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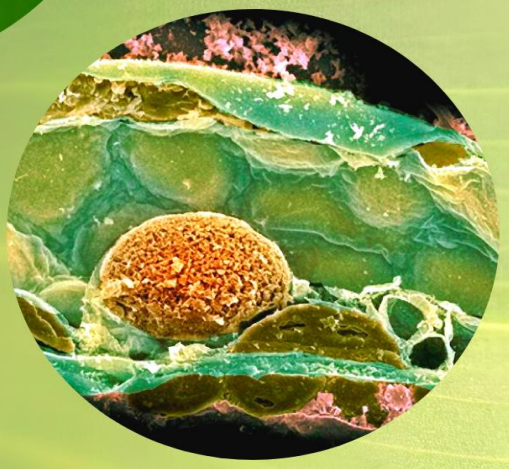
RESEARCH INTERVENTIONS AND ADVANCEMENTS IN PLANT SCIENCES



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Foreword

Dr. Dattatraya K. Gaikwad

Director, Dr. Babasaheb Ambedkar Marthwada University Sub Campus,

Osmanabad, M.S. India

Over the past decade, progress in plant science has grown considerably.In this regards, Research interventions and Advancements in Plant Sciences (RIAPS) provides succinct updates, opinion, and discussion on the most exciting and fascinating current research in all aspects of plant science. It contains brief, readable articles and thoughtful articles that keep readers up to date on the recent and latest trends, important developments and new innovative ideas within and outside their specialist area. The original, peer reviewed articles from the leading and upcoming scientists ensures balance and accuracy. In addition RIAPS edited book series contains a shorter articles that intended to serve as a forum for scientific discussion. I am happy to mark that, this book is covering topics at the interface of science and technology. Most of the budding researchers can no longer simply turn to the older strategies, and new innovative ideas are needed to accomplish their research with spotlight, this book series a right plat form to them. The book covers new information in the area of plant science research. The topics in the book are practical and user-friendly. They allow practitioners, students and academicians with specific background knowledge to feel confident about the research presented on a new generation of Plant Science Research.

I congratulates to the editors for this initiatives and wish them all the very best for their upcoming edition of the research series book.

DKGaikwad

Dr. D. K. Gaikwad

PREFACE

Plant Science research in last few years has made major contribution to our understanding of biology.The research interventions and innovative research ideas benefited from insights gained from studies on various aspects of plant science. Our edited book brings together expert authors under the skilled editorship of leading scientists to produce state-of-the-art compendiums of current research. Aimed at the research scientist, graduate student, medical researcher and other professionals, this book is highly recommended for all plant science researchers. Research Interventions and advancements in plant sciences seek to provide all scientists, from the tenured to the tenderfoot, with concise and curated updates on the latest research. It is our aim to highlight new scientific developments in plant science. Our high-caliber articles are cutting edge, provocative, yet accessible and are written by the most authoritative voices in science today. They are intended not only to bring readers up to speed on recent progress in the field, but also to serve as platforms for debate and to push the boundaries of conventional thinking.

The articles in the book have been contributed by eminent scientists and academicians. Our special thanks and appreciation goes to our esteemed experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking efforts in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- ***Editorial Team***

Research Interventions and Advancements in Plant Sciences

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EXPRESSION OF VIRAL COAT PROTEIN: A TOOL FOR PLANT VIRUS DETECTION

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Abstract:

The central dogma of molecular biology is expression of protein from gene construct. Expression systems are widely used for the synthesis of proteins like human insulin, interferon, virus like particles and antiserum against viruses. Protein expression systems are of two types; cell based and cell free. Cell based expression system is the in vivo tool whereas cell-free system is the in vitro tool for protein synthesis. In vivo expression system comprises of an expression host and vector. The most widely used expression host for plant viral proteins is Escherichia coli. Commonly used expression vectors are pRSET, pET and pGAPZαA. Although in vivo protein expression system yields high, it involves tedious cell culturing steps and less efficient post translational modifications. In order to overcome these disadvantages, in vitro system was introduced. The post translational modifications like disulfide bond formation required for proper folding of secondary and tertiary structures of recombinant protein is taken care of well in this system. The in vitro protein synthesis can be carried out in batch type and continuous type formats. The protein expression system especially in vitro system ensures synthesis of high quality protein required for X-ray crystallography and nuclear magnetic resonance experiments. It is also essential in the synthesis of toxic proteins, membrane protein, envelope (coat) protein etc. which are difficult to produce in the in vivo system.

Keywords: Gene, virus, Coat protein, Expression, anti-serum.

Introduction:

The crop damages owing to viral diseases are viewed to be the second greatest contributor to yield loss (Hsu, 2002). The symptomless or confused symptoms of viral infection with those of abiotic stresses, challenges the symptomatic diagnosis and prediction of viral disease (Strange, 2005; van der Want and Dijkstra, 2006). Indexing the virus at early stage of

infection is the only amicable method for management (Agrios, 2005*; Hull, 2009*). As there is no direct treatment protocol for plant viruses so far, indirect strategies have to be adopted (Aboul-Ata *et al.*, 2011). Therefore, a lot of methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods and so on (Lopez *et al.*, 2008). Among which serology based techniques play inevitable role as they are very sensitive to the viral antigens. Coat protein mediated serological detection of plant viruses are widely accepted and followed (Hu *et al.*, 1995; Koohapitagtam and Nualsri, 2013).

The central dogma of molecular biology is the unidirectional flow of genetic information within a biological system. It is often stated as DNA makes RNA and RNA makes protein (Crick, 1958), which is the mainstay of protein expression system. DNA template or gene of interest will be transcribed to produce mRNA, which further translate and produce protein. Viral coat protein is a component of capsid which mediates the attachment of virus to the host cells, facilitating viral mRNA to take control over host ribosome for translation during infections. The most widely used genes to engineer virus resistance in plants were derived from coat protein (CP) genes, either as full length or truncated constructs (Reddy *et al.,* 2009).Viral protein can be expressed artificially using several different recombinant systems including bacteria, yeast, baculovirus or insect, mammalian cells, and more recently filamentous fungi such as *Myceliophthorathermophila* (Visser *et al*., 2011).In many viruses, in vitro expressed coat proteins have been used as immunogens and the resulting antibodies have been utilized for detecting the corresponding viruses (Kumar *et al*., 2018). The recombinant coat protein has been used to raise high quality antiserum (Hema *et al.,* 2003) thus reducing viral infection by the expression of coat protein genes (Quemada *et al*., 1991). Therefore the production of antibodies to recombinant coat protein with no background reactions and with the potential of working in all serological test can be used for diagnosis and developing of antibody based PCR tests (Hema *et al.*, 2003). Relatively high sequence conservation (around 90–100%) in the coat protein also makes it an ideal candidate for the development of diagnostics (Bhardwaj *et al.,* 2020).

Cloning of coat protein gene in Expression Vector:

Expression vectors possess sequence for affinity tags, which provide polyamino acid such as polyhistidine and polyarginine overhanging on expressed protein. Different expression vectors were used for cloning of coat proteins of different virus such as pMAL-c2 expression vector for *Citrus tristezavirus* (Nikolaeva, 1995), *Banana bunchytop virus* DNA- 3 (Wanitchakorn1997). pBluescript SK for *Grapevine leafroll associated closterovirus*-3(Ling *et al*., 2000). pRSET- A expression for *Sugarcane streak mosaic virus*(Hema*et al*., 2003). pGEM®-T easy vector

for*Grapevine leafroll-associated virus* 3(Kumar *et al*., 2012a),*Citrus psorosis virus* (Reda *et al*., 2018), *Apple stemgrooving virus* (Bhardwaj *et al.,* 2020). Plasmid pET28a (+) vector for *Grapevine leafroll-associated virus* 3 (Kumar *et al*., 2018). Expression vectors pET-30(+) for *Citrus psorosis virus* (Reda *et al*., 2018) pET-32a (+) and pHIS-Parallel for *Apple stem grooving virus* (Bhardwaj *et al.,* 2020), and so on.

Expression of Coat protein gene:

The recombinant clones were then transformed into different recombinant protein expression system depending on the type of protein, the requirements for functional activity and the desired yield (Nettleship *et al*., 2010). The selection of the Expression systems are summarized in to cell based (eg. mammalian, insect, yeast, bacterial, algal) and cell-free.

Cell-based protein synthesis- *in vivo* protein synthesis system:

The oldest and most widely used expression systems are cell-based and may be defined as the combination of an expression vector, its cloned DNA, and the host for the vector that provide a context to allow foreign gene function in a host cell, that is, produce proteins at a high level (Sambrook and Russell, 2001; *Chow et al., 2003*). Expression systems are normally referred by the host and the DNA source or the delivery mechanism for the genetic material. For example, common hosts are bacteria (*Escherichia coli*, *Bacillus subtilis*), yeast (*Saccharomyces cerevisiae*) or eukaryotic cell lines (Yokoyama, 2003). Recombinant proteins from the bacterium E. coli and the yeast S. cerevisiae make up about 40% of the therapeutic protein production market (Vaishnav and Demain, 2009). The best expression system depends on the gene involved, for example the *S. cerevisiae* is often preferred for proteins that require significant post-translational modification (Hillebrecht and Chong, 2008). Nevertheless, it is felt that *E. coli* is still the first choice as the host for heterologous gene expression, except in cases where special requirements such as protein modifications, glycosylation or unusual cofactors, must be met (Gellissen, 2006). The major hosts used in expression analysis and their peculiar characters are discussed in Table 1 As there is clearly no single system that is optimal for all possible proteins, predictions for a successful development can be made upto an extent, and as a consequence misjudgments leading to costly time- and resource-consuming failures cannot be excluded (Zemella *et al*., 2015). Both prokaryotic and eukaryotic organisams are used as expression host, which comprises bacterial systems, yeasts, mammalian and insect cell lines etc. Examples of

prokaryotic expression host are *E. coli*, *Corynebacterium* sp. and *Pseudomonas* sp. and eukaryotic hosts are yeasts, insect and mammalian cell lines etc.

Table 1: Major hosts used in expression analysis and their peculiar characters

Expression host	Peculiar characters	Reference
Escherichia coli	*E. coli* (gram negative) is one of the most widely used expression hosts, and DNA is normally introduced in a plasmid expression vector.	Swartz (2001); Baneyx (1999); Jacob and Usha *(2002).*
Corynebacterium	Non-pathogenic species of the gram-positive *Corynebacterium* are used for the commercial production of various amino acids.	An *et al*. (2013)
Pseudomonas fluorescens	The non-pathogenic and gram-negative bacteria, *Pseudomonas fluorescens,* is used for high level production of recombinant proteins; commonly for the development bio-therapeutics and vaccines	(Retallack *et al*., 2012; Oganesyan and Lees, 2018)
Saccharomyces cerevisiae, Pichia Pastoris	Systems using *P. pastoris* and *S. cerevisiae* allow stable and lasting production of proteins closer to mammalian cells, at high yield, in chemically defined media of proteins	(Miller *et al*., 2005)
*Leishmania*sp.	Protozoan *Leishmania tarentolae* (non-pathogenic strain) expression systems allow stable and lasting production of proteins at high yield	(Kushnir *et al.*, 2005)
Mammalian systems	Chinese Hamster ovary, Mouse myeloma lymphoblstoid, Human embryonic kidney cells	(Zhu *et al*., 2012; Almo *et al., 2014;* Hacker and Balasubramanian, *2016)*

Table 2: Strains of *E. coli* used for expression analysis

Strain	Significant features	Reference
BL21(DE3)	Expression vectors containing bacteriophage T7 promoter (DE) which is integrated with chromosome of BL21.	Studier and Moffatt (1986) ; Sambrook and Russel, 2001; Borollosy and Hassan (2014); Reda *et al*.,2018
DH5α	A recombination deficient and suppressing strain used for plasmids and cosmids. The LacZΔM15 promotor permits a complementation with amino terminus of β-galactosidase encoded in pUC vectors.	Hanahan (1983); Pandey (2015)
JM109	A recombination deficient strain which allows blue/white screening on X-gal and permits bacteriophage M13 superinfection.	Perron *et al*. (1985)
W3110	Wild-type strain.	Hill and Harnish (1981)
RB791	A strain that makes high levels of lac repressor and is used for inducible expression of genes under the control of the *Lac* and *Tac* promoters.	Brent and Ptashne (1981)
ED8654	A suppressing strain commonly used to propagate bacteriophage vectors and their recombinants.	Borck *et al*. (1976)
XL1 Blue	A recombination deficient strain that will support the growth of vectors carrying some amber mutations. It allows blue/white screening on X-gal and permits bacteriophage M13 superinfection.	Bullock *et al*. (1987); Ling(2000)

Most commonly used bacterial or prokaryotic expression host is *E. coli* and among which strain BL21 is widely preferred in plant virology researches since,the T7 RNA Polymerase system is the most popular approach for producing proteins in *E. coli* (Komorowska and

Malinowski, 2009). In this system, an expression vector containing a gene of interest, cloned downstream of the T7 promoter, is introduced into a T7 expression host. T7 expression hosts such as DE3 strains have a chromosomal copy of the phage T7 RNA polymerase gene. When an inducer such as isopropyl β-D-1-thiogalactopyranoside (IPTG) or rhamnose is added to the culture, T7 RNA polymerase is expressed and transcribes the gene of interest, followed by translation of the desired protein (Sabharwal, 2017). Apart from BL21, other strains of *E. coli* are also used and the features of major strains are discussed in Table 2.

Cell-free protein synthesis- *in vitro* protein synthesis system:

The expression of recombinant protein in live cells is a well known and widely used method of protein production. As an alternative to slower, more cumbersome E- coli cell-based method, simple and fast cell-free protein expression method is an accelerated avenue for the transcription and translation of specific protein in a quasi-cell environment. CFPS is advantageous over in vivo protein expression for its open system, the elimination of reliance on living cells, and the ability to focus all system energy on production of the protein of interest (Rosenblum G. and Cooperman, 2014; Gregorio *et al*., 2019). CFPS has the potential to allow for simplification of the antibody production process for more rapid manufacturing due to the open system, which can easily be modified from case to case for the production of active antibodies using rapid design–build–test cycles and modification of the redox potential of the reaction (Gregorio *et al*., 2019).

CFPS require a DNA template with promoter (P) mainly T7/ sp-6 promotor. Ribosome binding site (RBS) should be there, along with Shine dalgarno for prokaryotic (Blattner *et al*. 1997) or Kozak consensus sequence (Kozak, 1984) for eukaryotic translational initiation sequences. The sequence should be end with terminator (T). Linaear DNA templates are not used, as they are susceptible to nucleolytic cleavages. Also a solution containing all the necessary component to drive transcription and translation. Genetic material in combination with cell free extract begins the expression of target protein. Earlier CFPS use cell extract derived from *E coli* as it provide higher yields and more-homogeneous samples suitable for structural studies (Carlson *et al*., 2012). Presently, CFPS has been implemented using cell extracts from numerous different organisms dividing CFPS platforms into two categories: High adoption platforms for CFPS are based on extracts from *Escherichia coli*, *Spodoptera frugiperda* (lepidoptera), Saccharomyces cerevisiae (yeast), Chinese hamster ovary, rabbit reticulocyte lysate, wheat germ, and HeLa cells and Low adoption platform include *Neurosporacrassa*,

Streptomyces sp., *Vibrio natriegens*, *Bacillus subtilis*, Tobacco, Arabidopsis, *Pseudomonas putida*, *Bacillus megaterium*, Archaea, and *Leishmaniatarentolae* (Gregorio *et al*., 2019).

Applications of protein expression:

In this post-genomic era, high-throughput protein expression platforms are becoming increasingly important. Cell-free systems have many advantages for meeting this need. First, direct use of PCR templates avoids time-intensive molecular cloning steps. Second, improvements in cost-effective high-yield batch reactions make multiwall protein production feasible (Endo and Sawasaki, 2003). The method can be used as producers of valuable proteins that have already reached in the market, while others are newly defined systems that have yet to establish themselves but demonstrate a great potential for industrial applications (Papendieck *et al*., 2002). As the issues of cost, scale, and protein folding are no longer barriers to the adoption of cell-free technology, efforts to exploit CFPS for commercial production of therapeutics will be intensified (Tsuboi *et al., 2008).*

Purification of expressed coat protein and production of polyclonal antibody:

Expressed coat protein from the soluble fraction has to be purified using Protein Purification Kit. Separation of the protein of interest from all impurities is typically the most challenging aspect of protein purification. In the biopharmaceutical industry, chromatography is a critical and widely used separation and purification technology due to its high resolution (Liu *et al*., 2010). Purity and integrity of the fusion proteins have to be checked on 12 per cent SDS-PAGE (Laemmli, 1970) and concentration can be estimated by Protein Assay Reagent or Bradford method (Bradford, 1976) or UV Spectrophotometer. The expression of coat protein has to be confirmed by Western Blotting (Sambrook and Russel, 2001) using polyclonal antiserum against the respective virus strains (conventional antiserum). The purified recombinant coat protein can be used as an immunogen and in accordance with the biosafety concerns this specific type of antigen can be injected into rabbit to elicit an immune response. Serological testing requires a large amount of specific antisera which is produced by injecting the purified virus particles in the blood stream of rabbits (Hull, 2002).

