Vanellus indicus	Red-Watted Lapwing	C	R
23.	**Family-Laridae** Larus brunicephalus	Brown-headed Gull	C	R
24.	Sterna aurantia	River Tern	C	RM
25.	**Family:Accipitridae** Elanus caeruleus	Black-Winged Kite	C	R
26.	Milvus migrans govinda	Pariah Kite	C	R
27.	Spilornis cheela	Crested serpant Eagle	C	R
28.	Circus macrorus	Pallied Harriar	Un	W
29.	Accipiter badius	Shikra	C	R
30.	Gyps bengalensis	White rumped Vulture	O	R
31.	Haliatur indus	Brahminy Kite	O	LM
32.	**Family-Glareolida** Cursorius coromandelicus	Indian Courser	O	LM
33.	**Family-Faconidae** Falco tinnunculus	Common Indian Kastrel	O	M
34.	**Order-Galliformes Family:Phasianidae** Francolinus pictus	Painted Patridge	O	R
35	Coturnix coromandelica	Rain Quail	C	R
36.	Perdicula asiatica	Jungle Bush Quail	C	R
37.	Pavo cristatus	CommonIndian Peafowl	C	R
38.	Francolinus pondicerianus	Grey Patridge	C	R
39.	Cotumix cotumix	Common Quail	O	R
40.	Turnix suscitator	Barred Button Quail	C	R
41.	**Order-Anseriformes Family:Anatidae** Dendrocygna javanica	Lesser Whistling Teal	C	R
42.	Anas acuta	Northern Pintail	C	W
43.	Anas poecilorhyncha	Spot-bill duck	C	R
44.	Aythya ferina	Common Pochard	O	W
45.	**Order-Piciformes Family:Picidae** Dinopium benghalense	Lesser Golden-backed Woodpecker	O	W
46.	Dendrocopos nanus	Brown-Capped Pigmy Woodpecker	O	R
47.	Dendrocopos mahrattensis	Yellow-fronted Pied-Woodpecker	C	R
48.	Chrysocolaptes festivus	White napped Woodpecker	O	R
49.	**Family:Megalaimidae** Megalaima haemacephala	Coppersmith Barbet	C	R
50.	Megalaima viridis	Small Green Barbet	C	R
51.	Megalaima zeylanica	Brown-headed Barbet	Un	R
52.	**Order-Bucerotiformes Family:Bucerotidae** Ocyceros birostris	Indian Grey Hornbill	C	R
53.	**Order-Coraciformes Family:Coraciidae** Coracias benghalensis	Indian Roller	C	R
54.	**Family-Alcedinidae** Alcedo atthis	Common Kingfisher	C	R
55.	Halcyon smymensis	White breasted Kingfisher	C	R
56.	Ceryle rudis	Lesser Pied Kingfisher	C	R
57.	**Family-Meropidae** Merops philippinus	Green Bee-eater	C	R
58.	**Order-Cuculiformes Family-Cuculida**			
59.	Hierococcyx varius	Common Hawk	C	R

60.	Cuculus micropterus	Indian Cuckoo	C	R
61.	eudynamy scolopacea	Asian Koel	C	R
62.	Phaenicophaeus leschenaultii	Sirkeer Malkoha (Cuckoo)	Un	R
63.	**Family-Centropodidae** Centropus sinensis	Crow-Pheasant	O	R
64.	**amily-Psittacidae** Psittacula krameri	Rose-ringed Parakeet	C	R
65.	Psittacula cyanocephala	Plum-headed Parakeet	C	R
66	Psittacula eupatria	Alexadrine Parakeet	O	R
67.	**Order-Apodiformes Family-Apodidae** Apus affinis	Little (House)Swift	C	R
68.	**Order-Strigiformes Family-Strigidae** Otus bakkamoena	Collared scops-Owl	Un	R
69.	Bubo bubo	Eurasian Eagle-Owl	O	R
70.	Athene brama	Spotted Owlet	C	R
71.	Heteroglaux blewitti	Forest Owlet	Un	R
72.	**Family-Caprimulgidae** Caprimulgus asiaticus	Indian LongTailed Nightjar	Un	R
73.	Caprimulgus indicus	Grey (Indian Jungle) Nightjar	O	R
74.	**Order-Columbiformes Family-Columbidae** Columba livia	Indian Rock Pigeon	C	R
75.	Treron phoenicoptera	Yellow footed Green pigeon	C	R
76.	Streptopelia decaocto	Eurasian Collard-Dove	C	R
77.	Streptopelia chinensis	Spotted Dove	C	R
78.	Streptopelia senegalensis	Laughing Dove	C	R
79	Streptopelia tranquebarica	Red Collared-Dove	C	R
80.	Streptopelia orientalis	Oriental Turtle Dove	C	R
81.	**Order-Gruiformes Family-Rallidae** Amanrornis phoenicurus	White breasted Waterhen	C	R
82.	**Order-Passeriformes Family-Pittidae** Pitta brachyura	Indian Pitta	Un	LM
83.	**Family-Laniidae** Lanius vittatus	Bay-backed Shrike	C	R
84.	Lanius schach	Long-tailed Shrike	C	R
4.	Lanius meridionalis	Southern Grey Shrike	O	R
85.	**Family-Corvidae** Corvus splendens	House Crow	C	R
86.	Corvus macrorhynchos	Jungle Crow	C	R
87.	Oriolus xanthomus	Black-hooded Oriole	O	R
88.	Oriolus oriolus	Eurasian Golden Oriole	C	R
89.	Pericrocotus cinnamomeus	Small Minivet	C	R
90.	Pericrocotus flammeus	Scarlet Minivet	O	R
91	Coracina Melanoptera	Black headed Cukoo-Shrike	Un	R
92.	Coracina macei	Large Cuckoo-Shrike	Un	R
93.	Dicrurus macrocercus	Black Drongo	C	R
94.	Rhipidura aureola	White-Browed Fantail	C	R
95.	Rhipidura albicolis	White-throated Fantail	Un	R
96.	Dendrocitta vagabunda	Indian (Rufous) Treepie	C	R
97.	Terpsiphone paradisi	Asian Paradise-Flycatcher	C	R
98.	Aegithina tiphia	Commo Lora	Un	R
99.	Tephrodornis pondicerianus	Common Woodshrike	C	R
100.	**Family-Muscicapidae** Turdus merula nigropileus	Eurasian Blackbird	C	R
101.	Cyornis tickelliae	Tickell'sBlue Flycatcher	Un	LM
101.	Eumyis thalassina	Verditer Flycatcher	O	M
102.	Luscinia svecica	Blue throat	C	M
103.	Copsychus saularis	Oriental MagpieRobin	C	R
104.	Saxicoloides fulicata	Indian Robin	C	R
115.	Phoenicurus ochruros	Black Redstart	Un	M
106.	Saxicola torquata	Common Stonechat	Un	M
107.	Saxicola caprata	Pied Bush Chat	C	R
108.	**Family-Sturnidae** Sturnus roseus	Rosy Starling	C	M
109.	Sturnus contra	Asian Pied Starling	C	R
110.	Sturnus pagodaarum	Brahminy Starling	C	R
111.	Acridotheres tristis	Common Myna	C	R
112.	Acridotheres fuscus	Jungle Myna	C	R
114.	**Family-Paridae** Parus major	Great Tit	C	R
115.	**Family-Hirundinidae** Hirundo rustica	Dusky Crag Martin	C	R
116.	Hirundo smithii	Wire tailed Swallow	C	R
117	Hirundo daurica	Red-rumped Swallow	C	R
118.	**Famiy-pycnonotidae** Pycnonotus cafer	Red-Vented Bulbul	C	R
119.	Pycnonotus luteolus	White-browed Bulbul	Un	R
120.	Pycnonotus jocosus	Red-whiskered Bulbul	Un	R
121.	**Family-Cisticolidae** Prinia socialis	Ashy Prinia	C	R
122.	Prinia inornata	Plain Prinia	C	R
123.	**Family-Sylviidae** Chrysomma sinense	Yellow-eyed Babbler	C	R
124	Turdoides caudatus	Common Babbler	C	R
125.	Turdoides malcolmi	Large Grey Babbler	C	R
126.	Turdoides striatus	Jungle Babbler	C	R
127.	Acrocephalus dumetorum	Blyth's Reed Warbler	C	R
128.	Orthotomus sutorius	Common Tailor bird	O	R
129.	**Family-Alaudidae** Mirafra erythroptera	Indian Bush Lark	C	R
132.	Eremopterix grisea	Ashy-crowned Sparrow Lark	C	R
133.	Galerida cristata	Crested Lark	O	R
134.	Nectarinia asiatica	Purple Sunbird	C	R
135.	Nectarinia zeylonica	Purple-rumped Sunbird	Un	R
136.	**Family-Passeridae** Motacilla alba	White Wagtail	C	M
137.	Motacilla maderaspatensis	Large pied Wagtail	C	R
138.	Motacilla caspica	Grey Wagtail	Un	M
139.	Anthus rufulus	Paddy Field Pipit	C	LM
139.	Anthus rufulus	Paddy Field Pipit	C	LM
140.	Passer domesticus	House Sparrow	C	R
141.	Petronia xanthocollis	Yellowthroated Sparrow	O	R
142.	Ploceus phillippinus	Baya Weaver	C	BM
143.	Lonchura malabarica	White throated Munia	C	R
144.	Amandava a0andaa	Red Munia	C	R
145.	Lonchura punctulata	Spotted Munia	C	R
146.	Lonchura striata	White backed Munia	C	R
147.	Anthus hodgsoni	Indian Tree pipit	C	R
148.	**Family-Fringillidae** Melophus latham	Creasted Bunting	Un	R
149.	**Order-Upepiformes Family-Upupidae** Upapa epops	Common Hoopoe	Un	R

Occurrence-C-Common, O-Occasional, Un-Uncommon.Status:-R-Resident, M-Migratory, W-winter Migrant, LM-Local Migrant, BR-Breeding Migrant.

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Comparative Study and Tree Construction of Different Organism Based on Histone Family

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Abstract

Phylogenetics is one of the branch to study evolutionary study of living organisms, the classification of organism based on basis of similarity.Bioinformatics plays a key role to modulate and enhance the study of sequence similarity and provides the detailed study of each and every sequence and their evolutionary study using the concepts of Phylogenetic tree analysis, the concept help researcher to understand the importance of divergence, time factor, and pedigree of sample sequences.

Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes Genes encoding histone variants are usually not clustered, have introns and their mRNAs are regulated with polyA tails. Five major families of histones exist:H1/H5,H2A,H2B,H3, andH4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1/H5 are known as the linker histones.

Keywords:- Phylogenetic, Alignment, Histone, PHYLOGRAM, CLADOGRAM

1. Introduction

Histone are highly alkaline proteins found in eukaryotic cell nuclei that package and order the Dna into structural units called nucleosomes.They are the chief protein components of chromatin, acting as spools around which DNA winds, and playing a role in gene regulation. Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to 1 in human DNA). Histones are subdivided into canonical replication-dependent histones that are expressed during the S-phase of cell cycle and replication-independent histone variants, expressed during the whole cell cycle. In animals, genes encoding canonical histones are typically clustered along the chromosome,lack introns and use a stem loop structure at the 3' end instead of a polyA tail. Genes encoding histone variants are usually not clustered, have introns and their mRNAs are regulated with polyA tails. Five major families of histones exist: H1/H5,H2A,H2B,H3, andH4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1/H5 are known as the linker histones.

Our aim to study the similarity between different organism based on the marker (Histone family) using evolutionary studies. Phylogenetic inference is the process of developing hypothesis about the evolutionary relatedness of organisms based on their observable characteristics..when linaneaus developed the system of classification into kingdom, phyla genera, and species, the early biologists sorted living things into a symbolic tree of life. this tree based representation of the relationships among species is a phylogenetic tree.

Evolutionary distance between pairs of

sequence, relative to other sequences in an input data set, is one way to assign branch length. While a phylogeny of species generally has as root, assuming that all species have a specific common ancestor, a phylogenetic tree derived from sequence data may be rooted or unrooted.

2. Programs

Different protocols and methods are available by which information of common ancestor can be drawn for that firstly target sequence is obtained in specific fasta file format (developed by W. Pearson) form biological database, obtained sequence here Histone families (H1/H5) these sequence has processed for sequence alignment, similar sequence has been shortlisted on the basis of Expectation Value (E-Value) (Table 1), then those shortlisted sequence has been performed by using **CLUSTALX2** drawn in the form of fasta for tree construction program using ClustalX (Provides output in .dnd, .phy),Treeview and Phylip. shown in figure 1 and 2.

Table represent all species in phylogenetic tree:

Organism name	Scientific name	Accession number
Human	Homo sapiens	AAC61625.1
Cow	Bos Taurus	NP_001030449.1
Dog	Canis lupus familiaris	NP_001274021.1
Donkey	Equus asinus	XP_014707060.1
Horse	Equus caballus	NP_001243880.1
Tobacco	Nicotiana tabacum	NP_001312320.1
Oats	Avena sativa	AAA32718.1
Rat	Rattus norvegicus	NP_072169.1
Wheat	Triticum aestivum	AAB00193.1
Pig	Sus scrofa	NP_001116594.1
Rice	Oryza sativa	AAA33907.1
Cabbage	Brassica oleracea var. capitata	BAF63143.1
Barley	Hordeum vulgare subsp. vulgare	AEK21393.1
Pea	Pisum sativum	BAM74171.1
Maize	Zea mays subsp. Mays	AAQ62067.1

3. Phylogenetic tree construction

Those obtained output from clustalX in .dnd and .aln files are used as a input in phylip for Phylogenetic Tree Construction (rooted and unrooted form) **fig.1, fig.2, fig.3** and **fig.4**. Those outputs show the position of obtained sequence in evolutionary tree.

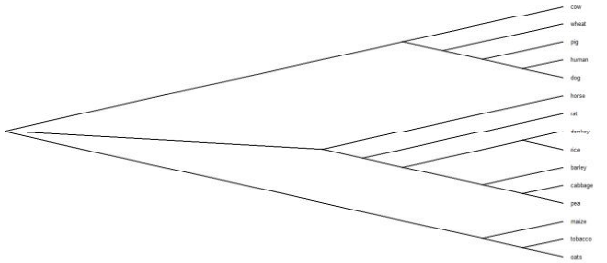

Figure 1 (slanted cladogram)

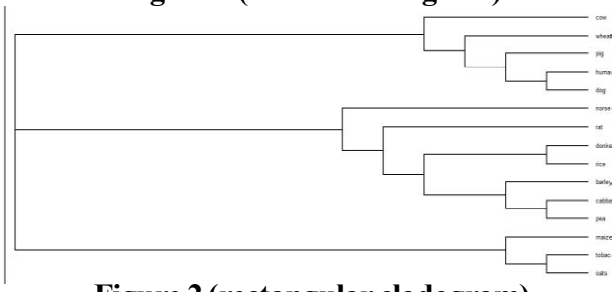

Figure 2 (rectangular cladogram)

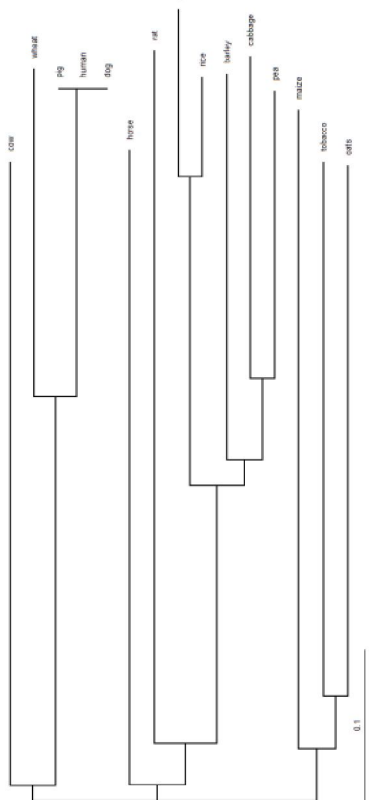

Figure 3 (phylogram)

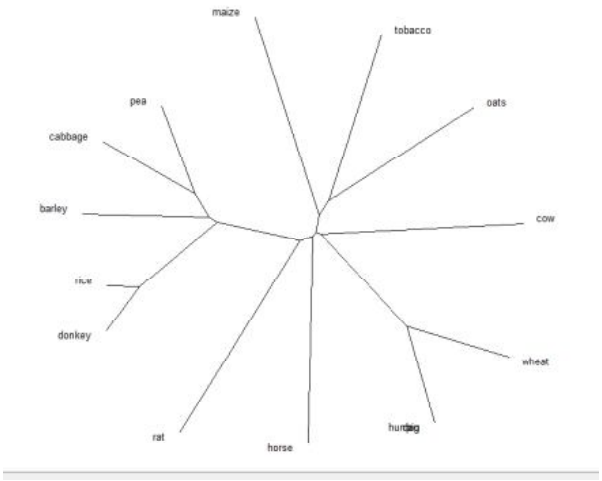

Figure 4 (unrooted)

4. Materials and Methods

Phylip:

PHYLIP (the PHYLogeny Inference Package) is a package of programs for inferring phylogenies (evolutionary trees). Methods that are available in the package include parsimony, Distance matrix, and likelihood methods, including bootstrapping and consensus trees. Data types that can be handled include molecular sequences, gene frequencies, restriction sites and fragments, distance matrices, and discrete characters. The data is read into the program from a text file, which the user can prepare using any word processor or text editor. Some sequence analysis programs such as the ClustalX2 alignment program can write data files in the PHYLIP format.

Clustalx2:

Clustalx2 is a new windows interface for clustalW multiple sequence alignment program. It provides an integrated environment for performing multiple sequence and profile alignment and analysing the results. The sequence alignment is displayed in a windows on the screen. The pull-down menus at the top of the window allow you to select all the option required for traditional multiple sequence and profile alignment.

TreeView:

TreeView is a program which is used to visualize a phylogenetic tree (**slanted cladogram, rectangular cladogram,phylogram, unrooted**), this is one of the application which is used to visualize a phylogenetic tree and able to understand different node, ancestor.

5. Conclusions

Phylogenetic trees contain a lot of information about the inferred evolutionary relationships between different organisms/species**. In fig.1, fig.2, fig.3** and **fig.4** the horizontal dimension gives the amount of genetic change. The horizontal lines are branches & are represents. Evolutionary lineages changing over time. The longer the branch in the horizontal dimension, longer the amount of change.