Detection of plant virus:

Serological detection systems use specific antibody developed in animals (mammals) in respond to antigens (Torrance, 1998). Several researchers have developed coat protein based detection strategies. Polyclonal antibodies from coat proteins of different viral strains such as

Citrus tristezavirus (Nikolaeva *et* al., 1995), *Banana bunchytop virus* (Wanitchakorn *et al*., 1997), *Bananabractmosaic virus* (Rodoni *et al*., 1999), *Grapevine leafroll associated closterovirus*-3 (Ling, 2000), *Cardamom mosaic virus* (Jacob and Usha, 2002), *Sugarcane streak mosaic virus* (Hema *et al*., 2003), *Grapevine leafroll virus* 2 (Ling *et al.*, 2007), *Grapevine rupestris stem pitting-associated virus*(Minafra*et al*., 2015) *Grapevine leafroll-associated virus* 3 (Kumar *et al.*, 2018), *Citrus psorosis virus* (Reda *et al.*, 2018), *Apple stem grooving virus* (Bhardwaj *et al.*, 2020) and so on has been developed for a large scale virus diagnostic.

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A REVIEW ON THE ETHNOMEDICINAL PRACTICES IN DIFFERENT PARTS OF ASSAM

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Abstract:

The rich biodiversity of Assam has always met the needs of the population in all aspects. The region is also noted for its richness in medicinal plants diversity. Since time immemorial different communities in Assam have been using different species of plants to cure their ailments and health disorders.

Ethomedicinal Knowledge is the knowledge of medicine from plants and various other sources that exists among the people in different communities of a particular geographical region, and which has been practiced over generations.Such indigenous knowledge has been known to be helpful and also worked wonders for many people. Nowadays very few people are known to prepare and provide such medicine and the method or recipe of preparation of such medicine is passed to the next generation of the owner .Various wild and cultivated plants are used in preparation of such medicine.

Keywords: Biodiversity, Communities, Ailments, Ethnomedicinal, Indigenous knowledge

Introduction:

Ethnomedicine is the study of traditional medicine which are mostly plant based and are practiced by the ethnic groups in a particular geographical region. Various wild and cultivated plants are utilized in the preparation of such medicine.Such knowledge is often seen to be preserved through oral tradition in which the knowledge is passed on through generations.

The state of Assam in the North Eastern region of India is a biological hotspot with many rare and endemic plant and animal species. Several ethnic groups which possess distinct social and cultural identities reside in this region and practice their own traditions. The Indigenous communities of Assam which have maintained their long followed tradition in medicine are seen to possess rich knowledge in the therapeutic use of plants and plant products.Their age old

interaction with nature has brought them in possession of vast knowledge about the medicinal uses of plants. These knowledge needs to be preserved for the benefit of the future generations and also for the study and possible extraction of medicine at an industrial level.

The methods of preparation of such medicine generally involves the extraction of the sap from the leaves, fruit or bark, and its application on the affected part or direct intake as whole fruit or leaves or as cooked. Very few studies have so far been carried out for proper documentation of the identification and method of preparation and application of such ethnomedicine.

The following review on Ethnomedicinal practices in different parts of Assam was done to document some of the plants used as ethnomedicineby people in different parts of Assam. Most of these plants are found in the household gardens and kitchen gardens and are readily available for use.The local names of the plants slightly vary in different places but the method and application mostly remains the same. Some of the commonly used ethnomedicine in various regions of Assam are as given below in the table.

Table 1: Table showing list of different plants used as ethnomedicine in Assam

Local Name	**Scientific Name**	**Family**	**Parts Used**	**Treatment**
Borthekera	*Garciniapendunculata*	Clusiaceae	Fruit	Dysentry
Kona-himolu	*Commelinabenghalensis L.*	Commelinaceae	Leaves stem	Eye soreness, stye
Jomlakhuti	*Costusspeciocus*	Costaceae	Stem	fever, asthma, bronchitis
Dalim	*PunicagranatumL.*	Punicaceae	Fruit, leaves	Inflammation
Nefafu	*Clerodendrumcolebrookianum Walp.*	Verbenaceae	Leaves, twigs	High bloodpressure
Duportenga	*Bryophyllumpinnatum*	Crassulaceae	Leaves	Kidney stone
Drun bon	*Leucasaspera*	Labiatae	Leaves, twigs	Bleeding nose, fever
Harjura bon	*Cissusquadrangularis Linn.*	Vitaceae	Leaves, vine	Bone fracture
Chalkuwari	*Aloe veraLinn.*	Liliaceae	Leaf extract	Burns, skin diseases

Narasingh	*Murrayakoenigii Spreng.*	Rutaceae	Leaves, roots, bark	Skin problems, vomiting, diarrhea
Kuhiya	*Saccharum officinarum*	Poaceae	Stem	Jaundice, urinary problems
Horumanimuni	*Hydrocotylerotundi folia Roxb.*	Apiaceae	Whole plant	Dysentry
Jaluk	*Piper nigrum Linn.*	Piperaceae	Fruit	Inflammation
Kordoi	*Averrhoacarambola*	Oxalidaceae	Fruit	Jaundice
Bormanimuni	*Centellaasiatica (Linn.)*	Apiaceae	Whole plant	Fever diarrhea
Matikanduri	*Alternantherasessilis (Linn.) R. Br. ex DC. DC.*	Amaranthaceae	Whole plant	Diarrhea
Paan	*Piper betle Linn.*	Piperaceae	Leaves	Fungal infection
Bhimkol	*Musa bulbisianacolla*	Musaceae	Fruit	Oxidative stress, diarrhea
Outenga	*Dilleniaindica Linn.*	Dilleniaceae	Fruit Bark, leaf buds	Stomach disorder Pneumonia
Amara	*Spondiaspinnata (L.f.) Kurz.*	Anacardiaceae	Fruit, young twigs	Dysentry
Mosundori	*Houttuyniacordata*	Saururaceae	Leaves, whole plant	Dysentry
Kola Tulokhi	*Ocimumamericanum Linn.*	Lamiaceae	Leaf extract	Fever, cough
Modhuri	*Psidiumguajava Linn.*	Myrtaceae	Leaves, twigs, fruits	Dysentry
Jetuka	*Lawsoniainermis Linn.*	Lyrthaceae	Leaf extract	Fungal infection
Sirata	*Andrographispaniculata*	Acanthaceae	Leaf extract	asthma, bronchitis

Conclusion:

These ethnomedicinal practices are claimed to be very effective and inexpensive and are therefore held to be highly beneficial for the society. However due to the lack of written records

such knowledge is seen to be slowly eroding away from the consciousness of the people. Moreover, the exact amount of plant extract to be used is seen to be uncertain in most of the cases. Proper studies need to be undertaken for the validation and documentation of such practices at the earliest. Also efforts must be made for the conservation of such beneficial plants and their cultivation at higher scales for the benefit of the society.

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STUDY OF THE DIVERSITY OF ETHNOMEDIFLORA USED BY TRIBAL COMMUNITIES OF HUSNABAD AREA IN TELANAGANA STATE

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Abstract:

Telangana is one of the tribal rich states in India. Among the different tribal communities of Telangana state, Lambada and koya tribal communities are living very much in surrounding area of Husnabad village, Siddipet district, Telangana state. These communities had a rich knowledge on the medicinal uses of the plants grown at their surrounding region and used them for various health care purposes. In our present study, found that 56 plant species are widely used by locally living tribal people such as Lambada, koya and others in their traditional medicinal system. These 56 plants are belongs to 32 families .This indigenous traditional knowledge is an integral part of their culture and history. It is one of the ancient traditional medicinal systems and it was developed by years of regular practices on day to day life and available resources are surrounded at their living places. These conventional Ethnomedicinal plants are hugely applied for treatment like skin diseases, diarrhea, jaundice, wounds healings, piles, infertility, abortions, urinary disorders, kidney stones, snake bite, earache, cough, asthma, fertility, toothache, leucoderma, bone joints. In our present paper, this conventional ethnomediflora used by local community of Husnabad area are arranged alphabetically following by botanical name, local name, family, ethnomedicinal parts and type of plants.

Key words: Husnabad area, ethnomediflora, tribal communities, local ailments.

Introduction:

India is rich in medicinal plant diversity that is extended to different geographical, environmental conditions and associated with tribal and folk knowledge systems. The tribal person mostly depends on forests for their survival and more than 70% of rural populations rely upon on traditional medicines as a primary healthcare needs. In India there are above 550 tribal communities, covering approximately 227 ethnic groups, living in about 5000 villages under different forest and vegetable types. In the developed countries, the 25% of medicinal drugs are based on plants and their derivatives (Principle, 2005) and uses of major role medicinal plants among the indigenous people in rural areas of many developing countries. Forests are covered more than 44% of the states of geographical area, and out of total forest, 56,448 Km^2 dense forests constitutes 67.10% and 32.89% is considered as open forests (Brij, 1993; Lewis and Lewis, 2003; Kala, 2006, 2007). Medicinal plants had played in human societies throughout history and pre history (Tirkey, 2004) with the development of modern civilization, allopathic drugs usage is increasing and employing of herbal drugs is restricted to few communities or areas only.

The ethno-botanical uses have immense significance (Kirtikar and Basu, 1988; Kala, 2005). India is very rich source of ethnobotanical information about 80% of Indian population, existing in villages and considerable proportion comprise tribal people living in remote forest areas. The population of several district of central and eastern India is predominantly covered by tribal. The different traditions beliefs, needs, cultures of various tribes and the diversity of flora in India richly contribute to plant folklore. The plant based traditional knowledge has become a recognized tool in search for new sources of drugs and wound healing properties and others (Nadkarni, 1986; Ayyanar, 2009). India is having rich biodiversity and it is one of the world's 12 mega diversity countries.

Telangana is the newly formed 29^{th} state of this country in southern region of India. There are many kinds of tribal peoples are living in different districts such as Karimnagar, khammam, Mahaboob nagar, Adilabad, Komaram bheem, Bhoopala palli districts. We had studied medicinal plants used for different ailments by local tribal peoples of lambada, koya, living in the area of Husnabad. In this connection found that 56 different plants are used by tribal people for various ailments and these plants are belongs to 31 families. The main objective this study is provides the information and documentation of medicinal plants used by tribal people of the different villages in Telangana state.

Methodology:

Visited Villages:

The present work has carried out in surrounding villages of Husnabad in Telangana state, on phytotherapeutic drugs in the health care systems of rural population. In this study ,survival of ethnobotanical knowledge is evident for the great economic important data collected from living elders of tribal people belongs to lambada and koya, etc. of study area and from previous published and unpublished sources from historic and contemporary times.

Data Collection:

The study area is a very prominence for ethno botanical studies and belongs to tribal communities of lambada and koya of Husnabad .The questionnaires (interview method) have devised to identify the indigenous knowledge of plant based remedies from local elder people. At the end of each interview, Plant specimens are collected and dried for identification and preservation. Samples of plants such as herbs, shrubs and trees and others had identified with the help of local floras and previous literature and also extensive studies has been conducted with the tribal people. The local name, part of plant used and their medicinal significance have been recorded. The botanical name of each plant is followed by Botanical name, local name, family, used plant part, ethno-medicinal importance ,mode of administrations, habit and study sites.

Findings and Analysis:

In this present work, found that 56 plants species are more commonly used by lambada (types of lambada such as Thakatla gadda, Lakma noik thanda) erukala, koya types of tribal people, who are living at present study regions of Telangana and used for curing different ailments. These 56 plants are belonging to the various types of Families like Fabaceae, Cucurbitaceae, Euphorbiaceae, Acanthaceae, Combritaceae and Asteraceae. We had scrutinized the medicinal plants used by tribal people with the ancient combinations of Bhava Prakasha Nigantu, Dhanvanthari Nigantu, Indian medicinal plants nadkarni, and Indian Meteria Medica kirthikar and Basu (Pakrashi and Basak, 1975; Mookerji Chunekar, 1982; Jain, 1991; Nadkarni, 1998; Ghosh, 2008; Yadav *et al.,* 2012). This flora is also found in our college campus and grown naturally. The above representing plants are applied mostly to treat for skin diseases, diarrhea, jaundice, wounds, piles, infertility, urinary disorders, kidney stones, antiseptic, snake bite, earache, cough, asthma, fertility, toothache, etc. Majority of remedies are prepared in the form of juice, followed by powder, paste form, fleshy collected, oily plant parts. In their treatment

medical administration includes inhalation, oral administration, paste applying, and oils for rubbing massage. Medicines are given mixed with milk, water and jaggery. From the interviews with tribal people, we found that oral absorption method is preferred for curing stomach ache, urinary disorders, jaundice, and diarrhea and External application of medicines is applied for skin infections, body swellings as antiseptic. These tribal people have not known the scientific knowledge of plant medicines to treat for various ailments and their treatment is based on their belief and confident .In present study we found that the most extensively used plant parts for preparation of medicines to treat various ailments are leaves, root, seeds, fruits ,and bark. And In their medicine preparation ,leaves are used widely 35 % and followed by roots 20%, seeds 18%, fruits 15% and bark is 10%.

Conclusions:

In this survey, we found that the selected area has plenty of medicinal plants that used to treat a wide spectrum of human ailments. Earlier studies on traditional medicinal plants also denoted that the economically backward local and tribal peoples of husnabad surrounding villages, Telanagana preferred folk medicines due to low cost and it is a part of their social life and culture. This is evident from the interview results of people in different surrounding villages of Husnabad where the study has conducted. This study concludes that even though the accessibility of medicine for various complicated diseases are readily available, many people are still depends on local traditional treatment from their community. These treatment mainly aim for curing the common ailments and Chronic diseases includes cold, fever, cough, asthma, fertility, infertility, jaundice, earache, skin diseases, urinary disorders etc. Due to lack of interest among the younger generation as well as their tendency to migrate to cities for jab purpose, there is great losing this wealth of knowledge in the near future. Thus it becomes much necessary to acquire and save these traditional systems of medicines by proper identification and documentation

Table 1: List of Ethnomedicinal plants used by tribal communities

Sr. No.	Botanical Name	Local Name	Family	Ethnomedicinal Uses	Type
1	*Achyranthus aspera L*	Khoruch	Amaranthaceae	Whole plant for Snake bite, Root decotion for stomachache,fever,cough., seeds for hydrophobia and skin diseases	H
2	*Aegle marmelos L*	Bel	Rutaceae	Leavesfor diabetes, diarrhea, dysentery and piles	T
3	*Aloe veera (L)Burm f.*	Kataban	Fabaceae	Pulp for piles, rheumatic pain,constipationand menstrual disorders and blood purifier	H
4	*Andrographis paniculata (Burm.f.)Wall ex Nees.*	Bhui Neem	Acanthaceae	Whole plant for blood purifier, skin diseases ,malaria and anti snake venom	H
5	*Annona squamosa L*	Sitaphal	Annonaceae	Leaf juice for antiseptic, wound healing, leaves and fruits for tumour and cancer.	T
6	*Azadiracta indica A de Jussieu*	Neem	Meliaceae	Bark for rheumatism, fever, diabetes, ulcer and bacterial infections. Leaves for toothache, skin diseases, tuberculosis. Oil is for leprosy, ulcer, rheumatism	T
7	*Bambusa arundinacea (L) Schreb.*	Bans	Poaceae	Whole plant for tuberculosis, wound healing, bronchitis and leprosy.	T
8	*Barlirea prionitis L*	Itola	Acanthaceae	Plant decotion for cough, toothache. Leaf juice for fever, wounds. Root paste for over boils.	H
9	*Bauhinia variegate L*	Kolyari phaji	Fabaceae	Flowers for piles, diabetes, obesity. Stem bark for	T

				asthma, skin diseases, intestinal worm infections, flower buds for dysentery, diarrhea.	
10	*Bombax ceiba L*	Semar	Malvaceae	Bark for rheumatic pain, scorpion bite, snake bite and leprosy, piles.	T
11	*Bryophillum pinnatum (L)Kurz*	Pathachtta	Crassulaceae	Leaves for kidney stones and antiseptic wounds.	H
12	*Butea monosperma .B. Lamark*	Tesu /Morod	Fabaceae	Gum for diarrhea, dysentery, piles, seed powder for scorpion sting, fruit for irregular menustration	T
13	*Calotropis procera W.T.Aiton*	Aak /shishudurw	Asclipiadaceae	Milk juice for dropsy, leprosy, rheumatic pain, ash leaves with sugar for asthma and bronchitis. Root paste for cuts, wounds.	Sh
14	*Carica papaya L*	papaya	Euphorbiaceae	Fruits for piles, antifertility ,liver enlargement, seeds for worm infections, leaf juice for anaemia, heart problems	T
15	*Cassia fistula L*	Amltas	Caesalpinaceae	Leaf juice for anti fungal and antiseptic, clearing cuts, wounds .leaves for fever, leprosy, cough.	T
16	*Chrysanthemum corinarium L*	Sevanti	Asteraceae	Bark for purgative, anti–helmintic, insecticidal propertynad sedative also	H
17	*Citrullus colocynthis (L) Schrad.*	Boda	Cucurbitaceae	Fruits for constipation.root for skin diseases.	Climber
18	*Clitorea ternate L*	Syahiful	Fabaceae	Leaf powder for urinary diseases. Root for headache, fever, cough, antidote for snake bite	Cl
19	*Coccinia grandis (L0J.OttoVoigt*	Kunuru	Cucurbitaceae	Leaves for high fever, diabetes, jaundice tuberculosis and skin diseases.	Cl