For our study Histone has been utilized as a genetic marker for similarity and various tree constructions using the applications like PHYLIP, TREEVIEW. clustal X2 gave alignment, distance matrix, to generate the phylogram, cladogram. samples of histone from various organisms extracted, based on they show the maximum similarity after tree construction. Four branches obtained after construction one shows large cluster of mammals (Human, pig, horse, Donkey, Dog, cow), small group belongs to other species (rice, wheat, barley, pea, maize)another group belongs some animals (rat), which concludes Histone (marker for this experiment) having similarity with different organism possess same fragment of sequence.

The molecular clock hypothesis states that Protein sequences evolve at a rate that is relatively constant over time and among different organisms.

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8. David Mount: Bioinformatics.

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Fish Biodiversity of the Katepurna River in Vidharbha, (M.S.) India

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Abstract:

The present study deals with fish biodiversity undertaken during period January-2015 to Decemeber-2017 to census and commercially important fishes in the Katepurna River. The present paper deals with the variety and abundance of fresh water fishes in the Katepurna River at Vidarbha (M.S) India. The results of present investigation reveal the occurrence of 124 fish species belonging to 10 orders, 20 families and 35 genera. Among the collected species, order Anabantiformes have 7%, Anguilliformes 3%, Beloniformes 2%, Cichliformes 2%, Cypriniformes 50%, Cyprinodontiformes 2%, Gobiiformes 2%, Osteoglossiformes 2%, Ovalentaria 2%, Siluriformes 21%, and Synbranchiformes 7% respectively.

Key Words: Fish biodiversity, Economic value , Nutritive Value, the Katepurna river

Introduction

Fishes are one of the important elements in the economy of many nations as they have been a stable item in the diet of many people. They constitute slightly more than one-half of total number of approximately 54,711 recognized living vertebrate species; there are descriptions of an estimated 27,977 valid species of fishes (Nelson 2006).

Biodiversity is essential for stabilization of ecosystem, protection of overall environmental quality for understanding intrinsic worth of all species on the earth (John Wiley and Sons 2011). Fish biodiversity of river essentially represents the fish faunal diversity and their abundance. River conserves a rich variety of fish species which support to the commercial fisheries. In India potential of fish culture is yet to be fully exploited. Fishes being rich source of proteins and have high nutritive value. Extensive development of aquaculture needs to be given priority after green revolution to feed ever growing population. Success of fish culture depends apart from other factors, on selection of suitable species. Secondly the country is rich in diversity of such important group of animals. Further, there is a need of a survey of diversity of fishes in different types of habitats of Rivers all over the country. The total length of rivers in India is about 29,000 km. All these rivers, their tributaries, canals and irrigation channels have an area of roughly 13,000 km. Reverine fisheries of India comprises of five major river systems.

- Ganga river system.
- Brahmaputra river system.
- Indus river system.
- East coast river system.
- West coast river system (Ehrlich, and Wilson, 1991)

The Katepurnariver, which flows through Akola and Washim district of Maharashtra. It rises from Ajintharenges near Kata village of Washim Tehsil. It is tributary of Purnariver, which flows

through central parts of Akla district. Its approximate length is 97km and its drainage basin has area of 1160sq.km. Further it flows through Mangrul, AkolaandMurtijapur Tehsils and merges with withPurnariver near Bhatori village. Coordinates: 20°46'50"N 77°19'12"E Present investigation was undertaken to study the fish biodiversity of Katepurna river District Akola (M.S) India. The objective of study was to give recent data regarding Fish diversity of the West coast river system, aiming to contribute a better knowledge of the fish diversity of Katepurna River and a tool for conservation planning of aquatic environments in this region. It is the first effort made in this direction, various indigenous, commercially important and economically valuable fishes were found in this area.

Materials And Methods

Fishes were collected from Katepurna River from the different sampling station with the help of local fishermen using different type of nets namely gill nets, cast nets, dragnets and Bhorjal. Immediately photographs were taken with help of digital camera. Fishes were brought to laboratory and preserved in 10% formalin solution in separate specimen jars according to the size of species. Small fishes were directly placed in the 10% formalin solution. While large fishes were given an incision in their abdomen and preserved.

The Meristic and morphometric characters collected fishes were measured and identified up to the species level, with the help of standard keys and books (Jayaram, K.C.et. Al 1999)(Pandey, K. and J.P. Shukla, 2007).

Table 1:

The fish diversity and Economic value of fish in Katepurnariver during January 2015-December 2017

+++ Most Abundant, ++ Abundant, + less Abundant, - Rare

1) LV- Larvivorous fish
2) PF- Predatory Food fish
3) MD- Medicinal Value
4) FD- Food fish
5) BT- Bait
6) WF- Weed fish
7) FR- Forage fish

Order	Family	Scientific Name	Common Name	Economic Value	Status
Anabantiformes	Channidae	Channamarulius	Spoted snake head	LF, PF	+++
		Channapunctata	Banded snake head	LF, PF	++
		Channagaucha	Dhok	LF, PF	++
	Osphronemidae	Colisalalia	Gourami	WF	_
Anguilliformes	Muraenesocidae	Muraenesoxbagio	Eill	WF	_
Beloniformes	Belonidae	Xenentodoncancila	Kola/Gar	WF	_
Cichliformes	Cichlidae	Oreochromismossambicus	Tilapiya	FD	++
Cypriniformes	Botiidae	Syncrossusberdmorei	botia	FD	++
	Cobitidae	Lepidocephalichthysguntea	Guntea Loach	LF,PF	+
	Cyprinidae	Amblypharyngodonmola	Mola carpet	LF<PF	+++
		Catlacatla	Catla	FD	++
		Cirrhinusmrigala	Mirgal	FD	++
		Cirrhinusreba	Bata/Aikhor	FD	+
		Cyprinuscarpio	Common carp	FD	++
		Devariomalabaricus	Danio	MD, FD	_
		Garramullya	Sucker fish	FD	+
		Labeobata	Bata	FD	++
		Labeoboggut	Kuria	FD	++
		Labeocalbasu	Calabus	FD	+
		Labeorohita	Rohu	FD	+++
		Puntiusticho	Kaoli	BT, LV, WF	++
		Puntiuschola	Barb	BT, LV, WF	+++
		Puntiusmahecola	Stigma barb	BT, LV, WF	++
		Puntiussophore	Pool	BT, LV, WF	++
		Rasboradaniconius	Rasbora	LV	+++
		Rohteeogilbii	Mirror	FD	+
		Salmophasiabacaila	Chelakani	LV	++
		Systomussarana	Sarputi	LV	++
	Nemacheilidae	Acanthocobitisbotia	Botia	FD	_
Cyprinodontiformes	Poeciliidae	Poeciliareticulata	Guppy	FD	+
Gobiiformes	Gobiidae	Glossogobiusguris	Goby	PF	_
Osteoglossiformes	Notopteridae	Notopterusnotopterus	Notopterus	PF, MD	+
Ovalentaria	Ambassidae	Chandanama	Chanda	FD	++
Siluriformes	Amblycipitidae	Amblycepsmangois	Mangois	WD	_
	Ariidae	Nematpteryxcaelata	Cat fish	FD,MD, PF	_
	Bagridae	Mystusbleekeri	Tengra	PF	++
		Mystuscavasius[	Shingta	PF	+
		Sperataseenghala	Giant catfish	PF,MD, FD	_
	Clariidae	Clariasgariepinus	Mangur	LV, FD	+++
	Heteropneustidae	Heteropneustesfossilis	Mangur	FD, PF	_
	Siluridae	Ompokbimaculatus	Puffta	PF	+
		Wallagoattu	Fresh water shark	PF	++
Synbranchiformes	Mastacembelidae	Macrognathuspancalus	Spiny eel	PF	+
		Mastacembelusarmatus	Baam	PF	++
		Mastacembelusshiranus	Baam	PF	++

Results

During the study period different fish varieties have been observed in the Katepurnariver. The results showed that the area was rich in fish biodiversity. Fishes belonging to ten orders and twenty families were collected during course of the study period. Many collected fishes having economic importance sold after collection in the local fish market.

35 different genera 20 families and 10 orders were recorded from the Katepurna River number of catches carried out during January 2015-December 2017. The members of Order Anabantiformes have [12 individuals][4 species], Anguilliformes [1 individual][1 species], Beloniformes [3 individuals][1 species], Cichliformes [2 individuals][1 species], Cypriniformes [63 individuals][22 species], Cyprinodontiformes [1 individual][1 species], Gobiiformes [2 individuals][1

species], Osteoglossiformes [2 individuals][1 species], Ovalentaria [3 individuals] [1 species], Siluriformes [17 individuals] [9 species], Synbranchiformes 3 species respectively.

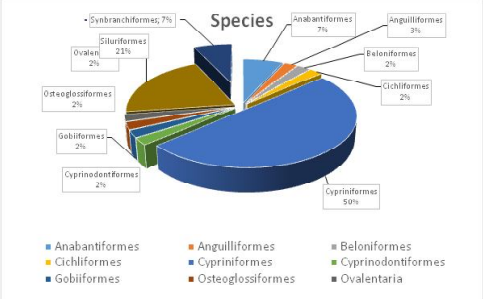

Figure 1: Order wise fish composition of Katepurna river dis. Akola

As mention above order Anabantiformes have Channamarulius, Channapunctate, Channagaucha are in abundant form and Colisalalia species is in rare form, in Order Anguilliformes have Muraenesoxbagio is also rare species, in order Beloniformes have Xenentodoncancila which is less abundant. order Cichliformes have Oreochromismossambicus species it also rare, order Cypriniformes have large no of verity of fishes Amblypharyngodonmola, Catlacatla, Cirrhinusmrigala, Cirrhinusreba, Cyprinuscarpio, Devariomalabaricus, Garramullya, Labeobata, Labeoboggut, Labeocalbasu, Labeorohita, Puntiuschola, Puntiusmahecola, Puntiussophore, Rasboradaniconius, Rohteeogilbii, Salmophasiabacaila, Systomussarana, Acanthocobitisbotia. Order Cyprinodontiformes have Poeciliareticulate species. Order Gobiiformes have Glossogobiusgiuris species which is abundant. Order Osteoglossiformes have Notopterusnotopterus which is less abundant species. Order Ovalentariahaverare Chandanama species. Order Siluriformes have Amblycepsmangois, Nemapteryxcaelata, Mystusbleekeri, Mystuscavasius, Speratаseenghala, Clariasgariepinus, Heteropneustesfossilis, Ompokbimaculatus, Wallagoattuspecies. Order Synbranchiformes have Macrognathuspancalus, Mastacembelusarmatus species.

Discussion Recorded abundance of catfishes in Katepurna reservoir (Talwar, andJhingran, 1991). Total 41 species were present in which 20 were commercially important. Reported 34 species of fishes in reservoirs of Parbhani Dist.Of Maharashtra (Sakhare, and Joshi 2003).Reported the Ichthyofaona of Harsool-Saving Dam Aurangabad (M.S.) India (Shinde, et al. 2009). Total 15 fish species belonging to 3orders, 4 family and 12 genera. The order cypriniformes found dominant with 11 species, followed by perciformes 3 species and siluriformes with 1 species.

Conclusion The work has been conclude with further strategies for development of fish fauna conservation of Katepurna River at VIdarbha Dist. Akola (M.S.)India. Resent data regarding Fish diversity of the East cost river system, aiming to contribute a better knowledge of the fish diversity of Katepurna River and a tool for conserving planning of aquatic environments in this region. To maintain Fish biodiversity has an immense important as it is not always possible to identify individual species critically to sustain aquatic ecosystem.

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Araneidae spiders acts as biocontrol agent for insect pests of agroecosystem.

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Introduction

Spiders are the venomous animal. Venom of spiders paralyzes the prey. Every year many farmers got loss in their cultivated crop due to the attack of insect pests .Therefore farmers has to increase the use of insecticide and pesticides. These chemical insecticide and pesticide To prevent harmful effects of insecticides and pesticides there is need to control insects pest by using biological agents such as spiders. Family- Araneidae spiders (Orb web spiders) . They are of economic value to man because of their ability to suppress pest abundance in agroecosystems. Faced with the need to reduce pesticide usage on the world's crops and optimize natural biological control, full investigation of the means by which spiders influence pest abundance is long overdue. Also, in recent years, there has been a realization by ecologists that components of agroecosystems are tractable to manipulate and that spiders are convenient model organisms (Sunderland 1999). The effect of spider predation on pest populations can be sufficient to reduce significantly levels of crop damage (Riechert & Bishop 1990; Carter & Rypstra 1995). Most of investigations on spiders are in agricultural ecosystems in Iran. For instance, some researches were performed on spider fauna and abundance of rice fields (Ghavami, 2004), and cotton fields (Ghavami *et al.*, 2007b and 2008a and Ghavami, 2007a).

Material and method

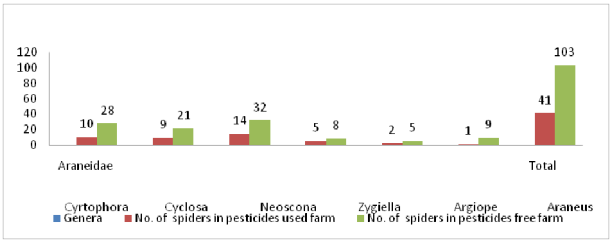
Present study is done in cotton field to see the role of spiders in Agro- ecosystem. Where we have observed number of spiders in two kinds of fields i.e.Chemically untouched field of cotton and pesticides used cotton cultivated crop. Sampling plots of 10 m × 10 m were made in both the fields to observe the spiders and to identify them. Sampling was done by visual search and collections were done by hand picking. After collection of the spiders, samples were taken to the laboratory and photographed with the help of stereozoom microscope. In chemically untouched field more density of spider recorded. Spiders identified morphologically. Pests population controlled by different species of Araneidae spider in agro ecosystem. rom their number we can say that Araneidae spiders are obligate .

Observation and Result

Table:-1. No. of individuals per plot of (10 m× 10m) of 6 different genera of the family Araneidae.

Family	Genera	No. of spiders in pesticides used farm	No. of spiders in pesticides free farm
Araneidae	Cyrtophora	10	28
	Cyclosa	09	21
	Neoscona	14	32
	Zygiella	05	08
	Argiope	02	05
	Araneus	01	09
Total		**41**	**103**

Effects of use of pesticides: Because of longer use of insecticide and pesticide, the insects



have developed resistant genes and these highly toxic chemicals destroying soil fauna and also affecting the soil quality. This insecticide and pesticides causes carcinogenic disorders in farmers and also in common man. While visiting the cotton field we can observe these kind of webs of spiders belongs to the family Araneidae.

Webs of Spiders in the Cotton field

Spiders along with their prey

Discussion

Araneids are orb-web spiders, constructing their webs on or between plants. Most are nocturnal and during their inactive period during the day they were collected from the leaves, stems and seed of cotton plants. Diurnal species were collected from webs between plants and leaves (Van den Berg 1989). Araneids were observed preying on *Helicoverpa armigera* (Hubner) moths and larvae, both in the laboratory and in the field. Members of the Araneidae were also commonly found on cotton in other countries. In Texas, Nyffeler et al. (1987, 1989) reported that 10% of the spiders collected were orb weavers belonging to five species in the families Araneidae, Tetragnathidae and Uloboridae. Most webs were small (40 cm in diameter) and intercepted relatively small prey, of which aphids were the most abundant (34.6-90 %). According to Dean et al. (1982), Araneidae were common on cotton in Texas where they preyed on various pests, while Lincoln et al. (1967) found that species of *Araneus* were one of the most important predators of bollworm moths. Other pest species preyed on by species of *Araneus, Neoscona* and *Argiope* included *Adelphocoris rapidus* (Say) (nymphs), *Brevicoryne brassicae* (Linnaeus), *Helicoverpa armigera* (larvae), H. *zea* (Boddie) (moths), *Myzus cerasi* (Fabricius), M. *Iythri* (Schrank), *Pseudatomoscelis seriatus* (Reuter) (adults), *Rhopa/osiphum padi* (Linnaeus) and spur-throated locust nymphs (Kagan 1943; Lincoln et al. 1967; Bishop & Blood 1981).

These following genus species controls the insects:

Araneidae Spider (Predators)	**Insects, Pests (Prey)**
Argiope aurantia	Cotton fleahopper
Araneus	Bollworm moth
N. mukherji & N. thesi	Hemipterans, Hymenopterans & Dipterans
Cyclosa	Small Dipterans, Small Hymenopterans, Aphids, leafhopper & Thrips
Zygiella & Cyrtophora	Lepidopterans, Dipterans &Homopterans

Conclusion

Araneidae spiders acts as a biological agent in cotton agro-ecosystem and it will control the population of insect pests into the field. Spider helps

to protect crop without damaging ecosystem and to maintain harmonious nature of ecosystem and their food chain. As the number of Araneidae spiders will be increased, insect pests will be decreased, which results into improved yield of crop. If the number of spiders are not sufficient so, we can inoculate more number of spiders by rearing them in laboratory for insect pest control.

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Phytochemical Analysisand AntimicrobialActivity of Methanol, Ethanol, and Acetone extract *Parthenium hysterophorus*in combination with Tetracyclin.

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Abstract

The present research work was carried out to study the phytochemical screening and antimicrobial activityof leaves extract of *Partheniumhysterophorus*. Ethanol, Acetone and Methanol extract leaves of *Parthenium hysterophorus* were tested against *Escherichia coli, Pseudomonas flurescens, Bacillus amyloliquefaciens*and *Staphylococcus aureus* which are known to be resistant to various antibiotics. These extracts were prepared from fresh*Partheniumhysterophorus*leaves. These extracts were evaluated for their part in increasing antibacterial activity of Tetracyclin against *Escherichia coli, Pseudomonas flurescens, Bacillus amyloliquefaciens*and *Staphylococcus aureus*. The antibacterial activity of Tetracyclin was enhanced against the test organism in the presence of these extracts. Tetracyclin in combination with these extracts showed maximum inhibition against test organism. Phytochemical analysis gave positive results for steroids, triterpenoids, alkaloids, glycosides, phenolic compounds, flavonoids, and tannins.

Keywords: *Partheniumhysterophorus*, phytochemical analysis, Tetracyclin.

Introduction

Partheniumhysterophorus is commonly called as congress grass, carrot weed, bitter weed, gajar grassetc. At present it occupies almost all part of India. It is native of sub tropics of north and South America and it accidently introduced in Indian subcontinent. It is an annual herb with a deep tap root system and an erect stem that becomes woody with age. The leaf proteins are reported to be better than cereal and legume proteins. It is used as spices in many parts of the world. Parthenin free dried fibers of plants are used as cattle feed (Narasimhan TR *et al.,* 1993). All parts of the plant are reported to be used as bitter tonic, febrifuge, emmenagogue, antidysenteric (Oudhia P., 2001). In Maharashtra and Gujarat (India) it is used in the treatment of diabetes mellitus (Patel VS *et al.,* 2008).It also shows antibacterial (Pandey AK. 2007), anti-tumor activity (Reddy DM *et al.,* 2011) and also used as folk remedy for the treatment of infectious and degenerative diseases (Knox J *et al.,* 2011; Kumar S *et al.,* 2013). The plant is used in the treatment of ulcerated sores, wounds, and fever, anemia and heart troubles. A decoction of the root finds use in treatment of dysentery (Kalsi*et al.,* 1995) and the lower concentrations of extracts might find use as antifungal agent (Bajwa*et al.,* 2003). Identification of novel therapeutic agents as new drugs for alleviation of the human suffering from cancer and other degenerative diseases is of prime concern (D. J. Newman *et al.,* 2003; A. Mishra *et al.,* 2013). For treatment of infectious diseases on Earth pharmacologists, microbiologists, and natural-products chemists are relying on plant derived dietary supplements as well as Phytochemicals (Pandey et al; 2015). Scientific investigations revealed that a

large number of plant products inhibit growth of pathogens (Deshpande and Deshpande 2017). *Partheniumhysterophorus*is considered as toxic plant but many medicinal allelopathicand industrial uses have been well documented in literature. It has been reported to have antimicrobial effects. It inhibits the germination and growth of several crop plants and trees. The allelopathic potential of *Partheniumhysterophorus*weeds results from the release of phytotoxic substance such as fernlic acid, caffeic acid, vanilic acid, anisic acid, chloragenic acid, p-courmaric acid, p- hydroxbenzoic acid, parthenin, ambrosin and coropilin.

In current study, screening the bioactive components and the antibacterial effects of the Ethanol, Acetone and Methanol extract and the extracts were evaluated in combination with tetracyclin to assess their antibacterial activity against pathogenic bacteria.

Material and Methods

Sample collection

The fresh leaves of *Parthenium hysterophorus* were obtained from campus of Badrinarayan Barwale Mahavidyalaya, Jalna (MS). They were washed under running tap water to remove surface dirt and impurities, followed by distilled water. These leaves were air dried. After drying the leaves were chopped to get fine pieces. These pieces were used for preparation of different plant extracts.

Preparation of leaf extracts

For Methanol extract 10gm of chopped pieces of *Partheniumhysterophorus* leaves were added to 100ml of Methanol and then kept overnight. This was filtered through Whatman's filter paper and filtrate was used as aMethanol extract. This extract was stored at 4^0C for further use. For ethanolic extract absolute ethanol was taken 100ml and to this 10gm of chopped pieces of *Partheniumhysterophorus* leaves was added and kept overnight. After that this was filtered through Whatman's filter paper and filtrate was used as an Ethanolic extract. This extract was stored at 4^0C for further use. For Acetone extract 100ml Acetone was taken and to this 10gm of chopped pieces of *Partheniumhysterophorus* leaves was added and kept overnight. After that this was filtered through Whatman's filter paper and filtrate was used as an Acetone extract. This extract was stored at 4^0C for further use.

Test organism

Bacterial cultures were selected from NCIM. The strain used for the study were *Staphylococcus aureus* (NCIM 5021), *Pseudomonas flurescens* (NCIM 2390)*, Bacillus amyloliquefaciens* (NCIM 2829) and *Escherichia coli* (NCIM 2995). These were grown on their respective selective media and purity was determined by morphological and biochemical characterization.

Inoculum preparation

Loopful of pure culture from selective media was picked up and inoculated in Muller Hinton Broth (Himedia). It was incubated at 37 0C for 3-7 hrs. until moderate turbidity develops. Inoculum turbidity was compared with that of0.5 McFarland standard.

Preparation of Disc

Whatman's filter paper no.1 was punched to get disc of 6mm diameter. These discs were sterilized under UV light. Each sterile disc was impregnated with ethanol extract, acetone extract, methanol extract and excess of solvent was dried in controlled temperature.

Antimicrobial activity of extract

The antimicrobial activity of the extract was evaluated by standard disc diffusion method (Baur*et al.,* 1966). Plates of Muller Hinton agar (Himedia) medium having media up to 4 mm were prepared. After solidification lawn of inoculum was prepared on to agar plates for each organism. Inoculum was taken by socking the sterile swab (Himedia) in prepared inoculum of test organism i.e. *Staphylococcus aureus, Pseudomonas flurescens, Bacillus amyloliquefaciens*and *Escherichia coli* and spread over the agar plates for respective organism. Ethanol extract disc, acetone extract disc and methanol extract disc of *Parthenium hysterophorus*was applied and incubated at 28-30

^{0}C for 16-18 hours.

Disc diffusion assay to evaluate combined effects

Disc diffusion method was used to evaluate in vitro antibacterial activity of tetracyclin disc (Whatman's paper disc saturated with tetracyclin) against test organisms on Muller Hinton Agar (Himedia). To determine combined effect, each paper disc was further impregnated with 10µl of each single extract. Muller Hinton Agar plates were inoculated with test organism. Standard antibacterial streptomycin disc were used as positive control and streptomycin disc impregnated with methanol, ethanol, and acetone extract were place onto Muller Hinton Agar plate inoculated with test organisms. These plates were incubated 16-18 hours. After incubation, the zones of inhibition were measured. The assays were performed in triplicate.

Well diffusion assay to evaluate combined effects

Muller Hinton Agar plates were inoculated with test organism and wells were prepares by using cork borer (6mm diameter). For the test organisms, standard antibacterial agent i.e. Tetracyclin was used in concentration of 100µg/ml, and the wells were filled with 50µl extract, 50µl single type ofextract, 50µl antibiotic plus 50µl respective extract, 50µl of control antibiotic. These plates were incubated 16-18 hours. After incubation, the zones of inhibition were measured. The assays were performed in triplicate.

Assessment of increase in fold area

The increase in fold area was assessed by calculating the mean surface area of the inhibition zone of each antibacterial agent (Tetracyclin) and Tetracyclin plus extract. The fold increase area of different test organism for streptomycin and for streptomycin plus extract was calculated by equation $(B^2 - A^2)/A^2$, where A and B were zones of inhibition for streptomycin and streptomycin plus extract, respectively.

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, glycosides, triterpenoids, steroids, tannin and phenols, reducing sugar, carbohydrates and protein and amino acids by the following procedure. (C.K. Kokate; 2000, J.B. Harbone; 1999, Prashanth Tiwari et.al.; 2011)

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

Molisch test

2 ml of aqueous extract was treated with 2 drops of alcoholic á-naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Tests for Reducing Sugars

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Tests for Flavonoids

Alkaline reagent test

The extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for Glycosides

Borntrager's test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was

added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinone glycosides.

Tests for Tannin and Phenolic compounds

Ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Test for Saponin

Froth test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Protein and Amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 minutes. Formation of blue color indicates the presence of amino acids.

Tests for Triterpenoids and Steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterol is present. Presence of golden yellow layer at the bottom indicates the presence of triterpenes.

Result

The antibacterial activity of Acetone, Ethanol and Methanol extract of *Partheniumhysterophorus* against test organism by disc diffusion and well diffusion methodwere shown in Figure 1and 2.

In disc diffusion assay, Ethanol extract shows highest 7mm zone against *B.amyloliquefaciens* among all test organisms, followed by acetone extract shows highest zone 4mm against *E. coli* among all test organisms , whereas Methanol extract shows 8mm zone diameter against *P. flurescens* among all test organisms. Standard antibiotic Tetracyclin shows 2 mm zone diameter against *E. coli*, 7 mm zone against *S. aureus*, 3mm *P. flurescens* and 8mm *B.amyloliquefaciens*. (Figure 1).In well diffusion assay, Ethanol extract shows highest 14mm zone against *P. flurescens* among all test organisms, followed by acetone extract shows highest zone 13mm against *B.amyloliquefaciens* among all test organisms , whereas Methanol extract shows 15mm zone diameter against *P. flurescens* among all test organisms. Standard antibiotic Tetracyclin shows 12 mm zone diameter against *E. coli*, 12 mm zone against *S. aureus*, 16mm *P. flurescens* and 12mm *B.amyloliquefaciens*. (Figure 2)

Figure 1.

Antibacterial activity of Ethanol, Acetone and Methanol extract of *Partheniumhysterophorusand* Tetracyclinby disc diffusion method

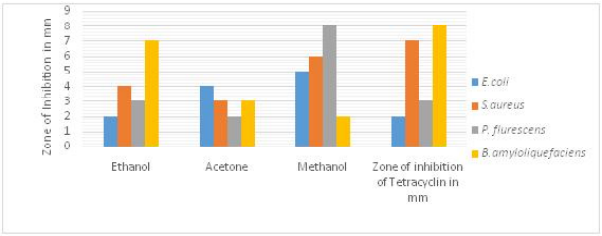
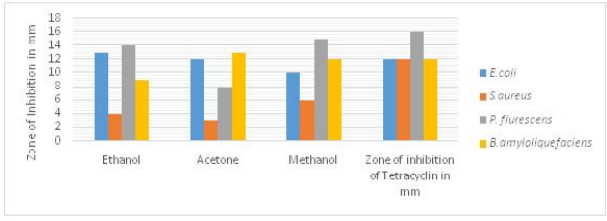


Figure 2.

Antibacterial activity of Ethanol, Acetone and Methanol extract of *Parthenium hysterophorusand* Tetracyclinby well diffusion method



In the in vitro antibacterial activity, Tetracyclin, an antibacterial agent that is widely used against many bacterial infection, was used as positive control for comparison with *Partheniumhysterophorus* extracts. The diameter of zone of inhibition and increase in fold area for all the test organism was measured. The antibacterial activity of Tetracyclin increased significantly in presence of Ethanol, Acetone and Methanol extract of *Parthenium-hysterophorus*.

Table 1. Zone of inhibition of Tetracyclin against test organism in absence and in presence of *Partheniumhysterophorus* extract at content 10µl per disc

Test Organism	*Partheniumhysterophorus* Extract	Tetracyclin +Extract (B)	Tetracyclin (A)(mm)	Increase in Fold B^2-A^2/A^2 Area
E.coli	Ethanol	6	2	8
	Acetone	4	2	3
	Methanol	5	2	5.25
S.aureus	Ethanol	8	7	0.3
	Acetone	7	7	0
	Methanol	9	7	0.65
P. flurescens	Ethanol	10	3	10.11
	Acetone	9	3	8
	Methanol	13	3	17.77
B.amyloliquefaciens	Ethanol	12	8	1.25
	Acetone	10	8	0.56
	Methanol	15	8	2.51

Table 2. Zone of inhibition of Tetracyclin against test organism in absence and in presence of *Partheniumhysterophorus* extract at content 50µl per well

Test Organism	*Partheniumhysterophorus* Extract	Tetracyclin +Extract (B)	Tetracyclin (A)(mm)	Increase in Fold B^2-A^2/A^2 Area
E.coli	Ethanol	14	12	0.36
	Acetone	15	12	0.56
	Methanol	13	12	0.17
S.aureus	Ethanol	15	12	0.56
	Acetone	16	12	0.78
	Methanol	19	12	1.5
P. flurescens	Ethanol	19	16	0.41
	Acetone	19	16	0.41
	Methanol	18	16	0.26
B.amyloliquefaciens	Ethanol	14	12	0.36
	Acetone	16	12	0.78
	Methanol	15	12	0.56

Phytochemical analysis The phytochemical analysis of plant extracts using Acetone, Ethanol and Methanol was showed in Table-3

Table 3. Phytochemical analysis of plant extracts (+) indicates presence while (–) indicates the absence of the components

Phytochemical test	Ethanol extract	Acetone extract	Aqueous extract
Tests for Alkaloids	+	+	+
Tests for Carbohydrates	-	-	-
Tests for Reducing Sugars	+	+	+
Tests for Flavonoids	+	-	+
Tests for Glycosides	+	+	+
Tests for Tannin and Phenolic compounds	+	+	+
Test for Saponins	+	+	+
Tests for Protein and Amino acids	-	-	-
Tests for Triterpenoids and Steroids	+	+	+

From the phytochemical analysis, reducing sugar were found in *Partheniumhysterophorus* in the solvents such as Acetone, Ethanol and Methanol. The Ethanol extract of *Partheniumhysterophorus* showed the presence of alkaloid, glycosides, flavonoids, saponins, tannin and phenolic compound, reducing sugar, triterpenoids and steroids. Alkaloid, glycosides, saponins, tannin and phenolic compound, reducing sugar, triterpenoids and steroids were observed in Acetone extract of *Partheniumhysterophorus* but there was absence of flavonoids.

Discussion

In the present study, we have investigated the qualitative phytochemical analysis of methanol extract of *Partheniumhysterophorus L.* leaves. Earlier Gupta *et al.* (1977) reported the presence of Amino acids, Carbohydrates and Saponins in the methanol extract of P. hysterophorus. In the present

study along with the above mentioned compounds we had found the presence of additional phytochemical constituents of Alkaloids, Cardiac glycoside, Flavonoids, Glycoside, Phenols, Tannins, Terpenoids and Steroids. Most of these compounds will play a major role in various biological activities right from inhibiting the microbial growth to destroying of cancer cells. Partheniumhysterophorus L. leaves extract exhibited significant activity against all the human pathogens studied. The present investigation proved that the extract of *Partheniumhysterophorus* L. leaves possess promising antibacterial potential against both the Gram positive and Gram negative bacteria. The activity of plant extract against both gram positive and gram negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds (Nagarsenker, KS.*et al.,* 2010). Teveswari K*et al.* (2016) evaluated the antimicrobial activity of methanolic extract of *Partheniumhysterophorus* by well diffusion method and according to them antibacterial potential showed varying degree of antibacterial activities against the *P. aeruginosa* and *S. aureus* but no activity against *E.coli*.

Conclusion

Partheniumhysterophorus possess pharmacological activecompounds. Phytochemical constituents are the source for useful drugs. Based on the results of the present investigation, it can be concluded that this plant is ideal for medical applications due to presence phytochemicals. Demand of the new therapeutic agents in low cost is very needful for the human beings as chemically synthesized drugs are very costly.Pathogens are becoming highly resistant to antibiotics therefore this study is beneficial for humans emphasizing on medical applications of *Partheniumhysterophorus*. Moreover, the combined effects of a standard antibacterial agent (Tetracyclin) with extracts against pathogenic bacteria is similarly a new finding. Further, it can be concluded that extract alone or their formulations (combination) can be used as effective agents against human bacterial pathogenafter toxicological studies.

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Vermicompost : An eco-friendly way for solid waste management

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Abstract :

Large quantity of agricultural and food waste is released in India without any treatment on it. Farmers burn these agricultural wastes in their farm itself which results in the pollution of environment. But this is not a proper way for solid waste management. Therefore an eco-friendly way for solid waste management is vermicomposting. In this earthworms are used to decompose the organic waste material and produces nutrients rich compost. We start a vermicompost project in our college campus to decompose the plant residues. The earthworms used for this purpose are *Eisenia fetida*which are purchased from Apharm Nursery , Latur. After 2 to 3 months a quality vermicompost is formed.

Key Words : Vermicompost, Respiratory diseases, *Eisenia fetida*

1. Introduction :

Agricultural wastes and waste food from hotels in the cities are the major components of solid wastes in India. Very less quantity of this solid waste material is drgraded by microorganisms naturally. But large quantity of agricultural residues is burned by the farmers . This burning causes air pollution which causes different types of respiratory diseases. To overcome this problem there is a eco-friendly

way to manage the solid agricultural wastes by producing vercompost. Vermicompost is a casting of earthworms consisting of many microorganisms. Vermicomposting is a managed process of earthworms which digest the organic waste material and convert it into a useful compost. Vermicomposting by using earthworms is an excellent way for recycling of agricultural and food wastes. The earthworm *Eisenia fetida* are used for this purpose. Vermicompost produced by earthworms is rich in many minerals and also different microorganisms which increases the soil fertility resulting into increased crop production. Total 3,920 species of earthworms are reported in world. In India 509 species of earthworms are reported. But the common earthworms used for vermicomposting are *Eiseniafetida* which has a wide range of temperature tolerance, very high reproductive potential and has good burrowing capacity. Earthworms are called as friends of farmers because they are involved in many activities such as soil aeration, turning of soil , acceleration of humification etc which results in farmer's crop production.

2. Materials :

i. Vermicompost bed :Four vermicompost beds of size 7 feet × 2.5 feet × 2 feet (length × breadth × height) are built by using cement and bricks. Small wholes are left at bottom of each bed for percolation of water and aeration.

ii. Earthworms : Earthworms of the genus *Eisenia fetida* are purchased at rate of 500 Rs per kg from the APHARM nursery, Latur.

Figure 1.
Earthworms of the genus *Eisenia fetida*

iii. Plant residues :

Plant residues and other solid wastes material is collected from the college campus with the help of students of B. Sc. Microbiology , NSS volunteers and peons of our college.

Figure 2 and 3. Collection of plant residues from the college campus.

Figure 4 and 5. Sorting plant residues.

iv. Cow dung:

It is made available from the animals of local farmer.

v. Water :

Water for spraying of vermicompost bed is made available from the bore well of college.

3. Methodology :

i. In the first layer 2 to 3 inches thick layer of biodegradable wastes is added at the bottom of each vermicompost bed.

ii. Above this layer 2 to 3 inch thick layer of cow dung or manure is spreaded.