20	*Cucurma longa L*	Kamka	Zinziberaceae	Whole plant for cough, skin diseases, diabetes worm infection,blood purifier,and used as spice.	H
21	*Cuscuta reflexa L*	Podha tonda	Convolvulaceae	Plant paste warm with mustard oil and wheat flour for joint pains, plant paste applied externally for headache.	Cl
22	*Cynodon dactylon (L) Christian Hendrikpers*	Doob rinda	Poaceae	Root powder for urinary tract infections, hypertension, diabetes and headache	H
23	*Datura metal L*	nelaarka	Solanaceae	Leaves for asthma, cough, seed for skin diseases, rheumatism	H
24	*Dalbergia sissoo Roxb*	Bahabija /Hermala	Fabaceae	Leaf decotion for gonnerhea.	T
25	*Emblica officinalisL*	Nelli	Euphorbiaceae	Fruit forjaundiceand wound healing	T
26	*Eucalyptus globules Labil*	Nilagiri ped	Myrtaceae	Leafpowder for antiseptic, diarrhea, cough and asthma	T
27	*Ficus racemosa L*	Dumar	Moraceae	Latex for piles, diarrhea,dysentery.fruits for urinary problems.roots for diabetes	T
28	*Hemidesmus indicus L*	Sugandi jad	Asclipiadaceae	Leaves for snake bite, scorpion sting and wound healing.	Cl
29	*Ipomoea carnea Jace*	Besrum	Convolvulaceae	Leaves paste for paralytic condition	H
30	*Ixora coccinia l*	Lalpul	Rubiaceae	Leaves for bronchitis, digestive problems and ulcers.	Sh

31	*Jatropa curcas L*	Rattan jat	Euphornaceae	Leaves for skin diseases	Sh
32	*Lawsinia inermis L*	Mehandi	Lythraceae	Leaves for good hair dye.	Sh
33	*Madhuca longifolia (j.Konig)J,F.Macbr*	Mahua	Sapotaceae	Flowers for nutrient richness, bark for toothache, seed oil for rheumatism	T
34	*Menthe spicata L*	Podina	Lamiaceae	Leaves for gastri problems, cold, cough, cholera	Sh
35	*Mimosa pudica L*	Uskadpoda	Fabaceae (mimosaidae)	Roots for snake bite, toothache. Leave for piles, wound healing	Cl
36	*Momordica charentia Descourt*	Kaerel	Cucurbitaceae	Fruits for reduce blood sugar, diabetes	Sh
37	*Moringa olifera Lamark*	Munaga	Moringaceae	Flower for bladder problems, fruits for heart disease bark for dental dis orders, fruits for liver problems and spleen problems	T
38	*Ocimum sanctum L*	Tulasi	Lamiaceae	Oil for antibacterial, insecticidal nerve tonic, leaves for fever, earache, mouth cleaner	Sh
39	*Phyllanthus amaras L*	Bhui korma	Euphorbiaceae	Leaves for diabetes .roots for jaundice, fever, whole plant decotion for malarial fever ans skin diseases.	H
40	*Plumbago zeylanica L*	Agnishikha	Plumbazinaceae	Root powder for abortive nad tonic	H
41	*Raphanus sativus L*	Mura	Brassicaceae	Root and leaf juice for jauncice	H
42	*Ricinus communis L*	Arandi	Euphorbaceae	Seed oil for purgative, skin diseases, rheumatism	Sh
43	*Rauwolfia sarpentina (L) Benthum ex W.S.Kurtz*	Bhuikurma	Apocynaceae	Leaves For High Blood Pressure, Mad Ness, Snake Bite	SH
44	*Semicarpus anacordium l*	Bhelwa	Anacordiaceae	Fruits for anti cancer	T
45	*Senna occidentalis (L) J.H.Friedrich*	Dandepadla	Caesalpinaceae	Seed for skin diseases, leprocy, leaveas for purgative.	Sh

46	*Sida cordifolia L*	Jhanti	Malvaceae	Roots for wound healing, diarrhea	H
47	*Sphaeranthus indicus l*	Mila phaji	Asteraceae	Whole plant for jaubdice, piles, uterus pain, vomiting	H
48	*Syzygium cumini (L) Skeels*	Jam	Myrtaceae	Fruits for diabetes .leaves for diarrhea, bark for dental problems	T
49	*Tamirandus indca L*	Itta	Fabaceae	Whole plant for diabetes, asthma, stem bark for fever.leaves for gastric dis orders, piles.	T
50	*Terminalia arjuna (Roxb) wight and Arn*	Kahua	Combritaceae	Bark powder for heart diseases, liver diseases.leaves for wound healing	T
51	*Terminia chebula A.J.Retzius*	Horra	Combritaceae	Fruits for gastric troubles and fruit paste applied for wounds, bark for skin diseases.	T
52	*Terminalia bellirica (Gaertn) Roxb*	Takha	Combritaceae	Fruits for gastic troubles, cough, cold	T
53	*Tinospora cordifolia (Thumb) Miers*	Guduchi	Menispermiaceae	Whole plant decotion for urinary problems, rheumatism, fever, heart problems	Cl
54	*Tephrosia purpuria (L) Pers*	Sarpunkha	Fabaceae	Whole plant for liver problems.seed oil for skin diseases.	sh
55	*Tribulus terrestris L*	Gukhru	Zygophyllaceae	Fruits for diuretic tonic, aphrodiasiac	H
56	*Zizypus jujumba Miller*	Ber	Rhaminaceae	Bark decotion for wounds .fruits for ulcer,fever,abdominal pain.leaves for asthma and wounds	T

Note: H=herb, Sh=shrub, cl=climber, T-tree

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ANTI BIOFILM ACTIVITY OF TRITERPENOIDS: AN OVERVIEW

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Abstract:

Phytochemicals, bioactive chemicals of plant origin, have long been used as herbal medicines in many countries but have become increasingly receptive in modern day biology for their non toxicity and easy availability. Among all the available groups, (like polyphenols, flavonoids, alkaloids) pentacyclictriterpenoids have received much attention for their wide range of therapeutic properties including anti-inflammatory, anti-cancer, anti-oxidant, anti-viral effects. They are further classified into three groups namely oleanane, ursane and lupane and Glycyrrhetinicacid (GA), ursolic acid (UA), and betulinic acid(BA) are three representative examples from each group respectively. All three compounds have been widely studied for establishing their anti-inflammatory, anti-cancer, anti-tumor and anti-microbial roles among many others. GA, UA and BA have also been studied for their anti-biofilm action. Anti-biofilm activity of various degrees have been established for all the triterpenoids against bacteria, particularly *Streptococcus mutans*, *Staphylococcus aureus* and other Gram positive pathogens and more recently against *Pseudomoans aeruginosa* though the precise mechanism of intervention remains elusive. Quorum sensing (QS) mediated alteration of gene expression has been associated with biofilm formation. In a systemic exploration for profiling action of petnacyclictriterpenoids against biofilms formed by Gram negative bacteria, GRA, UA and BA have been implicated in biofilm formation inhibition through impairing QS in *Vibrio choleare*, *Pseudomoans aeruginsoa* and *Acinetobacter baumannii* by interacting with various effectors of acylatedhomoserine lactone (AHL) based and autoinducer (AI) based QS-cascades. Within the scope of this review a brief retrospective of pentacyclictriterpenoid as candidate anti-biofilm agent is provided. Alongside, possible therapeutic applicability as a novel scaffold for drug development or in combination with antibiotics is also discussed.

Key words: Phytochemicals, Triterpenids, Quorum sensing, Biofilm, Pathogen

Introduction:

Phytochemicals, plant derived bioactive natural products, have been explored extensively for their therapeutic abilities since ages.Most prominent examples of such application includes curcumin from turmeric having anti tumor (Chang *et al.*,2020), anti microbial (Figueira *et al.*, 2020), anti inflammatory (Hasanzadeh *et al.*,2020) properties; gingerenone-A and shogaolfrom ginger with, grastroprotective (Haniadka *et al.*,2013) and anti obesity (Wang *et al.*, 2017) effects and catechols from tea with antioxidant and anti tumor potential (Tejero *et al.*,2007) to mention a few. Identification of structure function relationship of phytochemicals with biological macromolecules in combination with synthetic approach lead to tremendous augmentation of phytomedicine research.The biggest advantages of therapeutic application ofphytomedicines are its non toxic nature and apparent easy availability.With the receding life span of antibiotics due to the emergenceof resistance and the urge to find new anti-microbial agents have made the phytochemicals even more relevant for developing new-age antimicrobial interventions (Cowen *et al.*, 1999). Pentacyclictriterpenes, widespread in fruit peels, leaves, flower and stem bark have been identified as promising compounds with an array of pharmacological activities. This immensely potential group of molecules are broadly classified into three classes: the lupane-, oleanane-, and ursane, all harboring various pharmacologicaleffects.. Therefore, these triterpenes have been marked as promising lead compounds for the development of new multi-targeting bioactive agents (Jäger *et al.*, 2016). One of the major discoveries over the last few decades in the area of microbiology has been the realization that microbial growth and development takes place on a surface with the formation of a complex community like structure called Biofilm. Formation of biofilm structure allows the microorganism to withstand unfavorable environmental challenges, starvation, host immune system and other intervening agents like antibiotics, making them capable of causing a number of chronic disease conditions (Orazi *et al.*, 2019). Production of various pathogenic determinants as well as biofilm production is dependent on Quoramsensing (QS) (Yang *et al.*, 2020). Therefore disruption of QS has become a very useful alternate strategy to deal with the otherwise resistant pathogens. Any agents that can cause such disruptions are referred as quoramquenchers (QQ). Pentacyclictriterpenes, betulin and betulinic acid are being studied as candidate quorum quencher as these can perturb the QS response for biofilm formation by competitively inhibiting QS receptors in Gram negative pathogens like *Pseudomoans aeruginosa* (Rajkumari *et al.*, 2018). Antibiofilm action of petacyclictriterpenoids like oleanolic acid (OA), ursolic acid (UA) and maslinic acid (MA) has been expensively studied against Gram positive bacteria like *Staphylococcusaureus* and *Staphylococcus epidermidis* (Kurek *et al.*, 2014, Blanco-Cabra *et al.*, 2019)

Considering the immense potential of this group in albeit bacterial biofilms, within the scope of this review a comprehensive purview of research conducted to evaluate potential of pentacyclictriterpenoids as antibiofim agent so far is provided.

Petacyclictriterpenoid a significant bioactive phytochemical:

The number of bioactive phytochemicals synthesized by plants is enormous and most of them belong to phenols or their derivatives, flavonoid compounds, tannins, lignins and relatedcompounds. The main role of these being in plant defence against predatory attack of microbes, insects or others, these also provide odor (terpenoids), color (tannins, quinones)and flavor(terpenoids. Some are used by humans as spices (alkaloid in black pepper, terpenoids, tannins in cinnamon, terpenoidincloves, sulfated terpenoids in garlic, monosaccharide in coriander etc.) (Cowan, 1999) on a regular basis and impart immense health benefits. The gross categorization of phytochemicals with well documented antimicrobial action is summarized in Fig. 1.

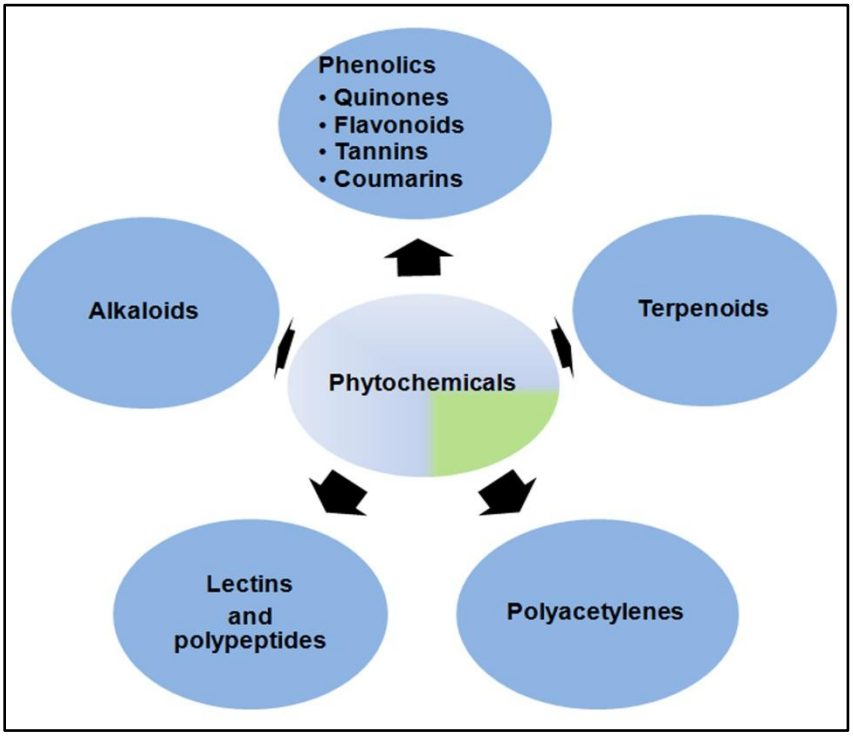

Figure 1: Major groups of phytochemicals with medicinal importance

Triterpenesare widely distributed in natureand are obtained either in free state, as esters or as glycosides. Triterpenoids are usually classified into three groups: acyclic, tetracyclic and pentacyclic (Alqahtani *et al.*, 2013). Pentacyclictriterpenoides are widely distributed in fruits (olive, sour cherry) fruit peels (tomato, apple, pear), leaves (rosemary, oregano, lavender), flower (clove, marigold) and bark (birch) (Sebastian Jäger et al, 2009).Pentacyclictriterpenoids are divided into three main classes, namely, oleanane, ursane and lupine, each of these classes comprising of bioactivecomponents (Jäger *et al.*, 2009). Each of these groups has received much attention due to their various biological pharmacological effects (Fig. 2, Table-1).