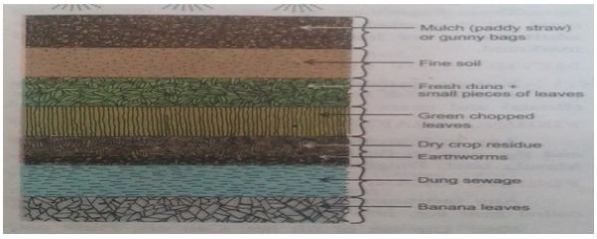
iii. This bedding is sufficiently moistened by adding water.

iv. After this earthworms *Eisenia fetida* are added over this bed.

v. It is again covered with cow dung or manure.

vi. Water is sprinkled regularly on the top of bed.

Figure 6. Methodology for vermicomposting.



4. Results :

After 2 to 3 months a very good quality of vermicompost is formed.

Figure- 7&8. Vermicompost after 2 to 3 months.

5. Conclusion :

Vermicomposting is an eco-friendly way foe management of agricultural and garden wastes. It reduces the environmental pollution and subsequently the health problems. At the same time vermicompost produced by this method is a rich source of nutrients for soil . Therefore every college , housing society , municipal corporations, Gram panchayats and farmers have to start their own vermicompost project and contribute in the prevention of environmental pollution.

6. Acknowledgement :

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Taxonomic study of ants of subfamily Myrmicinae (Hymenoptera: Formicidae) from Gautala Autramghat Sanctuary, Aurangabad-Maharashtra

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Abstract

The present communication deals with the taxonomic study of ants of subfamily Myrmicinae, around Gautala Autramghat Sanctuary located in Maharashtra India. Ants are widely distributed all over the world. Ants have been named ecological engineers due to their ability to modify the structure of their environment in ways that affect other organisms Lawton (1994), Kaspari, (2000). In the present study six species of ants of subfamily Myrmicinae were recorded from the study area.

Keywords: Taxonomy, Myrmicinae, Ants, Maharashtra.

Introduction

Ants are one of the groups of arthropods belonging to class Insecta and together with the social wasps and bees constitute 80% of the biomass. These ants are grouped in to single family Formicidae of the order Hymenoptera. Ant species assemblage has been used as a biological indicator of environmental conditions. These occur in all types of habitats. The branch of science which deals with the study of ants is called as "Myrmecology".

The Formicidae is subdivided into 21 subfamilies comprising 290 genera and more than 12,500 extant species Bolton *et al*., (2006). There are well known people are working on the taxonomy and diversity of ants like ., (1990), Bolton, (1994). In India the prominent researchers working on ants includes Bingham, (1903) Dolly Kumar, (2008) Rastogi, (1997), Varghese, (2003), S. Sheela, (2008), Ghosh *et al*., (2005), Tiwari, (1999), Bharti Himender, (2011) etc. India there is 652 species/ subspecies of ants under 87 genera in 12 subfamilies. Bharti Himender (2011). But in Maharashtra there is little work on the ant diversity and species richness. That include Chavan *et al*., (2010) who worked on ant diversity in and around Amravati city, and Gadagkar *et* al., (1993) studied the ant diversity from Western Ghats. As taxonomic knowledge of ants expands, some of these species will undoubtedly prove to be redundant; despite these redundancies, the current rate of discovery of new ants suggests their total species diversity could easily exceed 25,000 Ward *et al.,* (2010). Although most of these species occur in tropical bioregions, approximately 580 species have been described in the Nearctic region (North America north of Mexico) Holldobler and Wilson, (1990).

Ants are found on all continents except Antarctica, and only a few large islands such as Greenland, Iceland, parts of Polynesia and the Hawaiian Islands lack native ant species. Holldobler and Wilson, (1990) Jones, Alice S. (2008). Ants occupy a wide range of ecological and are able to exploit a wide range of food resources either as direct or indirect herbivores, predators and scavengers. Most species are omnivorous generalists but a few are specialist feeders. Their ecological dominance may be measured by their biomass, and estimates in different environments suggest that they contribute 15-20% (on average and nearly 25% in the tropics) of the

total terrestrial animal biomass, which exceeds that of the vertebrates Schultz T.R. (2000).

The Myrmicinae is the most diverse subfamily of ants on the generic level there is a huge amount of variety in the morphology and behavior of these ants. This subfamily owes its name to the genus *Myrmica*, which refers to the Greek term for ant: myrmeco. Many myrmicines are generalist predators or scavengers however there are several groups that are specialist predators that feed on Collembola and other soil dwelling arthropods. Some species obtain nutrients from harvesting. Seeds and are important in seed dispersal. Others tend fungal gardens as their sole source of food. Myrmicines have a well-developed postpetiole. They can be distinguished from other ants with developed postpetioles by their laterally expanded frontal carinae that partially or completely cover the antennal sockets. Myrmicines usually have a sting although in some genera this is absent or modified. This subfamily has a worldwide distribution. Representatives of the subfamily Myrmicinae can be found throughout the world on all continents except Antarctica Jason Allan Forster, (2003).

Material and Methods

The Gautala Autramghat sanctuary it is spread within Longitude 20^0.17'to 20^0.21N and Latitude 75^0.16'to 75^0.19'E. During the present study the ants were collected by handpicking method with the help of brush and forceps. For collection, preservation and identification of ants during the present study the standard methodology suggested by Agosti, (2000), Holldobler and Wilson, (1990), Mathew and R. N. Tiwari, (2000), S. Sheela, (2008), Bolton B. (1994), Bingham, (1903) was followed. Ants were collected from different habitats and microhabitats, such as in soil and leaf litter, in rotting logs, on and nesting in various plants, etc. After the collection of ants were preserved in the vials with 70% alcohol and brought to the laboratory. Vials were then filled with ethanol and a field label. Each sample, of ants were sorted from debris and identified to genus level. Sorted specimens were placed in vials with proper labels noting date, sampling site collection method, ecological information and collector. All specimens were identified to species level. The identified specimens were deposited in Entomology Research Laboratory Department of Zoology Dr. Babasaheb Ambedkar Marathwada University Aurangabad. As knowledge with the species in Gautala; specimens were identified to species level at the initial sorting step. Photography was done with the help of camera. The identification is made with the help of Olympus stereoscope trinocular microscope (MSZ TR); and ants were identified. The study was carried out during June 2010 to December 2010

Result and Discussion

Key to genera

1. Postpetiole attached to the dorsal side of gaster; gaster in dorsal view heart shaped ……..…***Crematogaster***
2. Club of antenna three-jointed ……..***3***
3. Antennae with 9 segments ..... ***Meranoplus***
4. Propodeum unarmed, evenly rounded; mandibles not as above ……***Monomorium***
5. Lateral margins of head and thorax denticulate and spiny……..….***Cataulacus***
6. Antennae7-jointed …… ***Myrmicaria***

Genus ***Crematogaster*** Lund

These ants are called acrobat ants. Because of the distinctive behavior that many *Crematogaster* species have of elevating their gaster up and over their heads when disturbed. The etymology of the generic name refers to this behavior (crema. Greek for hang or suspend and gaster referring to modified abdomen). These ants do not sting but instead exude noxious chemicals from their flattened modified sting Forel, (1928). This chemical defense is effective against ants but harmless to humans. Acrobat ants nest in a variety of areas including: living trees, soil, rotting logs or stumps, insect galls, acorns, and occasionally in woodwork. They tend to be omnivorous Scavengers but are commonly found associated with honeydew producing insects. Acrobat ants are found throughout the world.

Crematogaster species are easily recognized by their heart-shaped gaster (seen from above) that is attached to the post-petiole on the dorsal surface of the first gastral segment; in profile the dorsum of the gaster is relatively flat while the ventral surface is strongly convex. Their antenna is 11-segmented Jason Allan Forster, (2003).

Crematogaster subnuda (Mayr, 1879)

Diagnostic Characters:

Head, thorax pedicel, antennae and legs brownish red pilosity sparsely spread on thorax and apex of gaster, pubescence white appressed widely and regularly arranged all over head smooth with a small straite surrounding antennal hollows mandibles straite clypeus broad. Anterior portion almost transverse eyes lateral situated on the middle, more to posterior part scape clearly reaching top of head. Flagellum formed of apical three joints. Pronotum flat above rugolose anterolateral suture weakly and mesonotal suture clearly indicated. Propodeal spines straight and acute; apex of propodeum smooth petiole semicircular infront sides angular post petiole shallowly longitudinally grooved gaster broadly cordate. Size 4.5 to 5 mm

Habitat: on plants as well as on ground.

Material examined:

India, Maharashtra, Aurangabad, around Gautala, January 2010 – February 2012.

Distribution: Throughout India.

Elsewhere: Myanmar and Sri Lanka Sheela, (2008). Plate No-2

Genus ***Meranoplus*** Smith

Diagnostic Characters:

Head trapezoidal, broader posteriorly than in front, the front of the head from the posterior margin of the clypeus bent downwards, subtruncate; sides of the head slightly compressed, deeply grooved above the eye for the reception of the antennae, which fold back into the groove and when so folded are invisible from above; mandibles stout, the masticatory margin armed with 4 teeth; clypeus with the median portion broad, posteriorly well defined, frontal area triangular, depressed, and at times obscure; antennal carinae short, very widely separated, continued as lines along the upper border of the antennal groove; antennae of modrate size and thickness, 9 –jointed, the club distinct, formed of the apical three joints; eyes oval, always somewhat prominent. Thorax above more or less square in form, the pro-and mesonotum with no vestige above of a suture between them, more or less armed laterally and posteriorly with spines or teeth; metanotum depressed beneath the overhanging posterior portion of the pro-mesonotal shield, vertical, armed at its upper or anterior angles with two backward –pointing spines; legs somewhat short and stout, posterior tibiae with a single fine, simple, nonpectinate calar. Pedicel short, nodes large, sessile or only shortly petiolate; 1st node more or less conical; 2nd globose, in some species flattned anteriorly; abdomen cordate, lightly concave in front Bingham, (1903).

Meranoplus bicolor (Guerin-Meneville, 1844)

Diagnostic Characters:

Head, thorax, pedicel and legs red, gaster black; whole body covered with very long, and small hairs; head thorax and petiole coarsely striate, reticulate; proppdeum and petiole smooth, polished; gaster minutely reticulate; head trapezoidal; antennae 9 jointed; eyes prominent, lateral; pro-mesonotal shield fused, with an incision on sides at pro-mesonotal suture; pronotum dentate; mesonotum posteriorly projecting in two acute spines; propedeum with two slender spines; petiole node triangular in profile; post petiole globose, larger than petiole; gaster heart shaped. Size: 4-5 mm.

Habitat:

Sluggish, make subterranean nest with some outlets on surface.

Material examined:

India, Maharashtra, Aurangabad, around Gautala, January 2010 – February 2012.

Distribution:

India: West Bengal, Arunachal Pradesh and nearly throughout the country excepting hot, dry places.

Elsewhere:

Myanmar to Malayan subregion Ghosh *et*

al., (2005, Sheela, (2008) Bharti Himender, (2009). Plate No-10

Genus ***Monomorium*** Mayr

These ants are little and (usually) black *Monomorium* ants are common tramp species and are considered invasive pests in many areas. Most species are adaptable and can nest inside human dwellings. These ants are typical generalist omnivores but show a preference for sweet food items. Members of this genus are found worldwide. *Monomorium* species are small and have a 12-segmented antenna with a 3-segmented club. Their mandibles have 3-4 teeth and clypeus usually with 2 longitudinal carinae extending past the anterior border of the clypeus as teeth. Ants of this genus lack propodeal spines Jason Allan Forster, (2003)

Monomorium scabriceps (Mayr, 1879)

Diagnostic Characters:

Head, thorax, trochanters and pedicel brownish red; gaster, legs and antennae blakish brown; tibiae of legs and flagellum of antennae with a red tint, tarsi of legs and apex of terminal club segment reddish yellow; head except clypeus finely longitudinally striate, punctuate; clypeus smooth, polished and shining; mandibles striate, opaque; ventral side of head widely punctuate; pro-mesonotum granulate, rugulose; propodeum transversely rugulose, punctuate; prothoracic sternite and meso-sternite punctuate, latter posteriorly rugulose, metasternite basally longitudinally striate, becoming rugose above; pedicel rugoso-punctate; gaster smooth, polished; head and gaster with abundant long appressed white pubescence and pedicel and legs with sparse, short hairs; head posteriorly emarginate; mandibles with apical blunt teeth; anterior margin of clypeus with two minute clypeal teeth; antennae 12- jointed, club not thick, all segments elongate; eyes small, lateral, situated in front of midtranverse line; pro-mesonotum together form a single convexity; mesometanotal suture deep, postero-lateral corners prominent, carinate; pedicel, short, slender, ventral flange continuing throught peduncle; node of petiole subequal. Size: 4.8 mm.

Different Characters:

Mandibles ending in darker spiny end, gaster with distinct sutures on lateral and ventral side, delicate sting present.

Habitat:

They make small nests on ground by excavating soil.

Material examined:

India, Maharashtra, Aurangabad, around Gautala, January 2010 – February 2012.

Distribution:

India from Punjab to Kerala Sheela, (2008) Bharti Himender, (2009). Plate No-8

Monomorium indicum (Forel, 1902)

Diagnostic Characters:

Head, thorax and pedicel ferruginous red, the legs and in many specimens the head also verging to brown; abdomen dark brown or black; head, thorax and abdomen rugolose, opaque, the head and thorax anteriorly in certain lights appearing densely and extremely finely longitudinally striate; abdomen minutely reticulate; in some specimens the 2nd and following segments are smooth, polished and shining; pilosity entirely wanting. Head broad, almost as broad as long, broader anteriorly than posteriorly, the hinder margin slightly concave; mandibles narrow, obscurely longitudinally striate, when closed partially concealed under the projecting margin of the clypeus, the latter obtusely bicarinate; antennae moderately long, the scape not quite attaining the posterior margin of the head; eyes comparatively large and flat, placed about the middle of the side of the head. Thorax anteriorly rounded, moderately broad, the meso-and metanotum narrow and strongly compressed, the meso-metanotal suture, distinct; the thorax in profile emarginated at the suture, the basal portion of the metanotum broadening posteriorly. Pedicel: the nodes, seen from above, nearly equal, the rounded 1st node higher than the 2nd and petiolate anterirly; abdomen oval.

Habitat:

They make small nests on ground by excavating soil.

Material examined:

India, Maharashtra, Aurangabad, around

Gautala, January 2010 – February 2012.

Distribution:

India from Punjab to Kerala Bingham, (1903, Bharti Himender, (2009). Plate No-7

Genus Cataulacus Smith

Head broad, somewhat flat, very slightly convex above, posteriorly transverse or slightly emarginate, with the posterior lateral angles always prominent, dentate or spinous, anterioriorly somewhat narrowed, but the sides of the head for their posterior two-thirds are straight, the anterior third being suddenly curved to the base of the mandibles; sides of the head deeply grooved to contain the folded antennae, the latter when thus folded being invisible from above; mandibles somewhat broad from the base and armed with 4 or 5 teeth; the base partially hidden under the lateral laminate and slightly curled up; clypeus bent downwards at an angle to the front of the head, triangular, and generally emarginate anteriorly in the middle; frontal area triangular, fairly distinct; antennal groove placed below the eyes; the latter large, lateral and frontal, placed about the middle of the head; antennae short, stout; scape and flagellum subequal, the latter with 10 joints, the apical three long and incrassate, forming the club. Thorax broad and a little convex above constricted posteriorly, the basal portion of the metanotum with spines or at least with obtuse teeth at the lateral angles; the sides and apex of the thorax more or less vertical and concave; the margins of the head and thorax denticulate; legs stout, short, the tibiae flat above and laterally margined; claws dentate at base. Pedicel with the nodes more or less globose, not or very shortly petiolate, in some species dentate beneath; abdomen broadly oval, with the front emarginate at the junction of the pedicel Bingham, (1903)

Cataulacus latus (Forel, 1891)

Diagnostic Characters: Dull ink –black, with a mere touch of castaneous brown at the apex of the scape and of the flagellum of the antennae, and at the joints of the legs; pilosity almost altogether absent, merely a few very short white bristly hairs, chiefly at the apex of and beneath the abdomen; head, thorax and abdomen finely punctured, granulate and opaque, the legs and pedicel coarsely rugose, granulate, the margins, lateral and posterior, of the head and thorax studded irregularly with little blunts points; the sculpture on the head, thorax and abdomen in certain lights running into striate. Head much broader than long, lightly convex, the occiput widely emarginated, the posterior lateral angles prominent, slightly dentate; mandibles subtriangular, obscurely striate; clypeus large, widely emarginate anteriorly; antennae stout, when foiled completely hidden from above in the deep fossa beneath the eyes. Thorax: the pronotum broader than long, the anterior margin broad and transverse, the pro-mesonotal suture distinct and arched to the front; the mesonotum narrower than the pronotum, transversely oval, the meso-metanotal suture visible but not well marked, transverse; metanotum a little depressed, the basal portion about twice as broad as long, the sides denticulate and prolonged posteriorly into long laminate spines pointing backwards and curved a little upwards; legs stout and short, more thickly studded than the body with short stout white bristles. Pedicel short, the nodes not petiolate, thick, nearly subequal in length; front, broader than long; abdomen chordate, convex above, emarginated anteriorly Black, larger in size-5mm

Different Characters:

Postpetiole rounded, acidopore tapering at posterior end of gaster, surrounded by few long white hairs.

Habitat:

Mostly found on tree and sometime on trunk of the tree

Material examined:

India, Maharashtra, Aurangabad, around Gautala, January 2010 – February 2012.

Distribution:

West Bengal, Punjab Bingham, (1903) Bharti Himender, (2009). Plate No-5

*Genus **Myrmicaria*** Saunders

Head short, more or less rounded; mandibles thick, broadening only a little towards the

masticatory margin, this latter oblique and armed with 4 acute teeth; clypeus broad, arched in front, the medial portion convex, posteriorly somewhat narrowly rounded between the bases of the antennae; antennal carinae parallel, wide apart, front area not clearly defined posteriorly; antennae 7-jointed, the scape cylindrical, the joints of the flagellum slender, much longer than broad, no distinct club; eyes round, somewhat prominent, placed on the sides of the head a little to the front and closer to the top than to the anterior margin of the head. Thorax the pronotum more or less globose, convex and rounded above, with the anterior lateral angles above and below marked by distinct tubercles or spines; pro-mesonotal suture obsolete; mesonotum ending posteriorly in a more less thick, very distinct transverse carina often subdentate at the lateral angles; meso-metanotal suture deep, the thorax incised or narrowly emarginated at the suture; metathorax cubical, compressed, the basal and apical faces subequal, concave from one side to the other, and slender. Pedicel nodes conical, subequal, slightly compressed, with a distinct but narrow upper surface, the 1st node with a long petiole anteriorly and a very much shorter petiole posteriorly; abdomen broadly oval, subglobose Bingham, (1903)

Myrmicaria brunnea (Saunders, 1842)

Diagnostic Characters:

Dark red to brownish black; mandibles and scape of antennae finely longitudinally striate; head and thorax widely striate, vertex rugoso-reticulate; nodes of pedicel and gaster smooth, polished and shining; pilosity abundant, long, reddish yellow; very long setae also spread all over body; minute pubescence present on flagellum and coxae; head more or less rounded; mandible with 4 teeth; antennae 7 segmented; a median longitudinal carina running parallel to frontal lobes extending up to vertex; pronotum globose, convex and rounded above with anterior lateral angles above and below marked by distinct tubercles, prosternal tubercles developed into acute spines beneath; pronotum broad, pro-mesonotal suture obsolete, mesonotum ending posteriorly in a transverse carina, subdentate at lateral angles; metanotum in a level lower than mesonotum, narrow, cubical, dorsally concave, sides margined by a carina, meso-metanotal suture distinctly indicated; propodeal spines acute, oblique; nodes of pedicel conical, sub-equal, slightly compressed with a distinct but narrow surface; peduncle of petiole long; length of petiole and post-petiole subequal; abdomen broadly oval, subglobose. Size: 5.5-8 mm.

Habitat:

They are Sluggish, attract make subterranean nest with some outlets on surface.

Material examined: India, Maharashtra, Aurangabad, around Gautala, January 2010 – February 2012.

Distribution:

India, Punjab Bingham, (1903 Bharti Himender, (2009), Sheela, (2008). Plate No- 9

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Identification of Differentially Expressed Gene Due to Tobacco & Smoking In Normal Human Vs Lung Cancer patients

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Abstract

The purpose of this study was to identify the differentially expressed genes (DEG) in normal human and lung cancer patient. And also evaluate the relationship between buccal epithelium,nasal epithelium,lung tissues,lung cancer due to tobacco and smoking and related common genetic factors. R is a Programming Language and software environment for Statistical Computing and graphics. The R language is widely used among Statisticians and data miners for developing Statistical Software and data analysis. Polls, surveys of data miners, and studies of scholarly literature databases show that R's popularity has increased substantially in recent years. R is an implementation of the S programming languages , New Zealand, and is currently developed by the R Development Core Team, of which Chambers is a member. R is named partly after the first names of the first two R authors and partly as a play on the name of S.R is a GNU Project The Source code for the R software environment is written primarily in C, Fortran, and R.

Keywords:- DEGs, buccal epithelial, lung, R-GNU, graph.

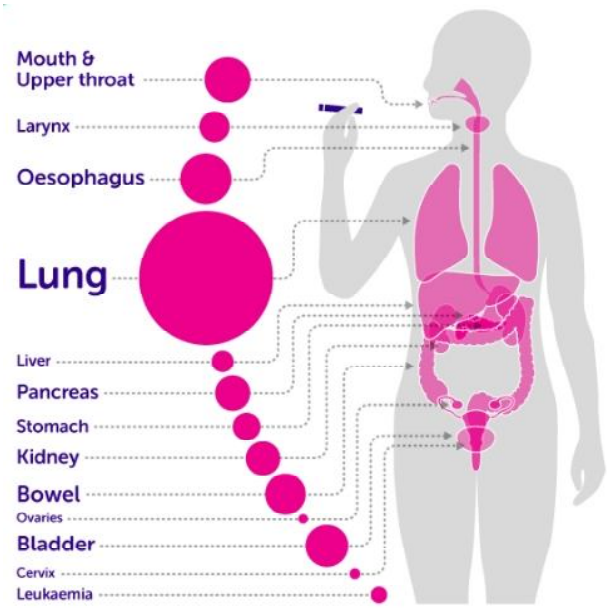
1. Introduction

Functional genomics involves the analysis of large datasets of information derived from various biological experiments. One such type of large-scale experiment involves monitoring the expression levels of thousands of genes simultaneously under a particular condition,called gene expression analysis. gene expression that responds to signals or triggers; a means of gene regulation, effects of certain hormones on protein biosynthesis. Different protocols and methods are used to identify the differentially expressed genes in normal person Vs lung cancer patients means which genes are present in Aerobic, Anaerobic and genes in both the condition.

A. How Gene are expressed

Gene expression is the process by which the instructions in our DNA are converted into a functional product, such as a protein. Gene expression is a tightly regulated process that allows a cell to respond to its changing environment.When the information stored in our DNA is converted into instructions for making protein or other molecules, it is called gene expression It acts as both an on/off switch to control when proteins are made and also a volume control that increases or decreases the amount of proteins made.Gene expression can be regulated by various cellular processes with the aim to control the amount and nature of the expressed genes. Expression of genes can be controlled with the help of regulatory proteins at numerous levels. These regulatory proteins bind to DNA and send signals that indirectly control the rate of gene expression. The up-regulation of a gene refers to an increase in expression of a gene whilst down-

regulation refers to the decrease in expression of a gene.



Pic.1

Effects of Smoking in different Organ

(Referred by Cancer research centre UK)

2.Materials & Methods

A)DEGs

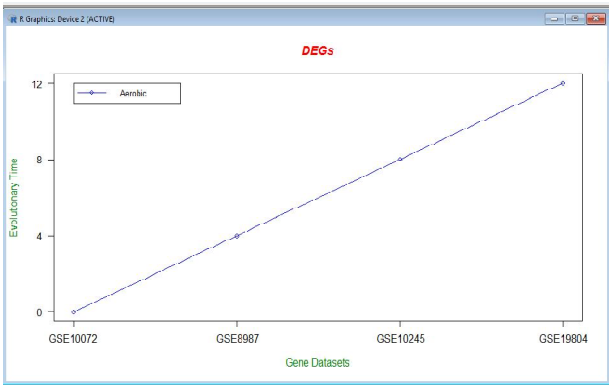
The translation of information encoded in a gene into protein or RNA structures that are present and operating in the cell. Expressed genes include genes that are transcribed into messenger RNA(mRNA) and then translated into protein, as well as genes that are transcribed into RNA, such as transfer and ribosomal RNAs, but not translated into protein. In this article, we tried to review the datasets of Some gene which are expressed in normal person and infected one due to the tobacco & smoking which causes lung, or may get infected to different parts of the body due to that carcinogens.

B.R-R is a language and environment for statistical computing and graphics. In this article we have used R language to show the Gene expression level in different datasets of normal person & infected ones in Aerobic and Anaerobic condition or in Both by using Graph.

C) Gene Expression Level

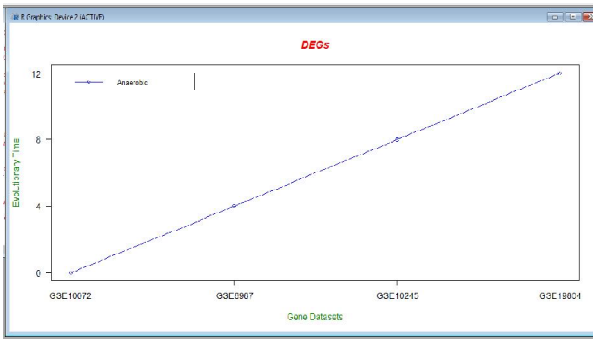
In Gene expression level we have used three criterions for identifying that how gene express in Normal human & Infected person due to Tobacco and Smoking in different conditions like Aerobic and Anaerobic or in Both conditions. When any particular person addicted to the Tobacco and Smoking then Some genes are expressed differentially than the gene in Normal person. Chemicals in cigarette smoke enter our blood stream and can then affect the entire body. This is why smoking causes so many diseases, including at least 14 types of cancer, heart disease and various lung diseases. How does smoking & Tobacco cause cancer? The main way that smoking causes cancer is by damaging our DNA, including key genes that protect us against cancer. Many of the chemicals found in cigarettes have been shown to cause DNA damage, including benzene, polonium-210, benzo(a)pyrene and nitrosamines. Smokers are also less able to handle toxic chemicals than those with healthy lungs and blood. Chemicals in cigarette smoke make it harder for smokers to neutralise or remove toxins, and can make their immune systems less effective too. Smoking also increases the risk of at least 13 other cancers including cancers of the mouth, pharynx (upper throat), nose and sinuses, larynx (voice box), oesophagus (gullet or food pipe), liver, pancreas, stomach, kidney, bowel, ovary, bladder, cervix, and some types of leukaemia Smoking could increase the risk of breast cancer, but any increase in risk is likely to be small.

D) Graphs for the DEGs in different Conditions



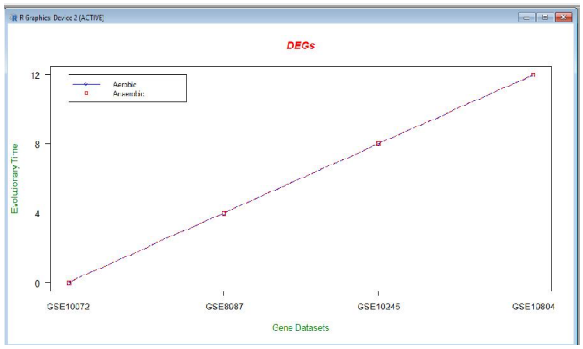
1. Aerobic Condition

Fig1



2. Anaerobic Cndition

Fig 2



3. In Both Aerobic & Anaerobic Condition

Fig 3

c) Table represent all DEGs in range of GSE datasets From GEO NCBI database:

DEGs Symbol	DEGs	GEO Accession
XIST	Xin active specific transcript	GSE10072
KRT7	keratin 7	GSE10245
HLA-DQA1	classII,DQ alpha 1	GSE19804
ADH6	alcohol dehydrogenase 6 (class V)	GSE8987
CYP1B1	cytochrome P450 family 1	GSE8987

Conclusion

Target of our research work was to achieve the expression levels of genes specifically studied for tobacco and smoking which gives different types of cancer like mouth, lung, etc.

Methodology implemented for our study was to extract those genes dataset from database, study based on their aerobic and anaerobic condition and sort there expression level, once obtained applied R statistical language to get the plot (fig no 1,2,3), which concludes that genes are responsible for diseases are expressive in both the conditions (aerobic and anaerobic) and implementing R gives graphical representation of our datasets, that R codes can further be used for any other datasets.

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Study of Tau Protein based on artificial neural network (ANN) associated with neurological diseases using Bioinformatics

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Abstract

ANN (Artificial Neural Network) generally referred as neural networks are the information or signal processing mathematical model that is basically based on biological neurons. It is a complex structure which consist of a group of interconnected neurons which provides a very exciting alternatives for complex problem solving and other application which can play important role in today's advancement of technology and computer world, so scientist and researcher from different streams are planning and designing the ANN to solve the problems for pattern recognition, sequence and structure prediction, optimization, associative memory and there control.

In this paper we have presented the basic study of the artificial neural network, its application and advantages also tried to study the GEO dataset based on neurological disease associated protein TAU and its relation to CNS central nervous system, also how the layers develop and signal processing takes place.

Keywords: *Neural Network, Neuron, Tau, NCBI GEO Dataset, Bioinformatics.*

1. Introduction

A neural network is an interconnected assembly of simple processing elements, *units* or *nodes,* whose functionality is loosely based on the animal neuron. The processing ability of the network is stored in the inter unit connection strengths, or *weights,* obtained by a process of adaptation to, or *learning* from, a set of training patterns.

1.1 What is a Neural Network:

An Artificial Neural Network (ANN) is an information processing paradigm that is inspired by the way biological nervous systems, such as the brain, process information. The key element of this paradigm is the novel structure of the information processing system. It is composed of a large number of highly interconnected processing elements (neurons) working in unison to solve specific problems. ANNs, like people, learn by example. An ANN is configured for a specific application, such as pattern recognition or data classification, through a learning process. Learning in biological systems involves adjustments to the synaptic connections that exist between the neurons. This is true of ANNs as well.

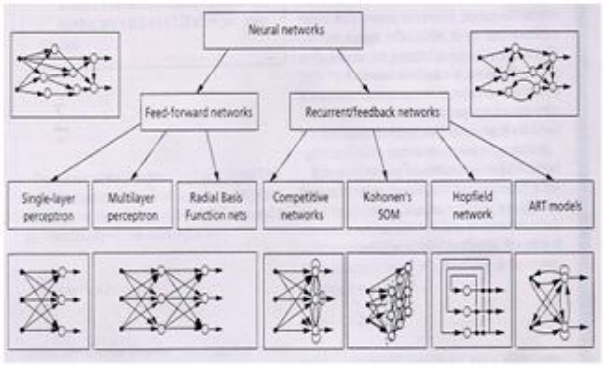

Fig: 1 Simple Neural Network

1.2. Type of neural network

Neural networks have the unique ability to derive meaning from complex and imprecise data.

Neural networks can easily extract trends and patterns that are way too complicated for humans or other computer techniques to extract. Neural networks that are thoroughly trained can be thought of as 'experts' in the areas of information that have been given to them for analysis.

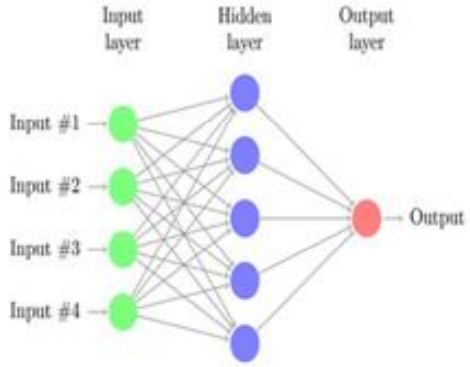

Fig:.2 Multilayer Artificial Neural Network

1.3 Feedforward Neural Network

The simplest of all neural networks, the feed forward neural network, moves information in one direction only. Data moves from the input nodes to the output nodes, passing through hidden nodes (if any). The feed forward neural network has no cycles or loops in its network.

1.4 Radial Basis Function Neural Network

The RBF neural network is the first choice when interpolating in a multidimensional space. The RBF neural network is a highly intuitive neural network. Each neuron in the RBF neural network stores an example from the training set as a "prototype". Linearity involved in the functioning of this neural network offers RBF the advantage of not suffering from local minima.

1.5 Kohonen Self-Organizing Neuralnetwork

Invented by Teuvo Kohonen, the self-organizing neural network is ideal for the visualization of low-dimensional views of high-dimensional data. The self-organizing neural network is different from other neural networks and applies competitive learning to a set of input data, as opposed to error-correction learning applied by other neural networks. The Kohonen self-organizing neural network is known for performing functions on unlabeled data to describe hidden structures in it.

1.6 Recurrent Neural Network

The recurrent neural network, unlike the feed forward neural network, is a neural network that allows for a bi-directional flow of data. The network between the connected units forms a directed cycle. Such a network allows for dynamic temporal behavior to be exhibited. The recurrent neural network is capable of using its internal memory to process arbitrary sequence of inputs. This neural network is a popular choice for tasks such as handwriting and speech recognition.

1.7 Modular Neural Networks

This interesting neural network comprises of a series of independent neural networks that are moderated by an intermediary. Each of these independent neural networks works with separate inputs, accomplishing subtasks that make up the task the network as whole hopes to perform. The intermediary accepts the inputs of each of these individual neural networks, processes them, and creates the final output for the modular neural network. The independent neural networks do not interact with each other.

1.8 Physical Neural Network

This neural network aims to emphasize the reliance on physical hardware as opposed to software alone when simulating a neural network. An electrically adjustable resistance material is used for emulating the function of a neural synapse. While the physical hardware emulates the neurons, the software emulates the neural network.

2. Main Architectures of Artificial Neural Networks

In general, an artificial neural network can be divided into three parts, named layers, which are known as:

(a) Input layer This layer is responsible for receiving information (data), signals, features, or measurements from the external environment. These inputs (samples or patterns) are usually normalized within the limit values produced by activation functions. This normalization results in better numerical precision for the mathematical operations performed by the network.

(b) Hidden, intermediate, or invisible layers These layers are composed of neurons which are responsible for extracting patterns associated with the process or system being analyzed. These layers perform most of the internal processing from a network.

(c) Output layer This layer is also composed of neurons, and thus is responsible for producing and presenting the final network outputs, which result from the processing performed by the neurons in the previous layers. The main architectures of artificial neural networks, considering the neuron disposition, as well as how they are interconnected and how its layers are composed, can be divided as follows:(i) single-layer feed forward network,
(ii) multilayer feed forward networks,

3. Tau:

Tau proteins are proteins that perform the function of stabilizing microtubules. These proteins are abundant in nerve cells and are present to a much lesser degree in oligodendrocytes and astrocytes. The tau proteins are the product of alternative splicing from a single gene that in humans is designated MAPT (microtubule-associated protein tau) and is located on chromosome 17. Tau proteins are mainly active in the distal portions of axons where they stabilize microtubules as well as providing flexibility. The proteins work together with a globular protein called tubulin to stabilize microtubules and aid the assembly of tubulin in the microtubules.

4. Methodology:

GEO (gene expression omnibus) datasets have been used for the said study, datasets have been shortlisted based on the criteria of TAU protein (found abundantly in neurons as compare to elsewhere in the body), also associated with Alzheimer's and Parkinson's Diseases. These datasets have been extracted from NCBI GEO and sorted according to the requirement of the application used for Neural Network Construction (JNN) (figure 3), which provides the connection between input layer and output layer and signal flow of from input to output using the hidden layers, also the energy level (i.e. minimum, optimum, maximum) (figure 4) in graphical format which shows how energy level increases or decreases at regular interval if level changes.