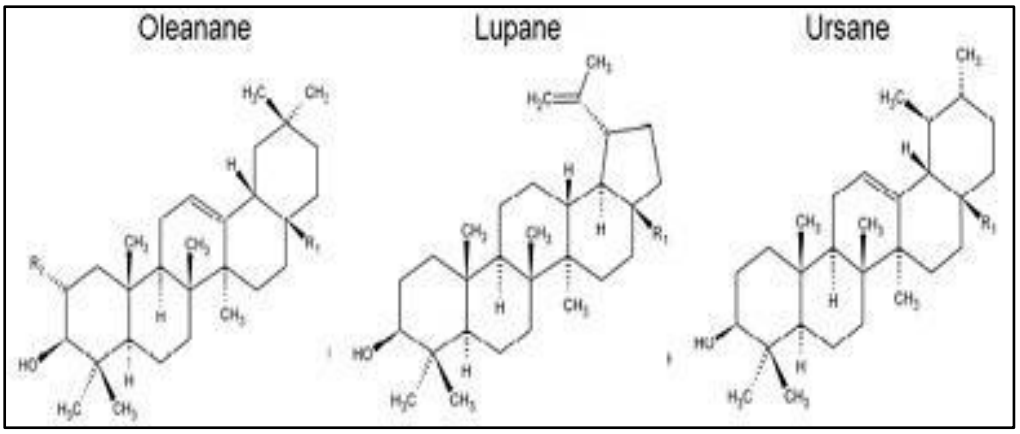

Figure 2: Structural depiction of three major classes of pentacyclictriterpenoids. (Details of R1 and R2 are provided in Table 1)

Table 1: Classification and examples of pentacyclictriterpenoids (Jager *et al.*, 2009)

Triterpene family	Example	R_1	R_2
oleanane	□-amyrin	CH_3	H
	oleanolic acid	COOH	H
lupane	lupeol	CH_3	
	betulinic acid	COOH	
ursane	□-amyrin	CH_3	
	ursolic acid	COOH	

Glycyrrhetinic acid (GA) is a triterpenoid belonging to oleanane family. It is obtained largely from licorice, a leguminosaefound in the Mediterranean region, south Russia, centralAsia, northern China and America. Licorice has long been apart of thetraditional medicine

with its antibacterial, anti-viral, anti-inflammatory and calming effects (Oyama *et al.,* 2016). Extract of licorice contains significant amount of GA and have been studied extensively as well. GA and its various derivative have been assigned with many properties, including anti-inflammatory as well as antitumor (Markov *et al.,* 2018), anti-allergic (Yang *et al*., 2015), anti-filarial (Tyagi *et al.,* 2019) anti-viral (Perelmuter *et al.,* 1988) and anti bacterial (Yamashita *et al.,* 2019, Oyama *et al.,* 2016) activities. GA and its derivatives have also found to work as a pro-apoptotic agent (Logashenko *et al.,* 2011) proteasome activator, agent decelerating aging and Alzheimer's disease progression (Papaevgeniou *et al.,* 2016). Glycyrrhetinic acid in combination with other bioactive compounds acts against Dopamin receptor D3 for Parkinson's diseaseis (Mirza *et al.,* 2014). It has also been effective, alone or in association with other antibiotics against *Mycobacterium bovis* (Zhou *et al.,* 2012) and methicillin-resistant *Staphylococcus aureus* (de Breij *et al.,* 2016). Anti fungal (Kim *et al.,* 2013) and anti leismanial (Gupta *et al.,* 2015) activity has also been reported in past years. Ursolic acid (UA) is a pentacyclictriterpenoid belonging to the ursanefamily. UA is a secondary plant metabolite exhibiting a wide range of pharmaceutical properties. Ursolic acid, usually present in the stem bark (eucalyptus, black elder) leaves (oregano, rosemary, sage etc) or fruit peel (apple) (Woźniak *et al.,* 2015). Amongst various pharmacological properties of UA it's pulmonary, hepato, kidney and cerebro as well as osteoporosis (Woźniak *et al.,* 2015) are worth mentioning. Besides, ursolicacid also exhibits antioxidant and anti-inflammatory mechanisms (Habtemariam *et al.*,2019, Kashyap *et al.*,2016), anti cancer (Yin *et al.,* 2018, Chan *et al.,* 2019) anti viral (Tohmé *et al.,* 2019) anti-microbial activities against different strains of bacteria (Park *et al.,* 2018, Zhou *et al.,* 2017). This triterpenoid has also been exploited to manage neurodegenerative and psychiatric diseases (Ramos-Hryb *et al.,* 2017), obesity-induced cardiovascular diseases (Lin *et al.,* 2016), cardiomyopathy with diabetic condition (Woźniak *et al.,* 2015). A representative molecule from lupine family is betulinicacid (BA), which has been investigated highly in the past decade for an array of beneficial properties. BA and its analogues have been studied for anti-cancer (Lee *et al.,* 2019), anti-inflammatory Ekuadzi E *et al.,* 2018), anti-angiogenic (Shin *et al.,* 2011), immune-modulatory (Takada *et al.,* 2003), antimicrobial (Haque *et al.,* 2014), anti malarial (Innocente *et al.,* 2012), anti-tumor (Zhang X *et al.,* 2016), and anti-HIV (Li *et al.,* 2016) effects among many others. Besides therapeutic potential, BA has been explored againstchemically induced hypothyroidism (Afzal *et al.,* 2014). Although already established as a potent antimicrobial (Carvalho Junior *et al.,* 2019) agent, the anti-biofilm potential of these molecules are explored mostly in past one decade (Kannan *et al.,* 2019**,** Feuillolay *et al.,* 2016).

Quorum sensing and biofilms:

Biofilm is an assemblage of microbial cells surrounded by a matrix formed by the extracellular polymeric substances (EPS) secreted by those residing cells. A biofilm community can harbour a pure culture, but a community of mixed microbial species is more common in nature. Biofilm formation is a developmental process in which a quorum sensing signal molecule, an auto-inducer, functions to induce the secretion of the EPS and leads to the formation of characteristic three-dimensional biofilm architecture (Flemming *et al.,* 2016). Biofilm formation provide protection from toxic compounds, such as antibiotics, host immune response and predation (Sharma *et al.,* 2016), thus serve as a survival mechanism for the inhabitants. Almost all microorganisms can form biofilm and microorganisms most often (>99%) exist in nature as biofilms. Biofilm formation also protects microorganisms from various environmental challenges such as pH, salinity, and metal toxicity (Koo *et al.,* 2017) and even confers resistance to antibiotics and microbicides (Hall-Stoodley *et al.,* 2009).

Clinical research also revealed the importance of biofilm in infectious diseases. An estimated frequency of infections caused by biofilms, especially in the developed world, lies between 65% and 80% as per reports from Centres for Disease Control and Prevention (CDC) and National Institutes of Health (NIH), respectively (Moser *et al.,* 2018). Biofilm is significant for pathogenic bacteria as it modulates the pathogenic potential of bacteria as evident from cariogenic bacteria in plaque biofilms. Microorganisms in biofilms have been reported to be less susceptible to antimicrobial agents and have reduced sensitivity to inhibitors (Jabra-Rizk *et al.,* 2006). Biofilm formation results in delayed penetration of tobramycin and colistin into *Pseudomonas aeruginosa* cells (Musken *et al.,* 2018) and *Escherichia coli* biofilms exhibited decreased susceptibility to antibacterials (Schiebel *et al.,* 2017). Similar reports are available in ESKAPE pathogens (Pletzer *et al.,* 2018), in addition to high persistence rate after drug exposure to biofilms (Michiels *et al.,* 2016). Moreover, the potentially pathogenic bacteria like *Staphylococcus aureus, Enterococcus faecalis, Streptococcus, Klebsiella, Pseudomonas,* tend to grow on catheters, artificial joints, mechanical heart valves, etc which lead to persistent infections as a result of periodic release from the said focus (Costerton *et al.,* 2003). Biofilm dispersion is also a matter of clinical concern as release of cells from biofilms initiates a new round of infection. In *P. aeruginosa*, the localized depletion of nutrition in a biofilm has been hypothesized as inducer for release or detachment of cells from the biofilm (Chambers *et al.,* 2017).

QS is dependent on production of specific signalling molecules in population density dependent manner. These small molecules called autoinducers (AIs) are secreted by the cell and

once reaching upon a certain concentration, can bind to certain receptors present on the cell sending a designated signal leading to significant alteration in gene expression often related to the pathogenicity of the bacterium. Different virulence factors are regulated by QS for different bacterium like pyocyanin, lectin and other factors in *P. aeruginosa*, hemolysin and enterotoxin production in *S. aureus*, biofilm formation in *Vibrio*sp etc.

QS was first identified in *Vibrio* sp.. For regulating QS cascade the bacteria possesses two independent QS-inducers. The CAI-1 ((S)-3-hydroxytridecan-4-one) system that acts intragenously is uniqe for *Vibrio* (Kelly *et al.,* 2009). The Other auto inducer, AI-2 ((2S, 4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate), which is conserved across Gram negative bacteria for inter species communication (Chen *et al.,* 2002). *Vibrio* proteins CqsS and LuxPQ, functions as CAI-1 and AI-2 receptors respectively. At low cell density, and lesser level of autoinducers, CqsS and LuxPQ act as kinases for LuxU. The phosphate is funnelled to LuxO the key response regulator for the bacteria. When phosphorylated, LuxO triggers transcription of an array of four small RNA - Qrr 1-4. Cumulatively these small RNAs repress translation of HapR which is the master QS regulator in high cell density. Parallely, Qrr 1-4 RNAs activates translation of AphA, regulator for low cell density QS regulator. At high cell density, when auto inducer the CqsS and LuxPQ acts as phosphatse with successive dephosphorylation of LuxUand LuxO. Hence under such a situation, qrr 1-4 is not expressed. This leads to removal of repression and activation of HapR translation. On the flip side, translaton of AphR stalls.Under such situation the aggregative behaviour is induced. Jung *et al.* recently reported that two other QS receptor CqsR and VpsS, with unknown ligands integrate signal into QS cascade via LuxU (2015). Recently a third biofilm modulatory Qs system was discovered in *V. cholera*e. Autoinducer, called DPO (3, 5-dimethylpyrazin-2-ol), binds to a transcriptional regulator called VqmA. This complex activates expression ofaregulatory RNA VqmRwhicheventualy represses genes required for biofilm formation (Bridges *et al.,* 2019).

The bacterial QS signals mainly consistof acyl-homoserine lactones (AHLs), autoinducing peptides (AIPs), and autoinducer-2 (AI-2). The QS signal differ for Gram positive and Gram negative organisms. Gram positives rely on AIP signaling and Gram negatives on AHL signaling and both on AI-2signals as well. These three types of signaling molecules have been found to regulate growth and infectivity in bacteria. AHLs once accumulated upto the threshold level, diffuse across the cell membrane and bind target transcriptional regulators leading to gene expression(Yang *et al.,* 2009). For AIP, they upon reaching threshold, are transported out and then enter the cell by the help of a histidine kinase sensor which upon

phosphorylation alter expression of target genes. AI-2 system is used by the bacteria to receive signals from other species present in the same environment. For most of the bacteria, AI-2 signaling is carried out by Luxsynthetase. As biofilm formation has been intricately linked with QS for most of the pathogens along with expression of many other virulence factors, blockage of QS signaling is therefore considered as an efficient intervention strategy. Application of QS suppressors or Quoram Quenchers (QQ) to inhibit the expression of virulence factors and thus making them susceptible to host immune system seems like an efficient alternative therapeutic strategy. Inactivation of receptors, inhibition of synthesis of the signal, degradation of the signal and blockage of the signal using antibody are few of the strategies applied.

Pentacyclictriterpenoid as quorum quencher:

Over the years unrestricted use of antibiotics have made the issue of resistance more and more complicated. Hence finding suitable alternative has become an urgent need of the hour. Among many agents tried and tested, tritepenoids have been one of the most readily accepted one due to its non toxic nature. Many researchers have not only established these as potent anti microbial agents, but also anti biofilm agent against various groups of microorganisms.

In a study with oleanolic aldehyde coumarate (OALC), a triterpenoidcoumarateester and novel bioactive compound obtained from dalbergiatrichocarpa bark not only inhibited the formation of biofilm by *P.aeruginosa*, but also affected its maintenance. The compound found to interfere with the expression of the *las* and *rhl* mediated QS systems, Consequently QS-mediated virulence factors.AHL production was affected and external supply of AHL was unsuccessful to restore the condition proving the extent of damage even beyond AHL production (Rasamiravaka *et al.*, 2015). In a study by Rajkumari et al.betulin and betulinic acid were found to be strong competitive inhibitors of QS receptors, LasR and RhlR. Another two triterpenes, ursolic acid and resveratrol were tested for their anti biofilm potential against Methicillin Resistant *Staphylococcus aureus*. Although ursolic acid seemed to inhibit biofilm formation by affecting amino acid metabolism, resveratrol affected QS related gene expression. *Hld gene that codes δ-hemolysin and located within the agr locus, one of the QS clusters in S.aureus,* was found to be up-regulated, indicating that the role of resveratrol and ursolic acid in MRSA *agr* function at the RNA level for inhibiting biofilm formation. A similar study by Quave CL et al with oleanene and ursene derivatives from European Chestnut leaf extracts showed biofilm inhibition in *Staphylococcus aureus* by targeting agr alleles (Quave *et al.*, 2015). Five limonoids isolated from sour orange were checked for their ability to interfere with QS and biofilm formation in *Vibrio harveyi*. Out of the five tested four, namely isolimonic acid, deacetylnomilinic acid glucoside

and ichangin were found to inhibit AI mediated QS. Moreover isolimonic acid and ichangin, both were identified as potent modulator of luxO expression (Vikram *et al.*, 2011). In a comprehensive study with three triterpenoids, glycyrrhetinic acid, ursolic acid and betulinic acid against *Vibrio cholerae* biofilm, all three were found to interfere with QS process and perturb biofilm formation (Fig.3). Molecular docking analysis hinted about probable interaction with cyclic di-GMP sensor VpsT, autoinducer-2 sensor kinase LuxP-LuxQ and transcriptional activator HapR (doi: https://doi.org/10.1101/2020.01.06.896183). Over all, comprehensive and better understanding of anti-biofilm potential of various pentacyclictriterpenoids has offered the scope of developing multi-faceted strategies to combat bacterial infections.

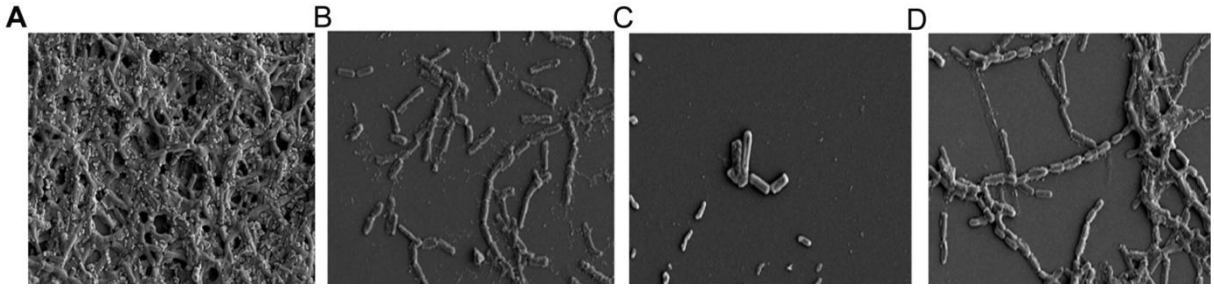

Figure 3: Effect if triterpenoids on biofilm integrity. Log phase *Vibrio cholerae* cells were allowed to form static biofilm in absence (A) or presence of three different pentacyclictriterpemoids representing oleanone (B), lupine (C) and ursane (D) family. Integrity of biofilms was visualized by scanning electron microscopy (Data: Paul Bhattacharya *et al.*, Unpublished).

Concluding remark:

A major concern for clinical implication of the pentacyclicterpepenoids is cytotoxic impact on various mammalian cell lines. However, the triterpenoids can offeran excellent bioactive scaffold to impart biofilm selectivity and diminishing cytotoxicity. Selective nano-delivery strategy for biofilm can also accentuate anti-biofilm action. Testing in combination with antibiotic for optimal triterpenoid-antibiotic composition can culminate into successful combinatorial therapeutics.

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POTENTIAL OF FOUR INDIGENOUS PLANT SPECIES IN THE REMEDIATION OF HEAVY METALS CONTAMINATED SOIL

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Abstract:

The present study aims to assess the heavy metals concentration and potential plant species present in the Thane-Belapur Industrial area, Navi Mumbai, Maharashtra, which is one of the biggest industrial areas in India. The soil and plant species which found abundantly in this area were collected in Pre-monsoon, Monsoon and Post-monsoon season from five collecting stations to cover most of the industrial area. The collected sample of soil and plants were analyzed for the heavy metals such as Cr, Fe, Co, Ni, Cu, Zn, As, Cd & Pb using ICP-AES. The phytoremediation indices such as bioconcentration factor and Translocation factors were also studied. The results show that the concentration of heavy metals in soil was in the sequence of Fe>Zn>Cu> Ni>Cr>Co>Pb>As>Cd. Based on the result of soil, five heavy metals Cr, Fe, Ni, Cu and Zn, which found in high concentration during seasons and at all stations were selected for analysis from four collected plant species *Ipomea carnea* Jacq., *Alternanthera sessilis* L., *Paspalum conjugatum* P. J. Bergius and *Commelina benghalensis* L. Roots of all four plants had a higher concentration of heavy metals as compared to shoots. The bioaccumulation of heavy metals were in the sequence of Fe>Zn>Cr>Cu>Ni while *I. carnea* was more potential than *C. benghalensis, A. sessilis* and *P. conjugatum.*

Keyword: Phytoremediation, Thane-Belapur Industrial area, Heavy metals

Introduction:

Heavy metals are a group of elements between copper and lead on the periodic table of the elements having atomic weights between 63.546 and 200.590 and have atomic densities greater than 4 g cm-3 or 5 times more than water (Duruibe *et al.*, 2007). Heavy metals are natural components of the Earth's Crust but the excessive amount in water and soil due to geological and anthropogenic activities causes pollution of it (Abii, 2012). Heavy metals in streams are as

results of flooding, erosion, weathering of rocks and minerals but industrialization, urban waste discharge, fertilizers, and personal care compounds are the most significant sources for soil and groundwater pollution (Maharia *et al.*, 2010). Contamination of heavy metals represents one of the most severe threats to water and soil resources as well as human health (Kabata-Pendias & Pendias, 2001). The most common contaminants are Iron (Fe), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Nickel (Ni) and Zinc (Zn) (EPA, 1997).