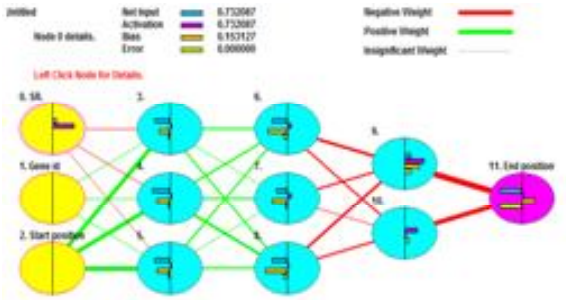

Figure 3:- JNN showing input layer, output layer, and hidden layer of neural network of TAU.

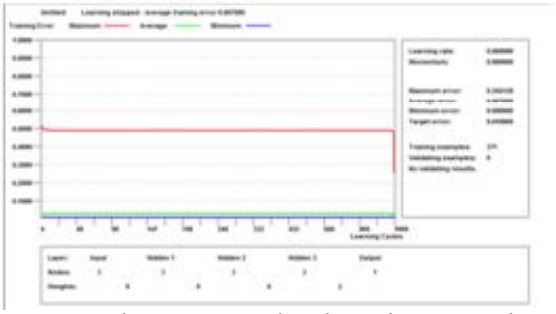

Figure 4:- shows energy level maximum, optimum, minimum.

Advantages of ANN:-

Various applications are available of ANN

a) Self organization: A neural Network can create its own representative.

b) RTO: Real Time Operations, in ANN computations can be carried out parallelly.

c) Pattern Recognition: one of the powerful technique, which helps for data security, which helps to learn to recognize the patterns if exist in data set.

d) Neural network are flexible in a changing environment.

e) ANN can build informative models whenever conventional approaches fail, because neural networks capable to handle complex interactions which can be easily model, which is difficult and tedious if processed with traditional approaches.

Limitations: There are some demerits of ANN which are as follows:

a) ANN or neural networks is not a daily life problem solver.

b) There is no structured methodology available.'

c) There is no single standard parameter/ protocol for neural development.

d) Description of problem solving method is not available.

Application of ANN:

a) Call control: answer the incoming call with a swipe of hand while driving.

b) Data pre-processing including filtering, clustering, blind signal separation and compression.

c) Classification, including pattern and sequence recognition, pattern detection, and sequential decision making.

5. Result:-

Obtained output as shown on figure 4 & figure 5, the sorted data of NCBI GEO dataset gives the 3 layer (Input, Hidden, and output) shows the transfer of signal from one end to another with minimum to maximum energy levels, which leads to the optimum path which has to follow to transfer the signals (messages) from one cell to another. For this process the sorted data which was utilised was of GENEID and the length of the gene sequences co-associated with neurological diseases (Parkinson's and Alzheimer's).

6. Conclusion:

In this research work we tried to explore and study the horizon of ANN (Artificial Neural Network), how it works, its advantages, limitations, and application of the topic.

There are various advantages of ANN over conventional approaches, depending on the nature of the data patterns which can generally expect a network. Which leads to solve the problem to conclude the relationships by dynamic or non linear one.

By exploring ANN we had concluded that as the technology is increasing the need of ANN is also improving because of parallel processing. Parallel processing allows us to proceed for multi tasking, which is in need due to advancement of technology.

For future it is need to develop algorithms and programs used for the study of ANN and make it more and more useful for the various kind of applications.

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Novel methods of chromate uptake using a screened consortium based on reusability & ease of storage.

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Abstract :

Chromate uptake using a consortium obtained from industrial effluents was studied using some novel techniques such as nutrient limitation , the use of biological contactors, immobilized consortium and dead cell biomass. The chromate uptake increase from 17.6 % to 25.8 % under nutrient limitation. Chromate uptake in a biological contactor was highest after 20 minutes of contact.(23.9%). Uptake varied from 7 to 58% over a period of 13 days in this contactor and efflux was also noted. The immobilised consortium also showed 28% chromate uptake at 10µg/ml of Cr(VI) by immobilized cells in 24h. At 20 and 50 µg/ml , 25 and 6 % uptake was noted respectively. Consortium lost its viability as a result of heating in boiling water bath for 20 min. In minimal medium 100% uptake has been demonstrated by live consortium while an average of 20.78% uptake has been observed by dead cell biomass in minimal media and no uptake was detected in Phosphate buffer.

Keywords : Cr(VI), consortium, biological contactor, immobilization.

Introduction :

The production of heavy metals depends on its industrial demand which are manifold with respect to chromium . The widespread use of chromate in industries such as leather tanning, metallurgy, electroplating, petroleum refining, textile manufacturing, and pulp production (Chung *et al.*, 2007) to chrome plating and polishing operations, ore processing (Ackerly *et al.*,2004), paints and pigments (Ackerly *et al.*,2004; Salunkhe *et al.*,1998) has resulted in large scale release of chromium into the environment. Hexavalent chromium is a common contaminant associated with nuclear reactors and fuel processing (Oliver *et al*, 2003; Ackerly *et al.*,2004). Further the biomagnification and the solubility of the metal species helps in its entry in the highest strata of the food chain which poses a serious threat to human health. This study aims at use of non nutrient media, reusability of the consortium and dead cell usage that minimizes the cost associated with storage and maintenance of the cultures.

Collection of samples:

Five different effluent and soil samples from disposal sites of electroplating industries were collected. Three of these were from metal plating industries in and around the industrial township of Ludhiana and one each from Waluj (Aurangabad, India) and Kalwa (Thane, India).

A bacterial consortium consisting of 10 organisms was raised from enrichments using above mentioned samples and selective microbiological techniques.(Bhattacharya and Lomte, (2009;2011).

Chromate uptake under conditions of nutrient limitation:

The possible cellular changes on account of nutrient limitations and its subsequent effect on chromate uptake was observed in appropriately diluted Glucose minimal media (Khare *et* al.1997). The consortium was grown in minimal medium spiked with 1µg/ml of sterile dichromate ($A_{6.00}$ =0.80). Glucose minimal medium was reduced to ¾ , ½ and ¼ of its original strength by diluting it with sterile distilled water. The consortium was inoculated at 5% of the total volume to which dichromate was added at 50, 100 µg/ml (not reported) and also at 150µg/ml. The flasks were incubated at room temperature (35^{0}C) for 24h

under static conditions. Uninoculated controls were used for recording initial dichromate throughout the incubation period. Flasks that did not contain dichromate were used for recording the extent of growth that occurred due to nutrient limitation. Residual dichromate was measured colorimetrically by diphenyl carbazide reagent (Urone, 1995) after 24 h of growth.

Chromate uptake using a biological contactor operated under static conditions:

Efficiency of chromate uptake in artificially entrapped biofilms was investigated in this study.

The cells were entrapped in the biofilm according to the procedure of Xu *et al* 1996.(Ganguli and Tripathi,2002) as follows: A static biological contactor was developed using inert matrix support of 4% agarose and 4% alginate . The slurry (50ml) was mixed with 5g (wet weight) of consortium (A_{600} = 1.0, total proteins 1100µg/ml) at 50 ^{0}C for 5 minutes and applied in a thin layer over a nylon sieve (20 cm diameter). Prior to inoculation, 50µl of toluene was mixed with the consortium and kept on a shaker for 30 min. at room temperature, so as to permeabilise the cells. The suspension was centrifuged at 10,000 g for 10 min. The pellet was washed twice in sterile distilled water and briefly vortexed before use. In the same manner an uninoculated contactor was also prepared. The matrix was allowed to settle on the nylon mesh overnight immersed in phosphate buffer pH 6.8.

Artificial effluent was prepared by adding dichromate at a final concentration of 50µg/ml in phosphate buffer pH 6.8. The consortium loaded nylon sieve was secured on both sides and suspended in a plastic trough. The effluent sample was sprayed through a sparger on to the sieve . After the entire content was sprayed, the effluent was recycled manually, allowing effective contact between the effluent and immobilized consortium. Finally the nylon sieve was submerged in the effluent and mildly agitated by using a fish tank aerator. Appropriate aliquots of the effluent were removed at intervals of 10 min. after spraying and estimated for residual dichromate using the diphenyl carbazide reagent. In all, observations were recorded for up to 40 min. In order to rule out significant adsorption of chromate by the supporting material, the same procedure was repeated concurrently with the unseeded contactor. Four samples were analysed and mean values considered for reporting of chromate uptake.

Reusability of the contactor :

Chromate uptake at 50µg/ml dichromate was monitored in a similar set up for up to 13 days to determine the reusability of the immobilized consortium. The sieve (29cms diameter) was bigger than the earlier one which therefore provided a greater contact area. The A_{600} of the consortium that was used for seeding of the agarose-alginate matrix was 1.50. After 24 h. the consortium loaded nylon sieve was thoroughly rinsed in phosphate buffer pH 6.8 and allowed to rest in the buffer for 1h. before resuming the uptake once again. Buffer containing 50µg/ml dichromate was replaced every day. Initial dichromate was estimated immediately after spraying the effluent and also sampled from unseeded controls. Residual dichromate estimation was done in triplicates after 24h of retention. Mean values have been reported**.** The same procedure was repeated with the unseeded contactor.

Cr(VI) uptake by immobilised consortium:

In order to observe chromate uptake in immobilized cells, the consortium was pregrown with 1µg/ml dichromate for 48h and harvested by centrifugation at 10,000g for 10 min. It was resuspended in sterile phosphate buffer pH 6.8 and briefly vortexed.(A_{600} = 1.10; Total proteins (lowry et al., 1951) = 750µg/ml).

Immobilization was done by a modified cell entrapment method using sodium alginate as described by Wuyep *et al.*(2007). 7g of alginate was transferred to a 250 ml conical flask containing 75 ml distilled water and mixed thoroughly. The mixture was allowed to settle and autoclaved at 121^{0}C for 15 min. The mixture was cooled to 40 ^{0}C to which 25ml of the consortium was transferred. The mixture was subsequently pumped through a 1ml micropipette tip, drop wise, into a flask

containing sterilized 100 ml of 0.12 M calcium chloride solution. The reaction, which was almost instantaneous, was allowed a retention time of 1 h for complete precipitation that formed spherical beads. The immobilized beads were removed and stored until use at 4^0C in 5 mM $CaCl_2$ solution. The beads were washed with doubled distilled water and used for Cr(VI) uptake. Comparative uptake in immobilized, free cells and unseeded beads was monitored in phosphate buffer.(Banerjee *et al*.,2004). Unseeded alginate beads prepared as above served as controls for monitoring chromate uptake by the matrix material. The number of beads formed per ml of the alginate-consortium mixture was 10. The beads (seeded or unseeded) numbering 40 per flask and equal amounts of free cells (1.32ml) were transferred to individual culture flasks to a final volume of 25 ml of phosphate buffer pH 6.8. The respective sets were kept at room temperature for 24h. Chromate uptake was monitored at 10, 20 and 50µg/ml.

Chromate uptake by dead cell biomass:

Overnight culture of consortium was developed in nutrient broth spiked with 1µg/ml dichromate, harvested by centrifugation and washed as before. The pellet was suspended in 5ml of sterile phosphate buffer and briefly vortexed. Appropriate amounts of the consortium was placed in a boiling water bath for 20 min. Phosphate buffer pH 6.8 containing 10µg/ml dichromate was inoculated with 10% of the treated consortium. Viability checks were carried out by spreading out 10µl of (1:10,000 diluted) previously boiled suspension (A_{600}=0.65) on nutrient agar plates. Consortium (A_{600}=0.65) that contained live cells was also inoculated in the same way. The sets were incubated at room temperature (39°C) for 18h. Total proteins/ml of the consortium was determined before and after cell lysis using Lowry's method with BSA used as standard protein. Metal containing uninoculated sets were used for sampling of initial chromate. Sets were prepared in triplicates and initial as well as residual dichromate concentration for both live and dead cell biomass were recorded colorimetrically using the diphenyl carbazide reagent. In another experiment dead and live consortium was obtained as mentioned above and set to an A_{600} of 1.00. These were also inoculated at 10% the final volume in GMM containing 10µg/ml dichromate and incubated at room temperature (39°C) for 18h. Initial as well as residual dichromate was estimated using the diphenyl carbazide method as mentioned earlier. Total proteins and viability checks were also carried out as before.

Results and discussion :

The cultures selected for the consortium were screened using plate assays and pure cultures were identified using biochemical tests and are as per table 1 (Bhattacharya and Lomte ,2009; 2011).

Table 1 : List of isolates selected for raising the consortium.

Sr.no	Culture used
1	*Bacillus brevis*
2	*Bacillus subtilis*
3	*Alkaligenes sp.*
4	*Listeria sp.*
5	*Caryophanon sp.*
6	*Cellulomonas sp.*
7	*Curtobacterium pusillum*
8	*Corynebacterium xerosis*
9	*Sporolactobacillus inulinus*
10	*Curtobacterium citreum*
11	*Bacillus laterosporus*
12	*Micrococcus luteus*

Chromate uptake under conditions of nutrient limitation:

When cells are shifted to nutrient limited conditions, an early observation is a reduced ribosomal RNA production, followed by reduction in the production of protein and DNA. These reductions take place while cell division is still rapid, so cell size also decreases. Later, the rate of cell division slows to a rate that maintains this smaller cell size. The end result of this is that cells grow smaller when shifted to nutrient limited conditions. It was reported by Vance.(2002) that starvation of cells reduced their size to less than 0.3 microns, thereby reducing overall filtration effects and allowing for penetration of a finer grained matrix.

There was a slight increase (10.04%) in uptake observed with 25% dilution of the medium (Table 2), however further dilution reduced chromate uptake. 50% and 75% dilution of medium with distilled water caused marked nutrient limitation that eventually led to poor growth and hence poor uptake.

Table 2: Chromate uptake at 150 µg/ml Cr(VI) under nutrient limitation

Strength of medium	A_{600} 24 h.	A_{600} 48 h	Initial Cr (VI) µg/ml (0 h)	Residual Cr (VI) µg/ml (24 h)	% uptake 24h.	Residual Cr (VI) µg/ml (48 h)	% uptake 48h.
Normal strength without chromate	0.96	0.83	–	--	–	–	–
Normal strength with chromate	0.32	0.38	147.56	121.52	17.65	133.68	9.41
¾ strength medium (control)	0.82	0.64	–	--	–	–	–
¾ strength medium (with chromate)	0.20	0.20	147.56	109.37	25.89	144.09	2.36
½ strength medium (control)	0.59	0.55	--	--		--	--
½ strength medium (with chromate)	0.18	0.20	159.72	138.88	13.05	154.51	3.27
¼ strength medium (control)	0.28	0.24	--	--		--	---
¼ strength medium (with chromate)	0.04	0.10	144.09	130.20	9.64	138.88	3.62

Chromate uptake using a biological contactor operated under static conditions:

Effluent treatment processes are designed to ensure that when waste waters are discharged into natural water courses, adverse effects are reduced or prevented. (Atkinson *et al.*,1998). LaPaglia and Hartzell.(1997), observed that a biofilm is a functional consortium of microorganisms organized within an extensive exopolymer matrix comprising mainly of hydrated polysaccharides. Ganguli and Tripathi (2002) observed the chromate-reducing ability of *Pseudomonas aeruginosa* A2Chr and compared in batch cultures, with cells entrapped in a dialysis sac, and with cells immobilized in an agarose-alginate films in conjunction with a rotating biological contactor. In all three systems, the maximum Cr(VI) reduction occurred at 10 mg Cr (VI)/l.

Efficiency of chromate removal in artificial biofilms was carried out using a modification that allowed a similar treatment under static conditions. Polymeric materials that have been used earlier by different researchers are calcium alginate (Babu *et al.* 1993), polyacrylamide, polysulfone and polyethylenimine to name a few. In this study , Sodium alginate and agarose mixture was used in preparation of the polymer which was seeded with an active consortium. The uptake was 9.42µg/ml Cr(VI) out of the 50µg/ml Cr(VI) within 40 min. of contact. With respect to this biological contactor the uptake was highest after 20 minutes of contact.(12.12µg/ml uptake)and higher than the earlier reported results.(Table 3)

Table 3:

Chromate uptake in a static biological contactor.

Time elapsed (min.)	Samples for residual chromate analysis (µg/ml)				Mean residual Cr(VI) µg/ml	% uptake
	I	II	III	IV		
0 (initial)	52.08	50.34	50.34	50.34	50.69	0
10	48.61	48.61	46.87	46.87	47.74	5.82
20	39.99	38.19	38.19	38.19	38.54	23.97
30	39.99	39.99	38.19	39.99	39.40	22.28
40	41.66	41.66	39.99	41.66	41.14	18.85

The reusability of the immobilized biofilm was investigated by observing chromate uptake over 13 days period. Chromate uptake varied from 7% to 58% (Table 3a).The efficiency of the metal removal process is directly related to a high surface to volume ratio of the confining system.(Paperi *et al.*,2006). The surface area of this contactor was

more than the one used previously which therefore showed a higher uptake. Thus it can be said that biological contactors are efficient in removing heavy metals , but the matrices also have an ability to chelate chromate which has to be monitored from time to time.

Table 3a: Chromate uptake in a biological contactor with respect to time.

Days elapsed	Initial Cr(VI)	Residual Cr(VI)	% uptake
1st	50.34	38.19	21.44
2nd	41.66	26.04	37.5
3rd	45.13	41.66	7.69
4th	45.13	46.87	Efflux
5th	52.08	36.45	30.02
6th	46.87	36.45	22.24
7th	50.34	43.40	13.79
8th	50.34	24.30	51.73
9th	48.61	36.45	25.02
10th	52.08	26.04	50
11th	50.34	17.36	65.52
12th	50.34	30.83	38.76
13th	52.08	35.4	32.03

Cr (VI) uptake by immobilized consortium :

Leung *et al*.(2000),noted that calcium alginate is a well known biopolymer, biocompatible, chemically resistant, inexpensive and easy to regenerate. Besides, it has a loose structure for overcoming diffusion limitations and provides a good model system for adsorption. Banerjee *et al*.(2004) observed that immobilization brings about increase in growth by producing direct contact between the cells and the suspending medium, thus increasing the surface area available for various cellular reactions. The feasibility of an immobilized *B.coagulans* bioreactor for hexavalent chromium reduction was investigated by Iyengar *et al*.(1999).The best immobilization matrices as reported by Humphries *et al*.(2005) were agar and agarose, where the initial rates of reduction of Cr(VI) (from 500 ■M solution) for *D. vulgaris* NCIMB were 127 (agar) and 130 (agarose) nmol /h/ mg cell dry weight, respectively.This consortium also showed chromate uptake and approximately 28, 25 and 6% uptake was observed at 10, 20, 50 µg/ml Cr(VI) by immobilized cells in 24h (Table 4). Chromate uptake by free cells at the same cell density and inoculum level as used for immobilized cells was found to be significantly higher. Chromate uptake that occurred in unseeded control beads was substantial and therefore the net uptake of chromate by immobilized cells was ultimately lower.