The metal contaminated soil can be remediated by chemical, physical and biological methods. Most of these conventional remediation technologies are costly, time-consuming and tedious, and may cause further degradation of the environment (Ghosh & Singh, 2005; Schmidt, 2003). The phytoremediation offers sustainable remediation technique by overcoming the conventional chemical and physical technologies. Phytoremediation is the set of technologies that use plants to treat contaminated sites (EPA, 2000). The categories of phytoremediation include phytoextraction (the use of plants to remove contaminants from the soil), phytovolatilization (the use of plants to make volatile chemical species of soil elements), Rhizofiltration (the use of plant roots to remove contaminants from flowing water), phytostabilization (the use of plants to transform soil metals to less toxic forms, but not remove the metals from the soil) and Phytotransformation (the use of plants to transform toxic metals into a nontoxic form)(Mahmood, 2010).

The soil of the Thane-Belapur Industrial area contains a high concentration of heavy metal contents like nickel, zinc, cadmium, copper, iron, arsenic and mercury. There is a need for regular monitoring of water resources and a further improvement in industrial wastewater treatment methods(Singare *et al.*, 2010). So the aims and objectives of the present research were to (i) To find out the concentration of heavy metals in the study area. (ii)To study floristic diversity and identify the number of plant species found abundantly throughout the year in the study area. (iii)To analyze the heavy metals bioaccumulated in the selected plant. (iv)To determine the portal in which heavy metals are bio-accumulated. (v) To find out potential plant species for remediation of selected heavy metals.

Material and Methods:

Study Area:

Thane-Belapur industrial area is referred in MIDC document as the TTC (Trans-Thane Creek) is one of the most industrialized area containing about 2000 industrial units. It covers an area of 2,546 hectares at 19004’22.52” N and 73001’08.40” E, and lies on the east of the Thane creek, Thane-Belapur road between the urban centres of Thane and Nerul. It has approximately 16 kilometres in length and flanked by the Mumbra-Parsik hills to the east. The industrial composition of this area has been dominated by petrochemical and engineering units. These

industries discharge polluted water into canals, rivers, creeks, and sea (Gajbhiye & Bhalerao, 2016). Five stations were selected so as to cover most part of this area (Fig 1).

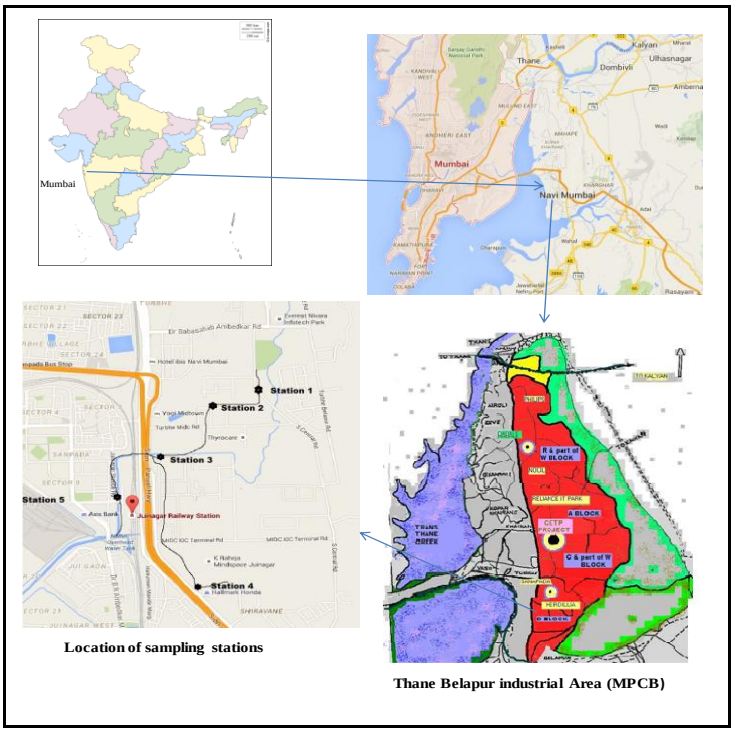

Figure 1: The geographical location of Thane- Belapur Industrial area

Collection and analysis of plants and soil Samples:

The plant and soil samples were collected from the 5 selected stations. The soil samples and the four plant species *Ipomea carnea* Jacq. *Alternanthera sessilis* L., *Paspalum conjugatum* P. J. Bergius and *Commelina benghalensis* L. were collected as per methods given by (Al-Farraj & Al-Wabel, 2007, p.) the sample preparation and analysis of heavy metals were done as per given by Gupta, 2004 using Inductive Coupled Plasma- Atomic Emission Spectrometer (ICP-AES) model ARCOS. The analysis of heavy metals Fe, Zn, Cr, Cu and Ni in plant materials were done on the basis of concentration in soil. The obtained data were multiplied by suitable multiplication factor to get accurate value of heavy metal. The experimental limits of detection (LOD) limits of quantification (LOQ) were also determined(Gajbhiye & Bhalerao, 2016)

Estimation of phytoremediation indices:

i. The Bioconcentration Factor (BCF) of metals was used to determine the number of heavy metals that are absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil(Ghosh & Singh, 2005) and is calculated using the formula:

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (Soil)}}$$

ii. Translocation Factor (TF) evaluate the potential of plants for Phytoextraction. This ratio is an indication of the ability of a plant to translocate metals from root to the aerial parts of the plants (Marchiol *et al.*, 2004). It is represented by the ratio:

$$\text{TF} = \frac{\text{Metal concentration (Stem + leaves)}}{\text{Metal concentration in roots}}$$

Descriptive statistics (mean, standard deviation) of the data obtained, Correlation coefficient (r) and ANOVA was calculated by using software Microsoft excels.

Results and Discussion:

Heavy metals in the soil sample:

The concentration of heavy metals present in the soil from the study area at five different stations is given in table 1 while the Pearson's coefficient of correlation matrix between heavy metals is given in table 2.

Table 1: Heavy metals concentration in Soil (mg/Kg) (n=3) and p-value Significance between the seasons (at5%)

Area	Cr	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
Station (S1)	516.67± 3.28	2087.8 ±11.21	337.33 ±3.89	904± 5.98	731.067 ±11.54	1684 ±12.38	110 ±7.34	8.33 ±0.84	0
Station (S2)	323.67 ±5.64	1089.1± 8.68	229.67± 7.24	163.33 ±2.68	438.67 ±8.44	615.33± 8.49	0	0	0
Station (S3)	399.67± 1.05	1678.4 ±6.48	424.00± 5.67	166.0 ±6.47	416.26 ±6.87	634.67± 5.36	0	0	0
Station (S4)	259.67± 9.21	1669.6 ±9.63	374.33± 3.87	189 ±5.86	531.67± 5.97	1297.33 ±13.8	0	0	747 ± 3.68
Station (S5)	597.00± 4.98	547.36 ±4.57	607.33 ±9.45	808 ±10.05	654.13 ±3.28	1339 ±11.06	0	0	1024.33 ± 9.07
ANOVA P value	0.3969	0.2115	0.003222	0.9207	0.0007751	0.1263			

The results show that the concentration of Fe was highest in all stations followed by Zn, Cu, Ni, Cr, Co, Pb, As and Cd. The Station 5 was found more polluted while the station 2 was found to be less polluted. This is because the station 5 was big nalla downside of Thane-Belapur industrial area where all small nallas join together while the station2 was small nalla upside of

this area. The high value of Fe, Zn, Ni, Cu and Cr at station 1 was due to the fact that it was uppermost sites at the hilly region where the stone mining was predominant activity and all these are earth metals. It is found that there were no significant seasonal variation ($p>0.05$) in the presence of Cr, Fe, Zn and Ni while significant difference ($p<0.05$) found in case of Co and Cu (table1). There was a strong positive correlation between the presence of Co and As (r=1.00) while other metals were weakly correlated with each other. Co was found to be negatively correlated with other metals (table2). The high concentration of heavy metals which was above the permissible level was due to the industrial composition of the study area (Krishna and Govil, 2005).

Table 2: Coefficient of correlation (n=15) between the heavy metals of soil collected from study area

	Cr	**Fe**	**CO**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Pb**
Cr									
Fe	0.0134								
CO	0.513	0.283							
Ni	0.765	0	0.133						
Cu	0.223	0.018	-0.470	0					
Zn	0.399	0.089	-0.254	0	**0.839**				
As	0.515	0.030	-0.245	0	0.678	**0.835**			
Cd	0.515	0.030	-0.245	0	0.678	**0.835**	**1.00**		
Pb	-0.240	-0.425	-0.065	0	-0.189	-0.129	-0.104	-0.104	

Heavy metals bioaccumulation in collected plants:

Phytoremediation technologies are largely dependent on the physiological characteristics of the selected plants and the kind of pollutants (Megateli *et al.*, 2009). The bioaccumulation was found to be highest for Fe which was 11,159.6±14.0 mg/kg in roots by *C. benghalensis*. Fe was highly accumulated by all the plants followed by Zn, Cu, Cr and Ni. The highest concentration was accumulated in roots than shoots. Even though *C. benghalensis* is seasonal herbaceous plant but accumulates the highest concentration of metals followed by I. *carnea*, *A. sessilis* and *P. canjugatum* (table 3). The normal heavy metal contents of terrestrial plants growing in uncontaminated soils were found to be in the range of 0.2-8.4 mg kg-1 for Cr, 0.1 to 3.7 mg kg-1 for Ni, 0.4-45.8 mg kg-1 for Cu and 1-160 mg kg-1 for Zn (Kabata & Pendias, 1984). Therefore heavy metal concentration above these limits in plant body may be helpful to decide the

capabilities of plants to remediate contaminated soil. Results showed that the concentrations of heavy metals in all these plants were found to be very much above the limits.

Table 3: The bioaccumulation of five heavy metals in mg/kg by the four plant species from the study area (n=3) ± SD

Metals	*Alternanthera sessilis*		*Ipomea carnea*		*Paspalum conjugatum*		*Commelina benghalensis*	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
Cr	40.0± 1.56	2.90± 0.58	56.83± 6.41	15.6± 2.66	22.20± 6.39	13.3± 5.78	53.4± 2.47	5.70± 2.11
Fe	2323.0± 16.12	1063.4± 8.66	2592.0± 13.98	1126.0± 8.58	1329.9± 9.05	526.5± 8.57	11159.6± 14.0	1628.6± 10.24
Ni	3.70± 1.08	24.6± 6.74	6.23± 1.25	8.82± 2.22	4.90± 1.16	2.0± 0.03	0.141± 1.04	4.40± 1.09
Cu	21.0± 2.47	9.80± 2.34	105.3± 6.86	19.26± 3.48	26.20± 4.77	5.7± 0.14	123.6± 5.68	26.5± 2.88
Zn	161.6± 5.32	101.4± 3.69	284.62± 5.75	135.46± 6.71	241.5± 6.83	87.30± 5.36	244.0± 5.71	124.6± 1.86

Table 4: The Bioconcentration Factor (BCF) and Translocation factor (TF) of studied plants for heavy metals

Metals	*A. sessilis*		*I. carnea*		*P. conjugatum*		*C. benghalensis*	
	BCF	***TF***	***BCF***	***TF***	***BCF***	***TF***	***BCF***	***TF***
Cr	0.083	0.072	0.114	0.106	0.068	0.599	0.140	0.275
Cu	0.153	2.142	0.746	0.214	0.158	0.217	0.619	0.183
Fe	1.622	0.457	6.125	0.145	0.889	0.395	1.781	0.435
Ni	0.033	6.648	0.005	1.415	0.007	0.408	0.016	31.205
Zn	0.155	0.629	0.218	0.510	0.195	0.361	0.249	0.475

Translocation Factor (TF) is the capacity of plants to translocate metals from roots to shoots while the Bioconcentration Factor (BCF) is a measure of metal accumulation efficiency (Zhang *et al.*, 2002). The plant species which has a value of TF and BCF higher than one are used for phytoextraction (Ashraf *et al.*, 2011). *I. carnea* (6.125), *C. benghalensis* (1.781) and *A. sessilis* (1.622) showed values of BCF > 1 indicate the potential phytoextraction species for Fe while other metals showed BCF< 1(table 4). If the value of TF is above ten, then the plant species is a hyperaccumulator of that metal. From the study, it was clear that *C. benghalensis*

(31.205) is a hyperaccumulator of Ni (Table 4). The species which has values of TF above one but below ten then they are hyper tolerant and that are *A. sessilis* for Ni(6.648) and Cu(2.142), *I. carnea* for Ni (1.415) while values of TF less than one shows tolerance to metals (Ashraf *et al.*, 2011).

Conclusion:

The four dominated plant species *Ipomea carnea, Alternanthera sessilis, Paspalum conjugatum* and *Commelina benghalensis* and soil collected from this area shows a high concentration of all selected metals. Roots of all four plants have a higher concentration of heavy metals as compared to shoots. The bioaccumulation of heavy metals has a sequence of Fe>Zn>Cr>Cu>Ni while plants were in a sequence of *I. carnea* >*C. benghalensis* >*A. sessilis* >*P. conjugatum*. From the result, it is clear that *C. benghalensis* is a hyperaccumulator of Ni, and *I. carnea A. sessilis* and *P. conjugatum* along with *C. benghalensis* are suitable for phytoextraction of Fe, Zn Cr, Ni and Cu.

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ISOLATION AND IDENTIFICATION OF RHIZOBIUM FROM ROOT NODULES OF FENUGREEK PLANT COLLECTED FROM VILLAGE VANGAON AND TO STUDY ITS EFFECT ON SOIL FERTILITY AND PLANT GROWTH

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Abstract:

As the population of the world is increasing day by day, the demand for food is also increasing. Increase in population and increased food demand, impart stress on agriculture. To increase the productivity of crops, farmers prefer to use chemical fertilizers. But these chemical fertilizers are responsible for various types of pollution. To avoid pollutionand to prevent environmental damage, now there is a need of biofertilizers. One of the biofertilizers that can be used is rhizobium which is symbiotic nitrogen-fixing bacteria. In the present study, isolation and identification of rhizobium is done by using the isolation technique and by using biochemical tests. Isolated and identified culture is used to inoculate soil, then the nitrate content of the soil is determined by the colorimetric method and also its effect is checked on plant growth by measuring its height. It was found that rhizobium shows an increase in soil nitrate content as well as plant growth. So it can be used alternative to chemical fertilizers.

Keywords: Rhizobium, biofertilizers, Isolation.

Introduction:

The population of the world is increasing drastically; therefore there is a demand for food. To satisfy that need agricultural productivity should get increased. For that purpose, farmers prefer to use chemical fertilizers. In agriculture, for better product yield, soil fertility plays an important role. Basic requirements for soil fertility include nitrogen, phosphorus, sulfur, etc. The concentration of atmospheric nitrogen is nearly 71% but unfortunatelyplantsas well as animals can’t use it directly. To convert this atmospheric nitrogen into a usable form of nitrogen,

certain microorganisms play an important role. One of such microorganisms is Nitrogen-fixing bacteria. These nitrogen-fixing bacteria have one enzyme called Nitrogenase which will convert atmospheric nitrogen into a usable form of nitrogen by plants like nitrate, nitrite, ammonium salts, etc. Leguminous plants include many important species that are used as food and fodder crop throughout the world. They can provide their nitrogen requirements through nitrogen fixation in symbiosis with soil bacteria collectively known as rhizobia. These bacteria form root nodules on leguminous plants and convert atmospheric N2 into a form usable by plants. Application of effective rhizobial strains as biofertilizers to improve legume production is an important approach in sustainable agriculture (Olivera, 2011). Nitrogen-fixing bacteria are of two types- symbiotic nitrogen-fixing bacteria and non-symbiotic nitrogen-fixing bacteria. Symbiotic nitrogen-fixing bacteria are those who live in symbiotic association with leguminous plants. The symbiotic association is also called mutualism because both the member in the association get benefited from this association.Fenugreek is an important legume crop for consumers as a popular spice in Indian cuisine and has long been used in both Ayurvedic and traditional medicine to induce labor and lactation. It aids in digestion and as a general health and wellness tonic (Basch, 2003). Its crop contributes an important nutrient N for the soil. Fenugreek is also a good soil renovator and widely used as green manure. Fenugreek was reported to fix 48% of its total N_2during the growing season. It is also a good source of atmospheric nitrogen fixation by *Rhizobium* present in its root nodules(Desperrier,1985).In the present study, bacteria present in root nodules of fenugreek plants collected from different localities of Vangaon were collected, isolated and then biochemically identified. The effect of these isolates was tested for plant growth and soil fertility.

Materials and Methods:

Study Area:

Vangaon is a village in the Palghar district of Maharashtra, India. It is located in the Dahanu taluka. Its Latitude is 19° 51' 59.99" N and Longitude is 72° 44' 59.99" E. Fenugreek plants (*Trigonella foenum-graecum*) were collected from three different locations nearby to Vangaon village that is Kotim, Kolavali, Bavada respectively having healthy root nodules. Then these samples are brought to the laboratory in clean, polythene bags.

Methods:

Surface sterilization and isolation of Rhizobium:

Roots of Fenugreek plants were washed with tap water to remove soil from it. Then pink, healthy root nodules were removed carefully by using sterile forceps. Root nodules were treated with 20% tween 20 detergents, followed by washing with sterile distilled water. Next root nodules were washed with 0.1% mercuric chloride for 3 minutes. Then washed with sterile distilled water for 3 times. Then root nodules were washed with 75% ethanol for 3 minutes. Finally, nodules were washed with sterile distilled water for 8 times. Nodules were taken on slide and teased with sterile forceps. White exudates from nodules were streaked on sterile CRYEMA plate (Congo red Yeast extract Mannitol agar pH 6.8 ± 0.2) by using a flame sterilized nichrome loop. The inoculated plate is then incubated at 37^{o} C for 3- 4 days. Rhizobium will form white-colored colonies while other organisms will give red colonies. Rhizobium does not allow Congo red inside its cell andtherefore give white colonies. White translucent colonies developed on the plate were then morphologically observed for size, shape, color, opacity, elevation. Marginand then gram staining was carried out.

Gram staining:

Colony obtained after incubation was then subjected for gram staining. Smear was prepared on a microscopic slide. Then smear was treated with 1% crystal violet for 1 minute. Wash smear with distilled water. After that, 1% of Gram's iodine was addedand kept for 1 minute. The slide was washed with distilled water followed with washing with decolorizer that is 75% Alcohol for 30 seconds. The slide was counterstained with 1% Safranine for 1 minute. The slide was washed with distilled water. The slide was air-dried. One drop of cedarwood oil (immersion oil) was added on the smear. The slide was observed under the microscope (100X). Gram nature and shape was noted.

2. Biochemical tests:

i) Sugar fermentation test:

To find out types of sugar utilized by rhizobium, a sugar fermentation test was carried out. Loopful of culture was inoculated in sterile test tubes having 1% Glucose, 1% lactose, 1% mannitol, 1% maltose. Test tubes were incubated 37^{0} C for 24 hours – 48 hours. Change in color of media and gas production in Durham's tube was noted.

ii) IMViC TEST:

a) **Indole test:** To determine the ability of the organism to convert tryptophan into indole due to the presence of enzyme"tryptophanase"; an indole test was done. Loopful of culture was inoculated in sterile 2% tryptone water and tube was incubated at 37^{o} C for 24 hours. 2 to 4 drops of Kovac's reagent was added in inoculated, incubated tube. The Colour of the ring was noted.

b) **Methyl Red test:** To find out acid production ability of microorganisms,Loopful of culture was inoculated in sterile 1% Glucose phosphate broth. The tube was incubated at 37^{o} C for 24 hours. 5 drops of methyl red reagent were added after incubation. A Colour change was noted.

c) **Voges Proskauer Test:** To determine whether an organism can synthesize acetoin or not, Loopful of culture was inoculated in sterile 1% Glucose phosphate broth. The tube was incubated at 37^{o} C for 24 hours.4 drops of omera's reagent was added. The result was noted.

d) **Simmon Citrate agar Test:** To find out whether an organism can use citrate or not, loopful of culture was streaked on the surface of sterile Simmon citrate agar slant and slant was incubated at 37^{o} C for 24 hours. Change in color was noted.

iii) Triple sugar ion test:

Loopful of culture was taken on sterile straight loop and then it was stabbed in sterile TSI slant incubated at 37^{0} C for 24 hours. The change in color was recorded. This test will help to find out which sugars were utilized by bacteria.

iv) Urease test:

To determine whether isolated bacteria is urease producer or not, loopful of culture was inoculated in sterile Christensen's urea broth and incubated at 37^{o} C for 24 hours and change in color was noted.

v) Catalase test:

One colony was picked from the plate and kept on the slide. Then 2 drops of hydrogen peroxide were added on it. The presence or absence of effervesces was noted. The Presence of effervesces indicates an organism is a catalase synthesizing organism.

vi) Starch hydrolysis test:

Sterile 1% starch agar plate was taken. One loopful of culture was streaked on the plate. The plate was incubated at 37^{o} C for 24 hours. After incubation, few drops ofLugol's iodine was added on a plate. Change in color due to the presence or absence of starch was recorded. If the clear zone was observed around a colony, indicate, bacteria can produce an amylase enzyme.

vii) Glucose peptone agar test:

Loopful of culture was streaked on sterile Glucose peptone agar plate and incubated at 37^{o} C for 24 hours. The presence or absence of colonies after incubation was recorded. Rhizobium cannot grow on this media because this organism cannot utilize nutrients available in media.

Effect of rhizobium on soil nitrate content and plant growth:

Sterile soil was taken. It was divided into two equal half. One was inoculated with 100 ml of rhizobium suspension having density $1*10^{9}$cells/ ml while second was uninoculated and used as control.

i. The nitrate content of soil:

1gm of soil was taken and it was mixed with 10 ml of water. Then it was mixed properly and allowed it to settle down. The supernatant was transferred to the new test tube. One pinch of zinc dust was added and allowed to stand for a few minutes.Then the supernatant was mixed with 0.5ml of NEDD (*N*-(1-Naphthyl) ethylenediamine) and0.5 ml of sulphanilamide reagent. O.D. was measured by colorimeter at 540 nm. The same procedure was repeated for uninoculated soil also. Soil nitrate content was measured daily for 10 days. Standard reading was taken by using standard nitrite solution with a concentration of 100 ug/ml. Reading was taken at 540nm using colorimeter.The nitrate content of the soil was calculated using the following formula:

$$\text{The nitrate content of soil } (\mu g\ NO_3\text{-}N/lit) = \frac{\Delta \text{ O.D. of sample}}{\Delta \text{O.D. of a standard}} \times \frac{\text{concentration of standard}}{\text{volume of sample}} \times 1000$$

ii. **Plant growth**:

25 healthy surface-sterilized fenugreek seeds were added in both inoculated and uninoculated soil. Both inoculated and uninoculated soil with seeds was incubated in light for 16hours and in dark for 8 hours. Growth germination and height of plant was measured for 10 Days.

Results:

Isolation and Gram Staining:

After 48 hours of incubation,

Sample 1: Fenugreek plants collected from Kotim showed two white, translucent colonies.

Sample 2: Fenugreek plants collected from Kolavali showed three white, translucent colonies

Sample 3: Fenugreek plants collected from Bavada showed two white, translucent colonies.

Colony characteristics: All colonies were white, translucent, having entire margin, convex elevation, 3mm in size, consistency was smooth. Gram nature observed was gram-negative rods.

Table 1: Biochemical tests

Name of the test	Kotim Isolate	Kolavali Isolate	Bavada Isolate
i)Sugar fermentation test:			
1% Glucose	+	+	+
1% Lactose	+	+	+
1% Maltose	+	+	+
1% Mannitol	+	+	+
ii)Indole test	-	+	+
iii)Methyl red test	+	+	+
iv)Voges Proskauer test	-	+	+
v) Simmon citrate test	+	+	+
vi) Triple sugar ion test	+	+	+
vii) Urease test	+	+	+
viii) Catalase test	+	+	+
ix)Starch hydrolysis test	+	+	+
x) Glucose Peptone Agar test	-	-	-

Key: + = Growth, - = No growth

Effect of rhizobium on nitrate content of soil:

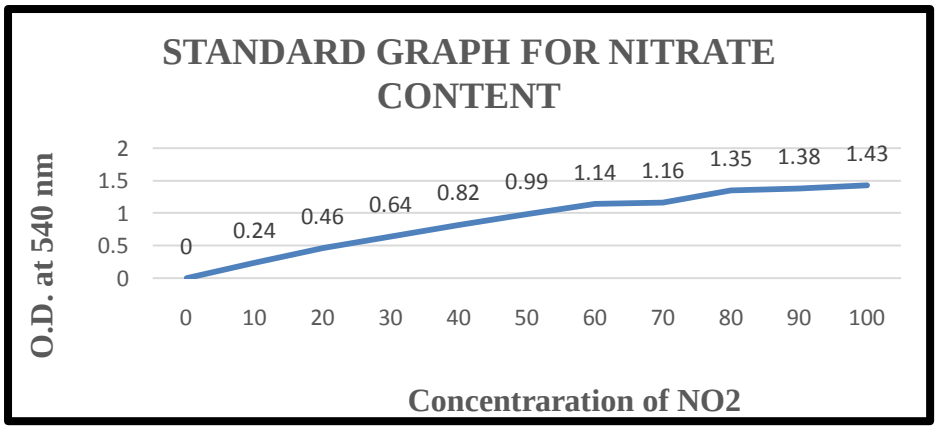

Figure 1: Standard graph for the Nitrate Content

Table 2: The nitrate content of the soil was calculated and it was as follows

Day	Kotim soil Nitrate content (µg NO3-N/lit)		Kolavali Soil Nitrate content (µg NO3-N/lit)		Bavada Soil Nitrate content (µg NO3-N/lit)	
	Inoculated soil	Uninoculated soil	Inoculated soil	Uninoculated soil	Inoculated soil	Uninoculated soil
1	260	253	198	196	169	160
2	457	242	406	180	384	158
3	856	221	752	175	679	155
4	1986	180	1647	156	1456	148
5	2797	160	2013	151	1987	146
6	2450	152	1856	132	1723	145
7	1932	139	1665	119	1470	132
8	1761	127	1400	98	1198	114
9	1340	126	1240	97	887	99
10	945	107	1188	97	800	88

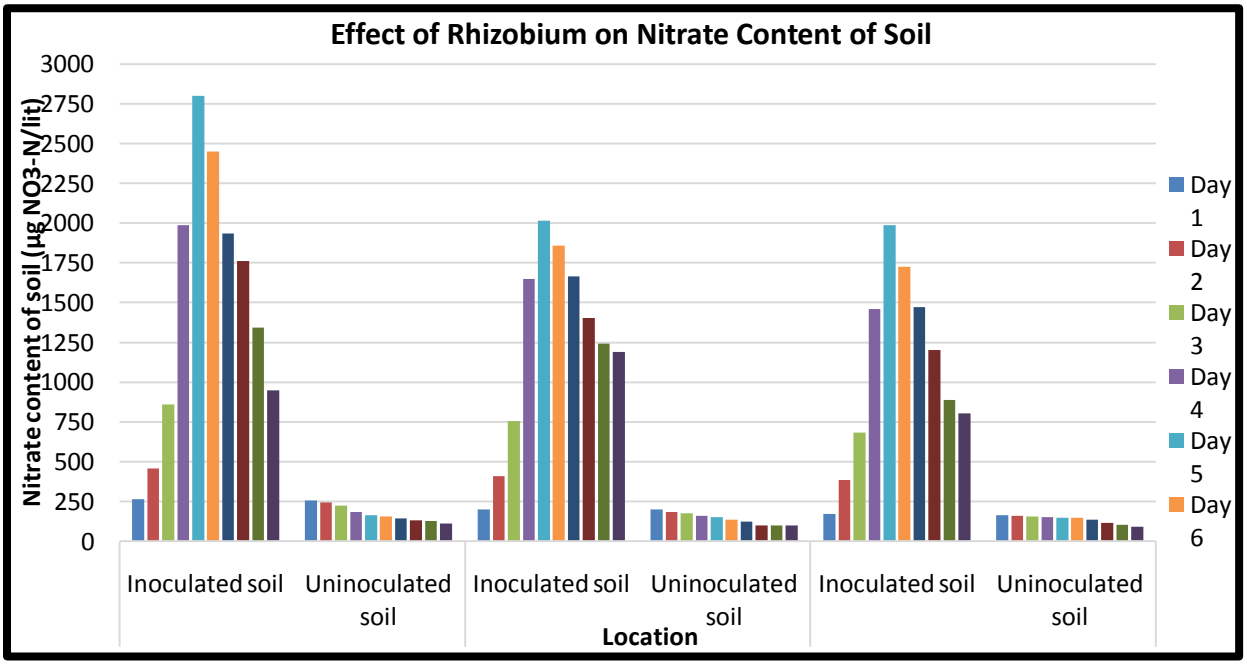

Figure 2: Effect of Rhizobium on the soil nitrate content

Table 3: Effect of Rhizobium on plant growth

Day	Kotim soil Height of shoot in cm		Kolavali Soil Height of shoot in cm		Bavada Soil Height of shoot in cm	
	Inoculated soil	Uninoculated soil	Inoculated soil	Uninoculated soil	Inoculated soil	Uninoculated soil
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0.5	0	0.4	0	0.3	0
5	2	0.2	0.8	0.1	0.5	0.1
6	3.5	0.8	3	0.3	1	0.3
7	5	1.9	5	0.8	1.8	0.7
8	8	4	6	2	2	1
9	10	6	8	4	3.6	2
10	10	7	9	6	5	4

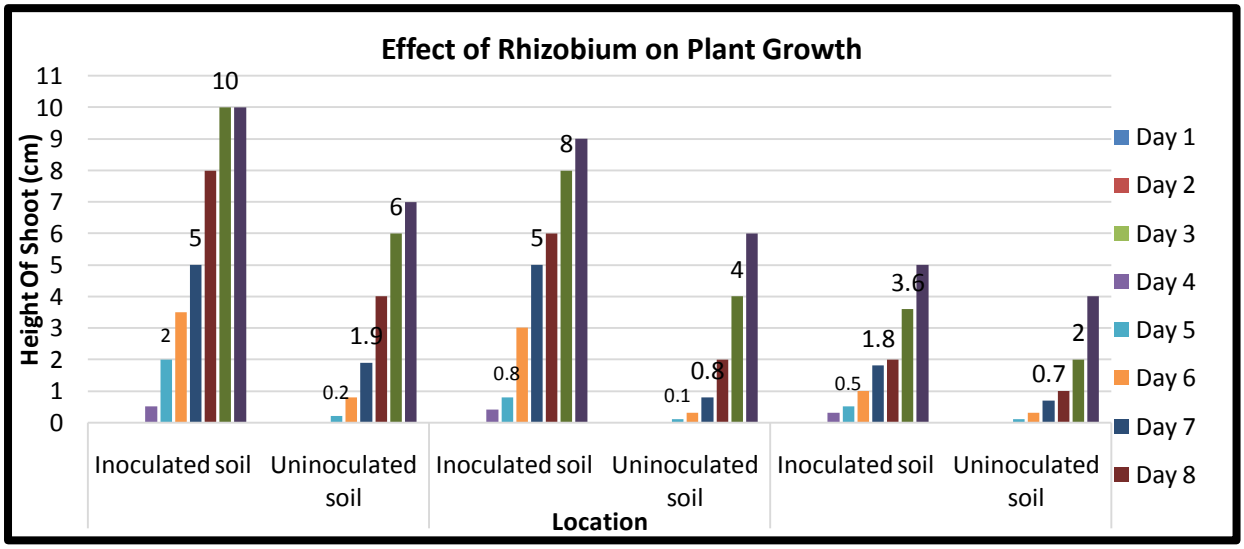

Figure 3: Effect of Rhizobium on the plant growth

Conclusion:

As after incubation there was the presence of white, translucent colonies on sterile CRYEMA plate but same isolates could not grow on Sterile Glucose peptone agar plate,thereforethere was the presence of Rhizobium in the root nodules of fenugreek plants which

were collected from three locations that were Kotim, Kolavali, Bavada nearby to the Vangaon village. The gram nature of isolated colonies was Gram-negative rods.

Rhizobium isolated from Kotim fenugreek sample wasable to use 1% glucose, 1% lactose, 1% maltose, 1% Mannitol as a source of energy. But this isolate did not have tryptophanase enzyme and also could not synthesize acetoin. This isolate was acid and it could utilize citrate also. This isolate was amylase, urease and catalase-positive organism. While isolate observed from KolavaliandBavada fenugreek plant sample was able to use 1% glucose, 1% lactose, 1% maltose, 1% Mannitol, citrate as a source of energy. The isolate was able to synthesize tryptophanase and acetoin. These isolates were acid producer, amylase, urease and catalase-positive organisms.