Table 4:
Chromate uptake using immobilized whole cells.

Particulars	10µg/ml Cr(VI) 1h.(residual chromate)	10µg/ml Cr(VI) 24h. (residual)
Phosphate buffer pH 6.8 (initial)	12.15	12.15
Immobilized consortium	6.94	0
% uptake	42.89	100
Unseeded beads	10.41	3.47
% uptake	14.33	71.45
Whole cells	7.6	0
% uptake	37.45	100
% net uptake by immobilized consortium	28.56	28.55

It has been reported by Leung *et al* .(2000) that non viable or dead biomass can be obtained through selective pretreatments of biomass. Prokaryotic and Eukaryotic microbes are capable of accumulating metals by binding them as cations to the cell surface in a passive process.(Beveridge and Doyle 1989).

Table 5:
Chromate uptake by live and dead cell biomass of the consortium.

Chromate uptake	State of the consortium	Total proteins µg/ml	% uptake (18h)		
			Set I	Set II	Set III
Phosphate buffer	dead	124.56	0	0	0
Phosphate buffer	live	144	80	80	100
Glucose minimal medium	dead	255.36	17.36	21	24
GMM	Viable	236	100	100	100

It has been reported by several researchers that dead cells can bind metal ions. This has an advantage which is the ease of usage and storage

along with the fact that dead biomass can be easily regenerated and also reused. However the same was not observed in the case of this consortium in phosphate buffer. (Table 5).Phosphate and acetate buffers of different pH have been tested , however phosphate buffer 6.8 has been found to be suitable for chromate uptake.

Consortium lost its viability as a result of heating in boiling water bath for 20 min. since no colonies developed on nutrient agar. In minimal medium 100% uptake has been demonstrated by live consortium while an average of 20.78% by dead cell biomass which may be due to the presence of electron donors (e.g Glucose) in the medium used.

Conclusion :

Thus it may be concluded that biological contactors, immobilized consortia can be alternatives to the use of free cells and also has the advantage of reusability, ease of storage and above all a green technology for detoxification of a genotoxic pollutant.

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Impact of pesticides on Fresh Water Fish *Catla catla* of Mun Dam, District Buldhana (M.S.), India

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ABSTRACT

Industrialization of the world affects the faunal diversity of the water, as the waste water from these industries is directly dumped into the water bodies without any treatment. Water pollution is a global issue, as the rising population leads to a number of industries such as pesticides, paper, and fertilizers. *Catla catla* fish were catched from Mun Dam of Buldhana district. The fishes are nearly 100-150 gms. The LC50 value of Profenofos for *Catla catla* was 0.6 ppm. The subacute concentrations were 0.015 ppm, 0.030 ppm and 0.06 ppm were taken for concentrations. The total carbohydrate, protein and cholesterol concentration in the present study from Mun dam of Buldhana region (M.S.) India reveals a significant decrease in the level of carbohydrate, proteins and cholesterol in the fishes treated with profenofos.

Key words– Profenofos, Mun dam, pesticides, *Catla catla*

INTRODUCTION

Traditionally the problem of agricultural pest control has been dealt with by formulating new and

more potential pesticides. Very few pesticides kill the intended target but often lead to the death of many non target organisms. Due to increased public awareness of the potential of persistent pesticides that cause harm to environment and public health, great stress is being laid for developing least persistent and selective pesticides. Synthetic pyrethroids are one of wide variety of pesticides contributing to this situation. But these insecticides also tend to affect the biology of non target species along with pests These chemicals pollute the ponds, lakes, and river water. The chemicals from these industries directly affect the faunal diversity of our country and fish are mostly affected among them. Industrialization of the world affects the faunal diversity of the water, as the waste water from these industries is directly dumped into the water bodies without any treatment. Water pollution is a global issue, as the rising population leads to a number of industries such as pesticides, paper, and fertilizers. Pesticides are used worldwide in agriculture and aquaculture to control the pest and insects (Yonar *et al.,* 2012).

Organophosphate pesticides like ethion, dimethoate, monocrotophos and chlorpyrifos are widely used for paddy crop pests. The widespread use of synthetic organic pesticides over decades has let to their frequent exposure in the environment. Also acute and chronic exposures of humans to pesticides occur during their commercial production and their application. Fishes are a major source of proteinous food of our masses. Once the effluents are discharged into the water bodies, the toxins are incorporated in the bodies of fish resulting in bioaccumulation of the toxins and if these fish are consumed as food by the people, some of the toxins cause several health hazards in them due to biomagnifications. The pesticides, fertilizer chemicals can adversely affect the physiology, histopathology and biochemistry of fish fauna. These chemicals are sub-lethal for fish and their toxicity leads towards the mortality of fish. The aim of present study is to check out the sub lethal effects of pesticides and fertilizers chemicals on histopathology of fresh water fish *Catla catla*.

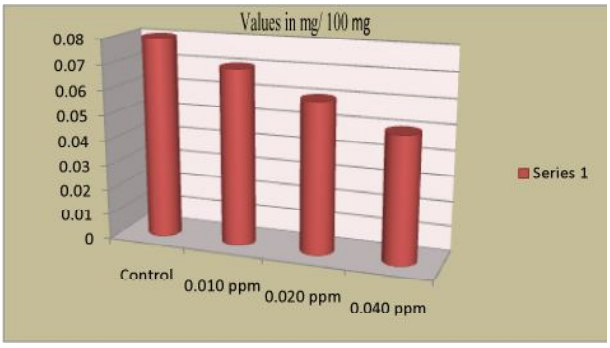
Material and Methods

Catla catla fish were cached from Mun Dam of Buldhana district. The fishes are nearly 100-150 gms. Healthy fishes were carefully packed in a medium sized polythene bag with sufficient oxygen which would help them to carry on their normal processes of metabolic activities during their period of transportation. When arriving in Laboratory fishes were carefully transformed into large glass aquarium. They were left for acclimatization in the normal laboratory conditions for a period of ten days. Feeding was stopped one day before commencing of experiments in order to minimize the quantity of excretory products in the test tank. After acclimatization the fish were weighted totally and individually, and then transformed into the treatment tubs carefully. The physico chemical conditions of well water used in the present study has the following characteristics; Dissolved oxygen 7.6-8 ppm; Salinity 0.5-0.7 ppm; Alkalinity 245 mg/l as CaCO3; Hardness 357mg/l; as CaCO3 PH: 7.2 to 7.5; Temperature : 27 ??2?C

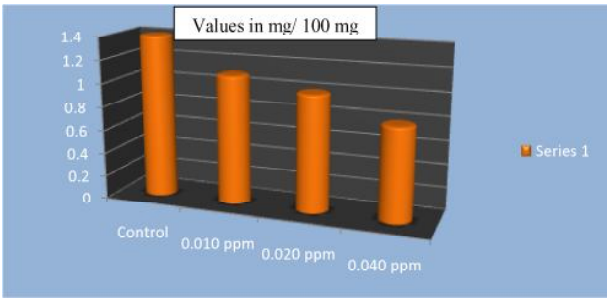
The fishes were exposed to different acute concentrations of Profenofos 50% Ec (0-4- bromo-2-chlorophenyl 0-ethyl S-propyl phosphorothioate) to arrive at LC50.The LC50 was determined following the procedure of Finney (1971). Based on the results of the acute toxicity study, with a LC50 value of 0.6 ppm doses of sub acute value of Profenofos 50%E.C. were selected for subacute exposure following the procedure of Desi et al. (1985). Fishes were divided in three treated and one control. In each aquarium 2 fishes are released. In the experimental set up profenofos sub acute values of 0.015, 0.030 and 0.06 ppm were added to the glass aquarium. Muscle sample from the experimental fishes were dissected out by sacrificing the fishes after 48Hrs time intervals. Muscle is the edible portion of the fish energy yielding substances like protein (Lowry et al. (1951) with Folin-Phenol reagent), carbohydrate (Anthrone method Roe, 1955) and cholesterol (Allain, 1974) in the muscle were analysed using standard procedures.

Results

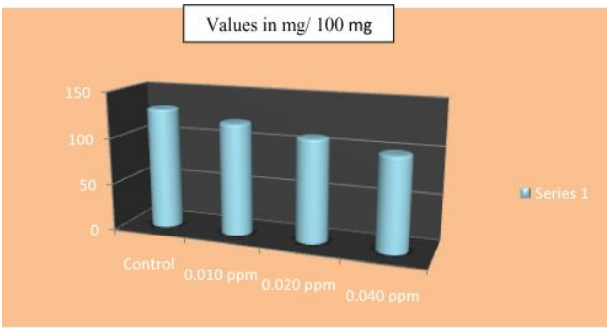
The LC50 value of Profenofos for *Catla catla* was 0.6 ppm. The subacute concentrations were 0.015 ppm, 0.030 ppm and 0.06 ppm were taken for concentrations. The concentration of Profenofos showed the spontaneous biochemical changes, and reduces the levels of biochemicals such as Protein, carbohydrate and Cholesterol.



Graph Plate No. 1
Conc. of profenofos
Estimation of Carbohydrate in the muscle sample of *Catla catla*



Graph Plate No. 2 Conc. of profenofos
Estimation of Protein in the muscle sample of *Catla catla*



Graph Plate No. 3 Conc. of profenofos
Estimation of Cholesterol in the muscle samples of *Catla catla*

Discussion

The biochemical analysis of the present study reveals a significant decrease in all the biochemical parameters. There was a significant reduction in the levels of carbohydrate content. Carbohydrates are the immediate source of energy in the cells. They play a major role in the cellular metabolism by serving as fuel and providing energy to the cells. Fluctuations in oxygen consumption reflect fluctuations in energy demands of the animal, changes in carbohydrate metabolism that would meet the changing energy demands may be expected to stress. The oxidation requirements of the living organisms require carbohydrate and they are used as chemical energy through the breakdown of glucose by the citric acid cycle (Quastel, 1969). The metabolism of carbohydrate decreases when the fish are exposed to toxicants is due to the fact that carbohydrate forms the immediate source of energy that increases the stress caused by the toxicant.

The total protein and cholesterol concentration in the present study from Mun dam of Buldhana region (M.S.) India reveals a significant decrease in the level of proteins in the fishes treated with profenofos. Protein and cholesterol is the most abundant carbon compound in all the living organisms (Parameswaran et al., 1987). It is the dominant biochemical constituent in the tissues of fishes (Pillay and Nair, 1973), Holbrook (1980) stated that the toxicant may directly cease protein synthesis.

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Production of an extracellular keratinase from *Bacillus amyloliquefaciens* KP015745 leather deteriorates

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Abstract

Extracellular keratinaseproducing bacteria wereisolated from deteriorated leather samples using feather meal as a substrate. From isolates, proficient keratinase producer was identified belong to genus *Bacillus.* 16s rDNA sequencing was done and sequence was deposited to GenBank with accession number *Bacillus amyloliquefaciens* KP015745. With 2% inoculum isolateproduced 38.59±3.72U/ml keratinase at optimum temperature 37°C and at pH 7 after72h using 1% feather meal as a substrate. Mg2+ and Mn2+stimulatedkeratinase production while mercaptoethanol completely inhibited the activity. The isolated bacterial keratinolytic protease was found to be metalloprotease, as the activity was inhibited by EDTA at the percentage 14.2% but not by PMSF. *Bacillus amyloliquefaciens* KP015745 also revealed the production of caseinase, gelatinase and collagenase. Investigationaloutcome propose that the isolate having ability to producevaried range

of enzymes can be competently used to treat keratin rich, leather wasteecofriendly.

Keywords: Leather,feather meal, metaloprotease, keratinase

Introduction:

Leather making is an important socio-economic activity for several countries throughout the world. Leather is used everywhere in daily life[1]. The most important for leather making is the protein. This protein may consist of many types: Collagen-on tanning gives leather; keratin- constituent of hair, wool, horn and epidermal structures. Approximate composition of a freshly-flayed hide: Water 64 % Protein 33 % (structural proteins and non-structural proteins) Structural proteins: Elastin 0.3 % Collagen 29 % Keratin 2 % Non-Structural proteins: Albumins, Globulins 1 %[2].LeatherTanning is a general term use for numerous processing steps involve in converting animal hide and skins in to final leather[3]. Traditional chemical leather processing generates huge amount of environmental pollutions. Whereas, enzyme biocatalysts were found to be effective in soaking, dehairing, bating and degreasing operations of environmental friendly leather processing[4].Leather industry has been categorized as one of the highly polluting industries and there are concerns that leather making activity can have adverse impact on the environment. The global production of about 24 billion m^2 of leather by 2005 presents a considerable challenge to the industry considering harmful nature of some of the chemicals used in leather processing .Solid waste generated in leather industries contribute mainly skin trimming, keratin wastes, fleshing waste, chrome shaving wastes and buffing waste. It constitutes protein as the main component [5].In poultry processing industries all over the world, chicken feathers are generally an unwanted waste by product. In Brazil 800,000 tons/ year of feathers are discarded by this sector. The accumulation of feathers can eventually lead to environmental pollution and can also be considered as a Traditional ways to degrade feathers such as alkali meal may destroy amino acids and they also consume large amounts of energy. Feathers are comprised essentially of keratin, an insoluble structural protein. Keratinous wastes are not degraded by commonly known proteases like trypsin, pepsin and papain due to presence of the disulfide bonds, but are easily degraded by keratinases. These peptidases are largely serine or metallopeptidases (EC 3.4.21/24) found in several microorganisms and have attracted a great deal of attention due to their multiple applications in industry for the development of nonpolluting processes of feather protein[6]**.** A significant amount of fibrous insoluble protein in the form of feathers, hair, nails, horn, and other are available as byproducts of agro industrial processing. These keratin-rich wastes are difficult to degrade as the polypeptide is densely packed and strongly stabilized by several hydrogen bonds and hydrophobic interactions, in addition to several disulfide bonds.If these protein and other chemicals, which are present in the chemical treated protein, are not utilized properly it will pose hazardous pollution problem to the environment the best remedy for this is to usedmicrobial keratinases are promising biocatalysts for several purposes, including applications in leather, fertilizer, feed, detergent textile industries, and also for biomedical and pharmaceutical applications[7].A number of keratinolytic microorganisms have been reported, including some species of fungi such as *Microsporum*, *Trichophyton* and from the bacteria *Bacillus,Bacilluslicheniformis*. *Burkholderia sp, Chryseobacteriaum sp, Pseudomonas sp*, *Microbacterium sp*.and *Streptomyces* and actinomycetes[8].

The present study objective for the proficientkeratinase production by *Bacillus amyloliquefaciens* KP015745 isolated from deteriorated leather samplesand its characterization.

Material and Methods:

Chemicals and Media: keratin azure (Sigma Aldrich, St. Quentin Fallavier and France), Nutrient broth (M002-100G), metal ions and organic solvents were obtained from Hi media Ltd., Mumbai. Phenylmethylsulphonyl fluoride [PMSF], Dimethylsulphoxide [DMSO], trichloroacetic acid

was obtained from Sigma Chemical Co. USA. Collagen peptide type I, TC343-Hi-Media. All other reagents used were of analytical grade.

Collection of sample:

Fifty deterioted leather samples were collected from Kedar leather industry, Aurangabad, Maharashtra state and different forms of naturally deteriorated finished leather's items like ladies footwear, belts, leather cases, gent's footwear, bags or purses from different places of Maharashtra. The samples were collected by the IS: 5868-1969 methods by Tran[9].

Feather meal substrate:

The feather meal as a source of carbon and nitrogen was prepared from native chicken feathers collected from Dept. of Animal Husbandry, Aurangabad, as described by Kate[10].

Assortment and identification of keratinase producer:

Keratinase producer bacteria were assorted from fifty deterioted leather samples using feather meal as a source of carbon and nitrogen. Initial isolation was carried out on nutrient agar. Among the different organisms isolated, one of colony showing highest zone of keratin hydrolysis on feather meal agar (feather meal 10g/L and agar-agar 2%) was selected for keratinase production [11]. According to the Bergey's manual of systematic bacteriology[12], morphological and biochemical characteristics of isolate was studiedand 16srDNA sequencing was done in GeneOmbio Technologies Pvt. Ltd., Pune, India.,and the sequences obtained was deposited to GenBank for accession number. Concurrently caseinase, collagenase and gelatinase activity were detected using 10% skimmed milk agar, 1%collagen agar and 1% gelatin agar respectively.

CrudeKeratinase production:

Proficient producers (B9) was selected for auxiliary study. The density of bacterial isolates (B9) of O.D600 1.0 [13] was used throughout the study. Triplicate set of 250ml flasks were inoculated by pure culture of the isolate into the production medium containingfeather meal (1%), yeast extract (0.01%), NaCl (0.05%), KH2PO4 (0.03%), K2HPO4 (0.04%) andMgCl2 (0.01%) of optimum pH7. Flasks were incubated at 37°C for 3 days at 180 rpm. The broth was centrifuged (REMIC-30 BL centrifuge, India) at 10,000 rpm for 10 min and the supernatant was used as a source of crude keratinase [10] and used further for assay.

Quantifying keratinase assay:

Quantification of keratinase assay was carried out in triplicates**,** the result was taken as an average of three replicates.Keratin from feather meal and keratin azure (Sigma Aldrich, St. Quentin Fallavier and France) were used as a substrate. The 5 mg keratin was suspended in 1 mL 50mmol/L Tris-HCl buffer (pH 8.0). The reaction mixture contained 1 mL keratin suspension and 1 mLappropriately supernatant. The reactions were carried out at 50°C with constant agitation of 200rpm/min for 1h. After incubation, the reactions were stopped by adding 2 mL 0.4M trichloroaceticacid (TCA) and followed by filtration to remove the substrate. The filtrate wasspectrophotometrically measured at 595 nm. One unit (U) of keratinase activity was defined as theamount of enzyme causing 0.01 increases in absorbance between sample and control at 595 nm afterone hour under the given conditions. The result was taken as an average of three replicates [14]. Both sources of keratin showed more or less same results hence, from economic point of view keratin from feather meal was used in further study.