In all three soil samples that are Kotim, KolavaliandBavada, Nitrate content of inoculated soil was found to be much higher than uninoculated soil. The nitrate content of Kotim soil was found to be much higher than the Kolavali soil sample as compared to the Bavada soil sample. In all three soil samples, nitrate content was initially increased as number incubation days increased, on the fifth day of incubation, nitrate content was found to be maximum which on further incubation decreased due to utilization of soil nitrate for plant growth. From that, it was concluded that Rhizobium inoculation in the soil will increase the nitrate content of the soil.

From results obtained for the shoot, height showed that rhizobium inoculated soil show faster plant growth as compared to uninoculated soil. Shoot height of plant grown in inoculated soil was more as compared to shoot height of plant grown in uninoculated soil. This indicates that rhizobium will show a positive effect on plant growth.

Future Prospectus:

In the future, isolatedand identified strain will be subjected to DNA sequencing.The growth curve has also to be studied to determine whether it is a fast or slow grower. Invasiveness (nodule formation capacity) of strain, Competitiveness of organism, the stability of organisms in carrier moleculeshas to be determined. To use it as biofertilizer.

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SCANNING ELECTRON MICROSCOPY TO STUDY STRUCTURAL VARIATIONS BETWEEN TWO *PHOMOPSIS AZADIRACHTAE* ISOLATES

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Abstract:

Phomopsis azadirachtae Sateesh, Bhat and Devaki is a Deuteromycetous fungus causing die-back of neem (*Azadirachta indica* A. Juss.). This is presently a devastating disease of neem in India reducing the life span and seed production of neem. Two isolates of *P. azadirachtae* collected from Kalaburgi and Mysuru districts of Karnataka State, South India were studied by scanning electron microscopy. Even though some of the features overlapped between the two isolates, some remarkable variations in the shape were noticed in the conidia of the two isolates.

Key words: Die-back of neem, *Phomopsis azadirachtae*, isolates, scanning electron microscopy, variability

Introduction:

Plant pathogenic fungi affect leaves, fruits and deciduous trees usually over summer as mycelium or resting spores or sclerotia and they are generally present on infected plant surface or on plant debris in the soil (Sateesh, 1998). The fungal species survive in the form of mycelia in the infected plant parts, in the case of perennial plants. Knowledge of structural details of the infection propagules of phytopathogenic fungal species is very much essential for the proper management of the diseases. The examination of infection propagules such as mycelia and conidia using scanning electron microscopy (SEM) would help in the characterization of these propagules into different mycelial and conidial types (Hobbs *et al.*, 1985). Further, SEM can be used to study the infection process in different phytopathogenic fungi (Dehpour *et al.*, 2007;

Matsuura 1986). SEM has been successfully used to differentiate various species of *Phomopsis* and also various isolates of *Phomopsis* species (Hobbs *et al.*, 1985). The present study was undertaken to understand the surface details of mycelia and conidia of two *Phomopsis azadirachtae* isolates, causing die-back of neem (Girish and Shankara Bhat, 2008; Sateesh *et al.*, 1997) collected from different agroclimatic regions of Karnataka State, South India, which showed marked variations in their colony morphology. Nonetheless the description of the morphology of these conidia has been limited to light microscopic level. In the present investigations efforts were made to determine the ultrastructural characteristics of mycelia and conidia of *P. azadirachtae* isolates as seen with scanning electron microscopy. SEM can provide the three-dimensional structure of growing pathogens (Yamada *et al.*, 2012). SEM has been widely used to provide high-resolution images from the surface of biological samples (Tang *et al.*, 2014).

Materials and Methods:

Isolation of *Phomopsis azadirachtae*:

The fungal isolates were isolated from naturally infected neem twigs and were maintained on PDA medium amended with 200 ppm of chloramphenicol (Sateesh, 1998). The Petriplates were incubated at room temperature (26 ± 2^{o}C) under continuous fluorescent light to induce sporulation (Uecker and Johnson, 1991). The two isolates of *P. azadirachtae*, one from Kalaburgi (Pa 02) and the other one from Mysore (Pa 03), which exhibited significant variations in their cultural characteristics were considered for scanning electron microscopic study. The mycelia and conidia of *P. azadirachtae* isolates were processed for scanning electron microscopy (Tokunaga *et al.*, 1973; Brown and Brotzman, 1979). The samples were fixed, washed, dehydrated and subjected to critical-point drying.

Preparation of mycelia for scanning electron microscopy:

The fungal isolates grown separately on PDA were incubated for 5-7 days at room temperature (26 ± 2^{o}C). For a successful study of the moulds, only young mycelium should be used (Weidenborner *et al.*, 1989). Thus young mycelia from the advancing margins of the colony were scraped and suspended in phosphate buffered saline (PBS), centrifuged and resuspended in an aldehyde fixative solution (3.0% formaldehyde and 2.6% glutaraldehyde prepared in PBS at pH 7.2). Fixation was done for 4 h at room temperature (26 ± 2^{o}C) while the hyphae were constantly agitated with a magnetic stirrer. This was followed by centrifugation and re-suspension in fresh fixative solution in which the material was stored at 4^{o}C for 1 or 2 days.

After further washing with 0.1 M sodium cacodylate buffer at pH 7.2, the samples were post-fixed for 1 h with 1% osmium tetroxide (Elad *et al.,* 1987; Quattlebaum and Carner, 1980) prepared in the same buffer at 4^{o}C. After another buffer washing, the samples were gently drawn down on 0.22 μm Millipore filters, where they were dehydrated using graded series of ethanol. The samples were then critical-point dried using liquid carbon dioxide at 31.5^{o}C and a pressure of 1100 psi. The samples were mounted on aluminium stubs using double adhesive carbon tape.

Preparation of conidia for scanning electron microscopy:

Newly formed conidial ooze was collected from the pycnidia after 2-3 weeks of incubation. The ooze containing both alpha and beta conidia was subjected to scanning electron microscopy. It was fixed in 2.5% glutaraldehyde solution for 1 or 2 days (Elad, 1988; Lifshitz *et al.,* 1984). Later the conidial suspension was mounted on rectangular microscope cover glasses. The cover glasses with conidial suspension were air-dried. The suspension was washed by passing through 0.1 M phosphate buffer at 4^{0}C. Three changes of 15 min each were given. The samples were dehydrated by passing through graded series of acetone such as 30%, 50%, 70%, 80%, 90% and 95%. In each grade the samples were left for 15 min. Then the samples were passed through 100% acetone or dry acetone. Three changes of 15 min each were given. The dehydration was carried out at 4^{o}C. The samples were critical-point dried using liquid carbon dioxide at 31.5^{0}C and a pressure of 1100 psi. The conidia of the two isolates were processed in the same way separately.

Scanning electron microscopy:

The mounted samples of both mycelia and conidia were coated with gold of around 30^{o}A thickness. The specimens were viewed using a scanning electron microscope LEO 435 VP from LEO electron microscopes Ltd., at 20 KVHT and various prints were recorded under different magnifications with a 35 mm camera system using ILFORD ASA 100 black and white film.

Results:

Isolation of *Phomopsis azadirachtae:*

The two isolates of *P. azadirachtae* developed colonies from infected neem twig explants. Isolate Pa 02 produced a dense mycelium when colony was young which later turned bright orange on PDA medium. The colony was characterized by pycnidia in concentric rings that often extended over the entire bottom of the Petri plate. Alpha and beta conidia were produced in the same conidioma. Beta conidia were absent in fresh cultures, but abundant in old

cultures. Isolate Pa 03 produced a floccose, dense mycelium that was initially white but became greyish as the culture aged. Alpha and beta conidia were formed within the same conidioma and were born on simple conidiophores. Beta conidia were produced during later stages of sporulation (Figure 1).

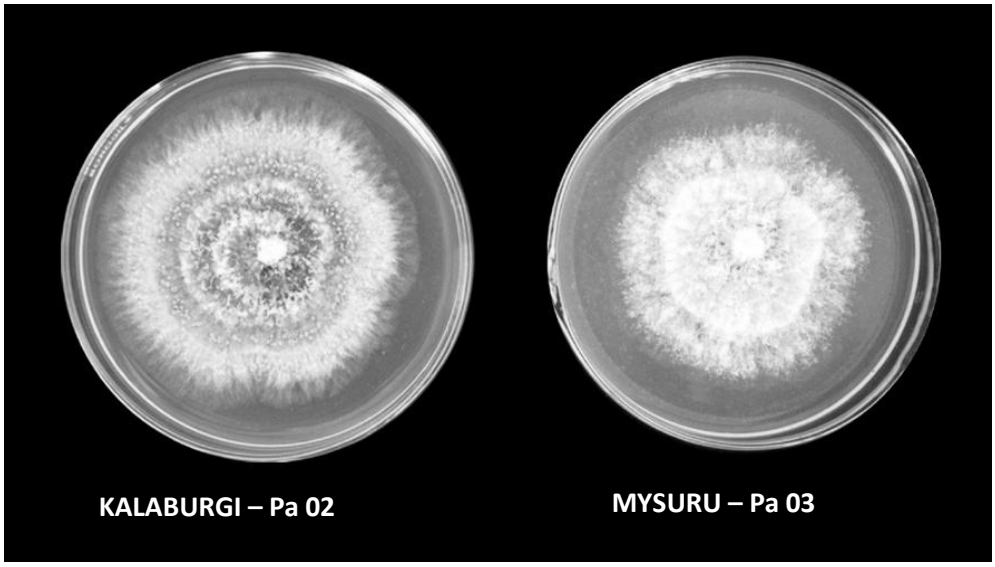
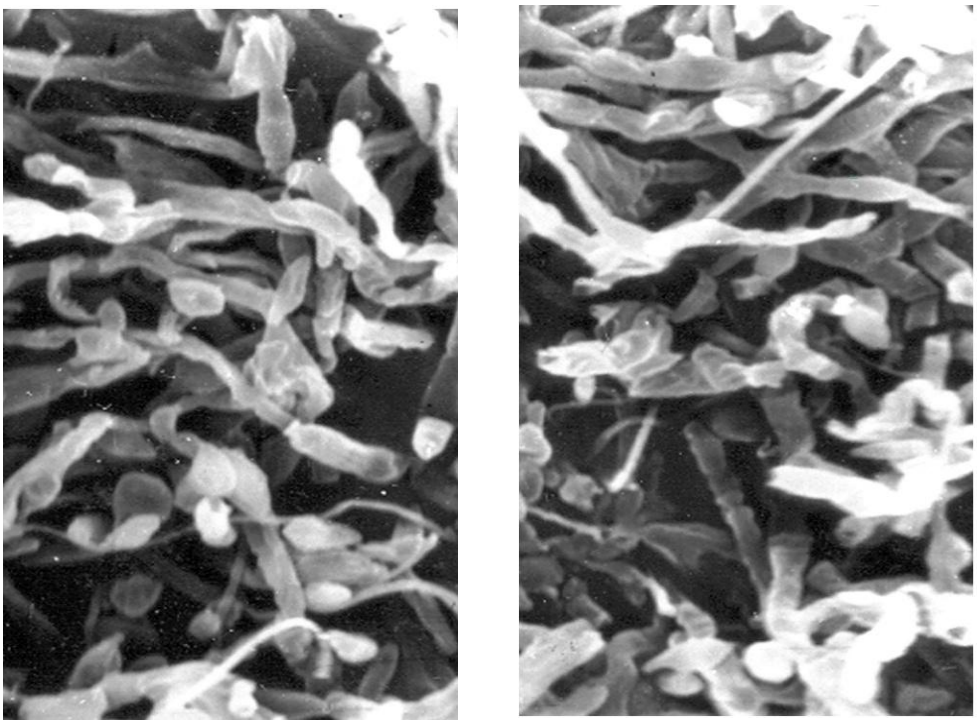


Figure 1: Morphological characteristics of colonies of *Phomopsis azadirachtae* from different regions of Karnataka on PDA (10-d-old)

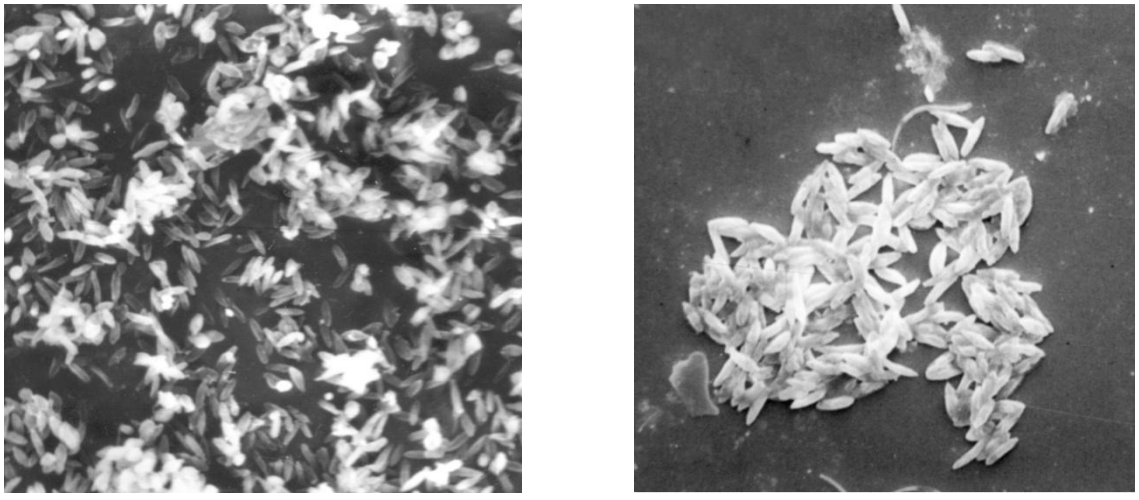
Scanning electron microscopy:

Profusely branched and septate hyphae constituted the mycelium in both the isolates of *P. azadirachtae*. The study revealed the presence of hyphae intermingled with conidiophores. The conidiophores were simple bearing alpha and beta conidia. Mycelium of the two isolates was almost similar (Figures 2). However, there was a slight difference in the conidial structure of the two isolates. The study revealed that the alpha conidia of isolate Pa 02 were fusiform and bulged at the centre. Further there were prominent deep depressions at the region of the guttules (Figure 3) whereas alpha conidia of isolate Pa 03 were elongated and fusiform without any bulging at the centre. Prominent deep depressions were not seen (Figure 4). The newly-formed alpha conidia in both the isolates were having a short stalk at the point of attachment of conidiophore. The beta conidia of both the isolates appeared similar having a smooth surface. However, beta conidia of isolates, Pa 02 showed the presence of slight depressions at one or two places on the surface and the beta conidia of Pa 03 were slightly slender and elongated (Figures 5 and 6).