Effect of culture conditions on the production of extracellular Keratinase:

The enzyme is extracellular which makes it particularly easy to recovery at the end of fermentation. The influence of percent inoculum was determined by adding cultures with different percent inoculum from 1, 2, 4 ,6 and10% to get optimum keratinase production in the medium at various incubation times such as 12, 24, 48, 72, 96 and 120 h. Feather meal was used as a substrate. The keratinase production by the selected bacterial isolates (B9) was studied by optimizing the media by adding different concentration of feather meal 0.5%, 1%, 1.5%, 2% and 2.5% in the media. Effect of pH and temperature on keratinase production was

individually tested by taking the production media at different pH ranging from 5, 6, 6.5, 7, 7.5, 8 and 8.5 and temperature from 10°C to 45°C, as mentioned as per method of Nagaraju et al.,[15] , Shivkumar et al.[16].Abiotic control (without bacteria) were always included. Amount of keratinase enzyme was checked every day till a decline was observed in the enzyme activity.

Determination of inhibitors and metals ions effect on keratinase production:

Byadding 1mM MnCl2, ZnCl2, HgCl2, CuSO4, MgCl2, sodium sulphite, cysteine, PMSF, EDTA, lead acetate, FeCl3, COCl2, CaCl2and 1% concentration of mercaptoethanol, glycerol, DMSO in the fermentation media,effect of inhibitors and metals ions on keratinase production was studied.The effect of them was measured by assaying activity every day for a period of three days at 50°C for 1h.[17, 18].Keratinase activity measured in the absence of any inhibitor or metal ions was taken as 100% relative activity. The aliquots of the samples were subjected to centrifugation and were used for assay. Amount of keratinase enzyme was checked every day till a decline was observed in the enzyme activity.

Result and Discussion:

Assortment and identification of keratinase producer:

Out of 91 isolates obtained in primary screening, 27 bacterial isolates were found to hydrolyze keratin, collagen, gelatin and casein. These isolates were subjected for secondary screening in terms of quantitative estimation of the amount of enzymes under study. Among isolates B9 Gram positive bacillus was found to be proficient keratinase producer.From morphological, biochemical, 16srDNA analysis and after submission of sequence to GenBank, the isolate was identified as *Bacillus amyloliquefaciens* KP015745 (strain JS518).

Quantifying keratinase assay:

After three days of incubation, supernatant obtained was subjected to keratin assay by using feather meal as a substrate.The *Bacillus amyloliquefaciens* KP015745 (strain JS518)(B9) was able to produce 38.59±3.72U/mlof keratinase after 72h at 37°C using feather meal as a substrate.*Bacillus cereus* produced 39.10±0.4 U/ml. *Bacillus* produced 29.5U/ml after six days incubation reported by Mozotto et al., [6]while Nagal et al., [19]in his study reported that,*Bacillus megaterium* KB008 produced 26.15±0.3 U/ml, *Bacillus subtilis* KB099gave 25.40 U/ml±0.1. In a present study, the isolate showed proficient keratinase production than the reports cited until after only three days of incubation.

Effect of culture conditions on the production of extracellular Keratinase:

The effect of various culture conditions such as inoculum size, incubation period, substrate, pH and temperature on keratinase production were studied by using *Bacillus amyloliquefaciens* KP015745 (strain JS518) (B9). All parameters were studied in triplicates.

Effect of Inoculum size -

In present work, *Bacillus amyloliquefaciens* KP015745 (strain JS518) (B9) showed keratinase production by using 1%-10% inoculum, initially after 24h. 2% inoculum showed maximum keratinase production (Figure 1) by*B. amyloliquefaciens* 38.1± 1.6 U/ml after 72h. Nagaraju et al., [15]reported that, keratinase production by *Pseudomonas aeruginosa* was detected at the 5% (112±2U/ml) of inoculum level. Shivkumar et al., [16]in their researchreported that, maximum keratinase production was recorded at 96 h in 4% of inoculum total activity 178.04±0.89 U/ml and minimum keratinase production was recorded at 12 h in 1% of inoculum 12.6±1.8 U/ml using *Bacillus thuringiensis* TS2 which was isolated from the feather.

Effect of incubation period-

The optimum keratinase production 38.9±1.6 U/ml was observed at 72 h by*Bacillus amyloliquefaciens* KP015745 (strain JS518) (B9), above this period the decline in the keratinase production was observed (Figure 2).This is because, the cells may reach the decline phase and displayed low keratinase synthesis[15].*Bacillus* strain SAA5, on

7th day [20]. The maximum activity for *Bacillus* TS2 in 96h was reportedbyShivkumar T.[16]B. *thurengensis* SN2 shown maximum production on 5th day [21], amongst the literature cited, isolates in the present study was shown highest keratinase production in minimum incubation period.

Effect of substrate concentration-

The efficiency of keratinase production was studied by using substrate concentration ranging from 0.5% to 2.5% (w/v).*Bacillus amyloliquefaciens* KP015745 (strain JS518) (B9) showed highest keratinase production (38.9±1.6 U/ml) at 1% concentration feather meal after 72h (Fig 3).Saibabu et al., [18] reported maximum keratinase production at 1.5% concentration within three days. *B. weihenstephanensis* PKD-5 showed 13.81U/ml at 1% within seven days[22].

Effect of pH-

In present work, *B. amyloliquefaciens* KP015745 (strain JS518) (B9) 38.9±1.6 U/ml showed keratinase production at pH 7 within 3 days(Figure 4). Above this level, the keratinase production decreased, because the metabolic activities of microbes depend on the pH change[15]. Maximum enzyme production was achieved at medium pH 7.5 for *Bacillus* sp. while *Bacillus licheniformis* gave 10.76U/ml of keratinase at pH 7 [23].

Effect of temperature-

B. amyloliquefaciens KP015745 (strain JS518) (B9) showed highestkeratinase production (38.9±1.6 U/ml) was at 37°C after 72h indicating 37°C as an optimum temperature for given isolate. Followed by this, 40°C temperature was the second best temperature for keratinase production. On the other hand, the minimum amount of keratinase production was observed at temperature 10°C and 45°C (Figure 5). Highest different temperature optimum temperature 60°C was reported by Shivkumar et al.[16] for*B. pseudofirmus* and 37°C for *Bacillus thuringiensis TS2* showed keratinase production of 41U/ml after 96 h. Mozotto et al.,[6] reported that, keratinase of *B. cereus* 1268 and *B. licheniformis* 1269were also optimally reported at 40°C.

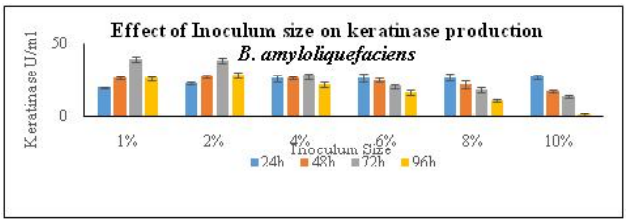

Figure 1:
Effect of inoculum on keratinase production by *B.amyloliquefaciens*

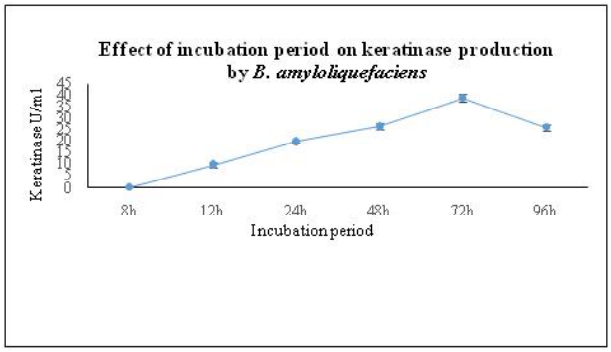

Figure 2:
Effect of incubation period on keratinase production by *B. amyloliquefaciens*

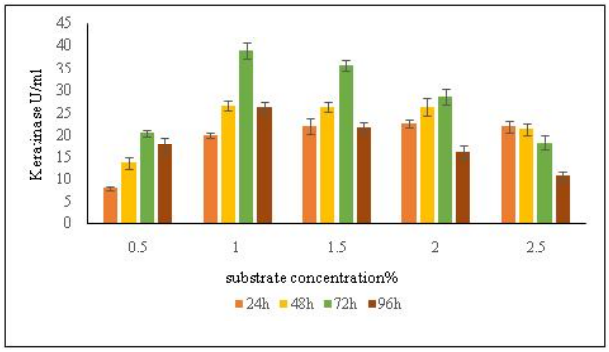

Figure 3:
Effect of substrate concentration on keratinase production by *B. amyloliquefaciens*

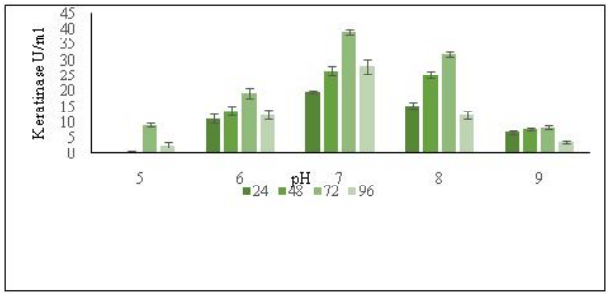

Figure 4:
Effect of pH on keratinase production by *B. amyloliquefaciens*

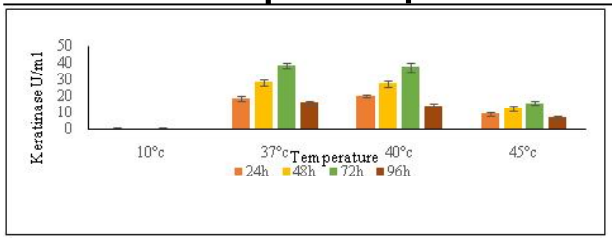

Figure 5:Effect of Temperature on keratinase production by *B. amyloliquefaciens*

Determination of inhibitors and metals ions effect on keratinase production:

The isolated keratinolytic protease was found to be metalloprotease, the activity was inhibited by specific metalloprotease inhibitors such as EDTA at the percentage 14% (Figure 6) but not by PMSF. The enzyme therefore seemed to be a kind of metalloprotease. Magnesium ions and Mn^{2+} stimulated keratinase production with relative activity 111%while copper, cobalt, lead, mercury,cysteine chloride,sodium sulphite and ferric chloride inhibited the activity, complete inhibition by â-mercaptoethanol, some keratinase in our study partially inhibited by glycerol, DMSO and Zn^{2+} while some partially activated by Zn^{2+}, similar results were reported by Hui Ni et al.,[24]have revealed that, the keratinase from *Bacillus* sp.SH- 517 tested with the metal ions and found that, 1mM of KCl and NaCl had a stimulatory effect, with a relative activity of 121% and 118%, respectively. Tork et al.,[25] indicated that, the activity of the enzyme was slightly reduced by the following metal ions; $CaCl_2$ and $ZnCl_2$ but EDTA decreased significantly the keratinase activity.The results in present study are in agreement with the cited reports.

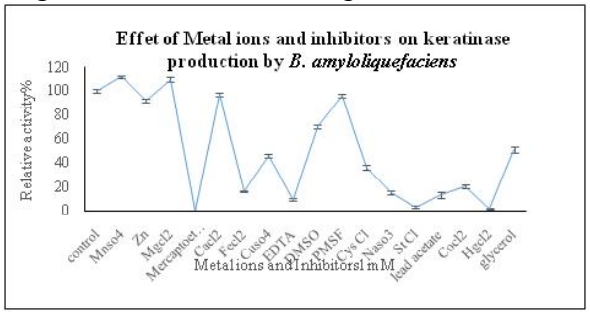

Figure 6:

Effect of Metal ions & inhibitors on keratinase production by *B. amyloliquefaciens*

Conclusion:

The leather deteriorates *Bacillus amyloliquefaciens* KP015745 (strain JS518) was found to be most proficient keratinase producer (38.9±1.6 U/ml) at 37°C after 72h . The isolates revealed maximum keratinase production at pH 7 using 1% feather meal as a substrate. Keratinolytic protease was metalloprotease as the activity was inhibited by EDTA. Investigational outcome propose that the isolate having ability to produce varied range of enzymes such as keratinase, collagenase, gelatinase and caseinasecan be competently used to treat keratin rich, leather waste ecofriendly.

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Foliar Anatomical characterization of Ecbolium linaenum (Forssk.) Alstone: A ethnomedicinal herb

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Abstract

Plants are the unending source for a number of compound for which can maintain the health of human being, plants have been the corner stone of pharmacy not only in ancient times but also in the area of modern drug discovery. In India different parts of medicinal plant use to cure the specific ailments has been in vogue from ancient time. Although the traditional system of medicine has large number of plants medicinal and pharmacological properties and are yet to be discovered which represent a priceless tank of new bioactive molecules. Ecbolium linaenum (Forssk.) Alstone synonym Ecbolium viride belonging to family Acanthaceae is a perennial, woody undershrub. In Sanskrit it is also called as 'Sahacharah'. Traditionally whole plant and all the parts of plant i.e. root, stem and leaves are used as folklore medicine. Though an important medicinal plant no data is available for drug characterization. Drug characterization is important to understand the purity of drug. Here an attempt to study the macro and micro morphology of plant organ i.e. structure of leaf, leaf architehcure and trichomes are studied.

Keywords - Ecbolium linaenum (Forssk.) Alstone, medicinal plant, morphology, leaf structure, leaf architechure, trichomes.

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Production and purification alkaline phosphatase from *Cocci* spp.

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Abstract

Alkaline phosphatase has become a useful enzymatic tool in molecular biology laboratories, since DNA normally possesses phosphate groups on the 5' end. Removing these phosphates prevents the DNA from ligating (the 5' end attaching to the 3' end), thereby keeping DNA molecules linear until the next step of the process for which they are being prepared; also, removal of the phosphate groups allows radio labeling (replacement by radioactive phosphate groups) in order to measure the presence of the labeled DNA through further steps in the process or experiment. The alkaline phosphatase from shrimp is the most useful, as it is the easiest to inactivate once it has done its job. The use of alkaline phosphatase is as a label for enzyme immunoassays. One common use in the dairy industry is as a marker of Pasteurization .This molecule is denatured by elevated temperatures found during pasteurization, and can be tested for via color change of a Para-nitro-phenol phosphate substrate in a buffered solution (Aschaffenburg Mullen Test). Alkaline phosphatase is an intracellular enzyme which has the optimum temperature 60°C, pH is ranging from 8-10. Especially the gram negative Cocci producing more amount of alkaline phosphatase enzyme than all the species which I have examined. This is the screening of microorganism has done with PNPP. Its activity was measured by increasing the concentration of the enzyme in spectroscopic analysis. The enzyme purification has done with ammonium precipitation, dialysis and ion exchange chromatography.

Key words:-Alkaline phosphatase,*Cocci*, pH, enzyme

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Effect of light on the production of chlorophyll in Wheat (*Triticum aestivum* L.)

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Abstract:

Chlorophyll is a green pigment, which is structurally similar to porphyrin pigments such as haeme and it is produced through the same metabolic pathway. The recovery of chlorophyll pigments by incubation method in which tender leaf tissue in 80% buffered acetone at room temperature give higher yield of pigments compared to other methods. The effect of various light intensity during 24 hours upon the production of pigments has been studied in plants under natural conditions in our laboratories. In the present study, the chlorophyll was extracted from the leaves from wheat plant and characterized by visible spectroscopy. It was found that light favored the production of chlorophyll pigments.

Key words: Chlorophyll, light intensity, spectroscopy, wheat.

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INDUCED SPAWNING, FECUNDITY, FERTILIZATION RATE AND HATCHLING RATE OF INDIAN MAJOR CARP *CATLA CATLA* BY USING SYNTHETING HORMONE AND CARP PITUITARY EXTRACT.

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Abstract:

In the present study during the period June –August 2009 applying the appropriate doses of the hormones ovaprim and carp pituitary extract was studied on the number of eggs/Kg body weight, fertilization rate and hatchling rate at Fish breeding center at Jaikwadi, Paithan Dist. Aurangabad. Fishes were spawned successfully by using appropriate doses of synthetic Ovaprim and carp pituitary extract. The percentage of fertilization ranged (92.00% - 95.00%) was found with ovaprim treatment and (69.58% - 85.29%) with pituitary extract treatment. The percentage of hatchling ranged (89.31 – 94.21%) with Ovaprim treatment and (58.82% - 78.82%) with pituitary extract treatment.

Key words: Synthetic hormone ovaprim, carp pituitary extract, fish breeding center, *Catla catla*.

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FECUNDITY OF *CYPRINUS CARPIO* IN RELATION TO TOTAL BODY LENGTH BODY WEIGHT AND TOTAL OVARY LENGTH & OVARY WEIGHT

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Abstract:

In the present study the fecundity of *Cyprinus carpio* was carried out during its peak breeding season December to February 2014. In the present study total twenty five mature female of *Cyprinus carpio* ranging from 15.0 to 45.0 cm in length and 370 gm to 1112 gm in weight examined for the study of fecundity. The result of correlation regression equation of fecundity and total body length, fecundity and total body weight, fecundity and total ovary length, fecundity and total ovary weight were, (r = 0.983), (r = 0.983), (r = 0.945) (r = 0.945) respectively. The study indicate that the fecundity increased with growth of fish and weight of ovary showed straight line relationship with body weight and, linear relationship with weight of gonad.

Key words: Cyprinus carpio, Fecundity, Body length, body weight, Ovary weight and ovary length.

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Diversity of Avifauna in Jivrekha reservoir Dist. Jalna

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Abstract:

A study to find out the bird diversity at Jivrekha reservoir Dist. Jalna, was carried out over a period of 10 months from June 2016 to March 2017. Jivrekha reservoir is having very good biodiversity having different types of flora and fauna. The reservoir is located 40 kilometers away from District Jalna. The majority of birds observed during the periods of December to February. The area is inhibited by 36 different types of birds, out of which 22 are residential migratory, 5 are migratory and remaining are resident, where Accipitridae is dominant over others.

Keywords: Avifauna Jivrekha reservoir, Accipridae.

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