A B

Figure 2: (a) Mycelium of Pa 02 (Kalaburgi isolate of *Phomopsis azadirachtae*); (b) Mycelium of Pa 03 (Mysuru isolate of *Phomopsis azadirachtae*)



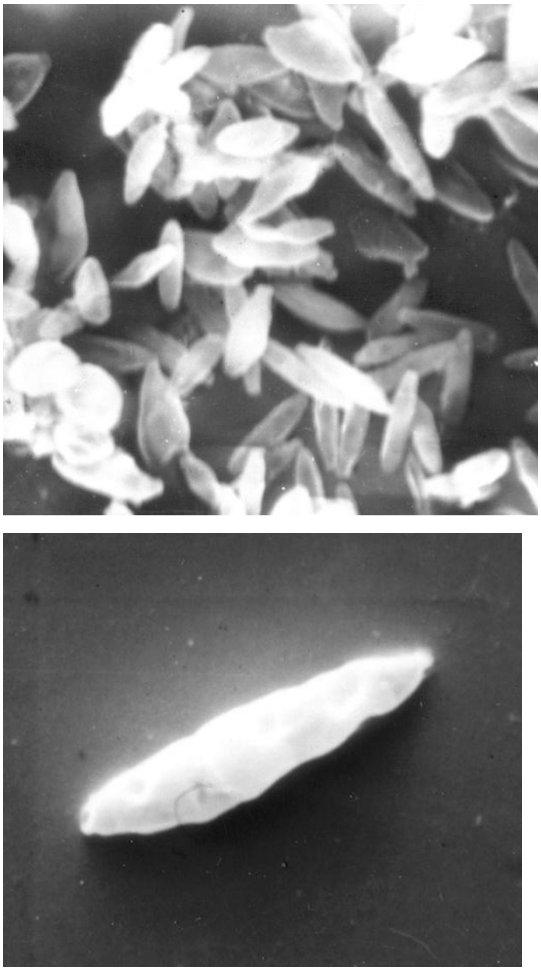


Figure 3. Alpha conidia of Pa 02 (Kalaburgi isolate of *Phomopsis azadirachtae*)

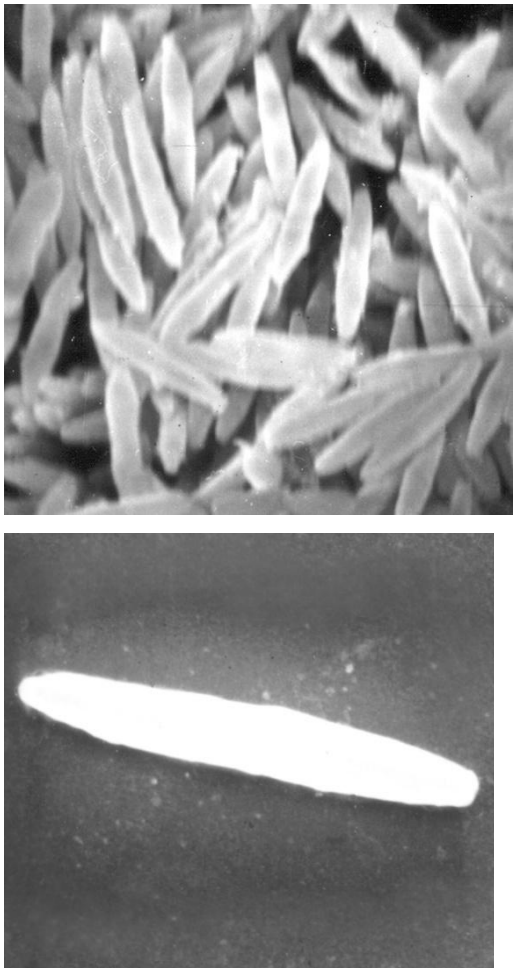


Figure 4. Alpha conidia of Pa 03 (Mysuru isolate of *Phomopsis azadirachtae*)

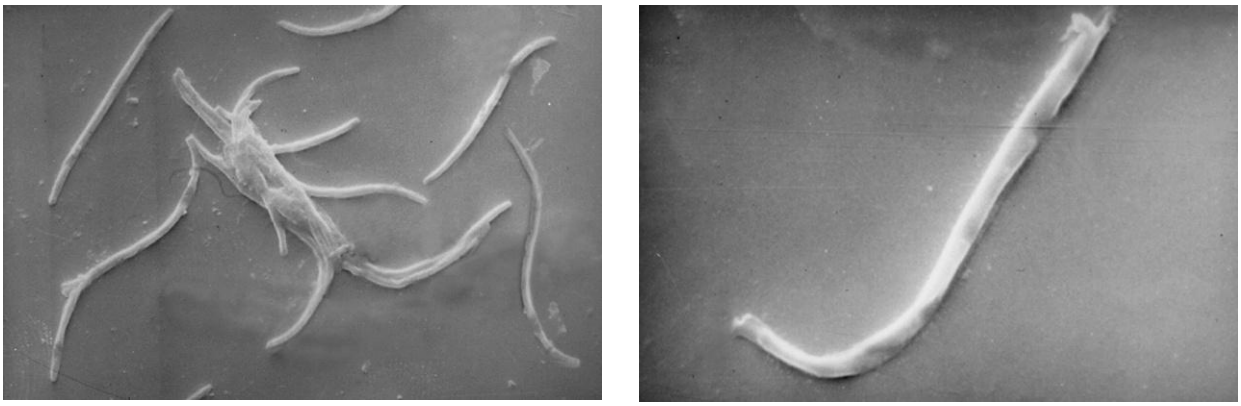


Figure 5. Beta conidia of Pa 02 (Kalaburgi isolate of *Phomopsis azadirachtae*)

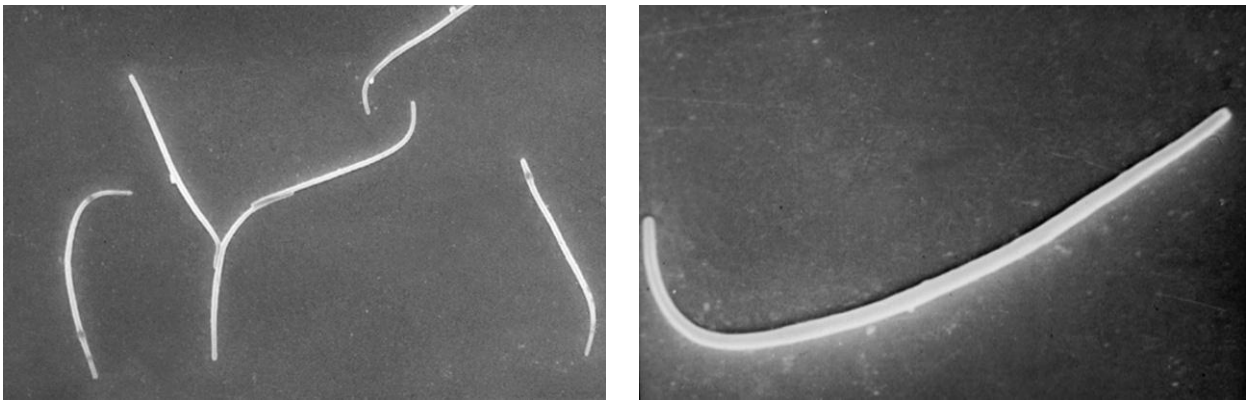


Figure 6. Beta conidia of Pa 03 (Mysusru isolate of *Phomopsis azadirachtae*)

Discussion:

Electron microscopy has contributed a lot to the fungal classification (Cole, 1979). Electron microscopy is still an important technique to diagnose pathogens and to identify microorganisms (Goldsmith and Miller, 2009). Ultrastructural analysis of cells by SEM can reveal valuable information about their morphological characteristics (Tang *et al.,* 2014). SEM has been used as a reliable tool to differentiate *Phomopsis* species and to group the isolates within a species (Hobbs *et al.,* 1985). Various species of *Phomopsis* such as *P. sojae, P. phaseoli, P. batatae and P. glycines* were identified based on colony character and morphological features supported by SEM (Hobbs *et al.,* 1985; Kulik, 1984). Hobbs et al. (1985) identified the species of *P. longicolla* considering the structural details of conidiomata, conidiophores and conidia as observed under SEM. Isolates of *P. longicolla* were further differentiated into groups considering the structural details of conidia produced by them.

In the present investigations a comparative study was made to understand the variations among the isolates of *P. azadirachtae* collected from two different agroclimatic regions of Karnataka State, South India by SEM. Even though some of the features overlapped between the two isolates, some remarkable variations in the shape were noticed in the conidia of the two isolates. The difference could be due to the difference in the geographical regions from where the two strains are isolated. *P. azadirachtae* isolates from Karnataka (Fathima *et al.,* 2004 and 2012) and Tamilnadu (Girish and Shankara Bhat, 2010 and 2011) were reported to exhibit variability in different characteristics. SEM technique has been widely used to investigate the morphological characteristics as well as variations among fungal pathogens. Mims et al. (1997) studied the ultrastructure of conidia of *Alternaria cassiae* infecting sunflower (*Helianthus annuus*). Morphology of infection structures of *Hymenoscyphus pseudoalbidus* on common ash (*Fraxinus*

excelsior) leaves and leaf petioles were studied using SEM (Cleary *et al.*, 2013). Morphological studies on dermatophytes and their infection process were carried out by SEM (Yamada *et al.*, 2012). García et al. (2012) used SEM to evaluate the diversity of endophytic fungi present in the leaves of *Sapindus saponaria* L. and their colonization of host plants. SEM studies revealed the intraspecific variation in the number of basidiospores produced per basidium in *Agaricus brasiliensis* (Herreira *et al.*, 2012). Variations among three isolates of entomopathogenic fungi *Beauveria bassiana* for infection on nymph of *Lipaphis erysimi* Kalt., were investigated using SEM (Sharma *et al.*, 2017).

This is the first report on the scanning electron microscopic study of mycelia and conidia of *P. azadirachtae*. Further study is warranted pertaining to the transmission electron microscopy of these and the other isolates of *P. azadirachtae*.

Acknowledgements:

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IMPORTANCE OF MEDICINAL PLANTS IN HUMAN LIFE

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Abstract:

The world is suffering due to Coronavirus pandemic with no vaccines or treatment available till now, so along with social distancing we need to focus on building our immune system because prevention is better than cure. Medicinal plants have been used for ages in traditional medicine to treat hundreds of ailments. India is repository of medicinal plants and about 8,000 herbal remedies have been codified in AYUSH system in India. According to WHO, 80% of people worldwide rely on medicinal plants for primary health care as they are in sync with nature and are considered safe. Medicinal plants like Moringa , Basil, Garlic, Ginger, Turmeric, Black pepper etc are commonly used in various Indian recipes as they impart flavour, taste, colour as well as health benefits such as relief from cold, cough, flu, aids better digestion, helps to develop immune system, relieves stress and the list is endless. The effectiveness of these herbal remedies, their easy availability, low cost and comparatively being devoid of side effects has made them popular worldwide. Medicinal plants are rich resources of ingredients and can be used in development of drug. Many medicinal plants are considered important sources of nutrition as a result recommended for therapeutic values others contain bioactive compounds and derivatives having various health benefits. In our fast life, we need to boost our health through natural ingredients rather than synthetic ones. It's time to promote beneficial effects of medicinal plants globally.

Keywords: immune system, medicinal plants, nature, health benefits, AYUSH.

Introduction:

Nature has blessed us with an array of amazing varieties of medicinal plants which not only helps us to improve our overall health but also become better version of ourselves.

According to World Health Organization (WHO, 2008) Medicinal Plants can be defined -as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (Shodhganga). As per the National Medicinal Plant Board (NMPB, 2018) "If the wisdom is herbal, many ailments are curable", hence keeping in mind the present world scenario resulting from the deadly Coronavirus pandemic with no specific vaccine or treatment available till now (World Health Organisation- WHO) all that we can do is boost our own immune system becauseprevention is always better than cure.India has been a rich repository of medicinal plants since time immemorial which has been extensively used in traditional medicine for their natural healing, therapeutic effectsand remarkable immunity boosting properties (Khodadadi, 2015). Famous ancient Indian herbalists like Charaka and Sushruta have reported use and benefits of a vast range of medicinal plants /herbs in the fundamental texts of medical tradition in India i.e the Charaka Samhita and the Sushruta Samhita (Waghmare and Nakanekar, 2016; Singh, 2017).

Herbal remedies have been so popular from ancient times that AYUSH system in India has codified about 8,000 herbal remedies (National Health Portal, 2016). From the past two decades medicinal plants have gained immense global popularity and their use has been growing rapidly since they are considered safe with no or minimal side effects and contain many ingredients that not onlyboosts our immunity and good health but also provides relief from many ailments. According to World Health Organization (WHO) 80% of people all over the world depend on herbal medicines for their primary health care needs. Looking at the immense popularity of medicinal plants in solving primary health care problems and growing global demands, the Government of India established - " The National Medicinal Plants Board" (NMPB) to coordinate all matters related to medicinal plants under the Ministry of Ayurveda, Yoga and Naturopathy, Unani , Siddha and Homeopathy in short known as AYUSH (Kala Sajwan, 2007).

According to Cordell (2004), plant extracts and products contribute to four major areas of human health and wellness:

1) Foodstuffs 2) Flavouring agents and spices 3) Perfumes and cosmetics 4) Pharmaceutical and biological agents.

Health benefits of some commonly used medicinal herbs/plants:

Medicinal plants contain many beneficial substances apart from antioxidant properties which in turn help to stimulate our immune system. Here we will discuss health benefits of a few commonly used herbal / medicinal plants like basil , moringa, garlic, ginger, turmeric and black

pepper which are regular ingredients in our kitchens for preparation of various recipes. The best part of these herbs/ plants are that we can very easily grow them in our houses to reap benefits of their magical properties forever and thus keep many ailments out of our lives with ease. NMPB emphasizes on maintaining herbal gardens in homes to promote the use of medicinal plants for primary health care at household levels.

1. **BASIL (*Ocimum basilicum*) and Tulsi (*Ocimum sanctum* Linn):**

According to the Central Institute of Medicinal and Aromatic Plants (CIMAP, 2007)- both Basil and Tulsi are the most widely distributed varieties which cover the entire Indian subcontinent. Ocimum sanctum / Tulsi is commonly known as the Holy Basil since worshipped by Hindus and also popularly known as 'The Queen of Herbs" as it is found to address physical, chemical, metabolic and psychological stress through its pharmacological actions(Cohen, 2014).

As per Ayurveda the whole plant has medicinal value including the leaves and flowers which is used in treatment of heart and blood disease, bronchitis. The leaf juice is specially used to provide relief from infantile cough, cold, diarrhoea, dysentery and skin diseases. It also exhibits diaphoretic, antiperiodic, stimulant and expectorant properties. An infusion of leaves is used to give relief from gastric disorders in children (CIMAP). Apart from that it has antimicrobial and antifungal properties too (Joshi, 2014). Daily consumption of tulsi promotes wellbeing and longevity and aids in dealing with stress due to its antioxidant properties (Cohen, 2014). Chewing of fresh (washed) basil leaves every morning or adding them in one's tea has been advocated by AYUSH for boosting of immune system.

Basil is popular culinary herbs, whose flower buds has a more subtle flavor and are edible whereas the seeds on soaking become gelatinous and are added to Asian drinks and desserts. It has therapeutic effects and is a source of essential oils mainly –eugenol, methyl chavicol and linalool which help in elimination of free radicals from body and boost immunity. Purple basil contains very high antioxidant activity due to anthocyanins. Apart from that they also contain key nutrients like vitamin A, C, calcium, phosphorus, beta-carotene etc. Water soluble flavonoids of basil, including vicenin and orientin exhibit anticancer activity (Lupton *et al.*, 2012).Other benefits includes-lowering of blood pressure, cholesterol and blood glucose level. Basil also boosts mental health (Brazier, 2019).

2. The Drumstick Tree (*Moringa oleifera*):

This medicinal plant is native to India and has been used in treatment of more than 300 conditions thereby is also referred to as Panacea. According to Dieye *et al.* (2008) moringa is famous by various names such as the Miracle tree, Tree of Life, God's Gift to man, Saviour of poor since every part of this plant is a storehouse of important nutrients like minerals for example- calcium, potassium, zinc, magnesium, iron and copper as well as vital vitamins like beta-carotene, C,D,E and vitamin B like folic acid etc and essential phytochemicals (Kasolo, 2010; Mbikay, 2012).

The leaf extracts contain potential antioxidants like Quercetin which helps to lower blood pressure, Chlorogenic acid – moderates blood glucose levels. It also exhibits- anti-cancer, anti – inflammatory, anti-diabetic, anti- microbial properties (Raimunda *et al.*, 2017). Moringa has shown to increase white blood cell counts and immunolevels as reported by Adedapo *et al.* (2005) and Rausch *et al.* (2006).

The nutritional as well as functional properties of moringa has made it a very widely used medicinal plant.Apart from the drumsticks the leaves and seeds of moringa are excellent source of protein containing several essential amino-acids like methionine and cysteine etc(Osman *et al.*, 2012). Leaves can be eaten raw but are best added to meals as a special ingredient. Rockwood, Anderson and Casamatta (2013) studied the health benefits of moringa and reported that it provides 7 times more vitamin- C than oranges, 10 times more Vitamin- A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach.

3. Garlic (*Allium sativum*)

Garlic has acquired the reputation of nature's most multipurpose medicinal plant and has been used as medicine since ancient timesin different traditions due to its endless therapeutic health benefits.

Garlic contains magnesium, selenium, vitamin A, vitamin C, volatile oil with sulphur compounds, zinc, water (65%), carbohydrate (28%), organosulphur compounds (2.3%) etc. The sulfur content of garlic has a strong therapeutic effect (Labu *et al.*, 2019). Allicin is the principal bioactive compound present in this bulbous plant which gives garlic its strong characteristic flavor and is responsible for the powerful pharmacological properties of the plant (Labu *et al.*, 2019) which exhibits beneficial effects on cardio-vascular system as it lowers blood pressure, reduces serum cholesterol, triglycerides and inhibits platelet aggregation. It also exhibits antioxidant properties, anti- bacterial activity, anti

diabetic, anti-cancer properties and a host of other benefits which helps in overall wellbeing (Chan *et al*, 2013). The active compounds of garlic have strong stimulatory effect on natural killer cell activity thereby boosts immunity (Bayan *et al.*, 2014). Garlic also helps in relief from many respiratory tract conditions such as asthma, breathing difficulties, bronchitis, colds, cough, sinusitis etc (Labu *et al.*, 2019). Consumption of raw garlic is more beneficial than cooking it, so it should be added when cooking is almost done or else the active compound is destroyed.

4. Ginger (*Zingiber officinale*):

Ginger is one of the most commonly consumed dietary condiments in the world. Ginger has been purported to exert a variety of powerful therapeutic and preventive effects and has been used for thousands of years for treating hundreds of ailments from cold, cough asthma, nausea, gastro-intestinal complications to cancer etc. Gingerol- is the bioactive compound present in ginger -the primary ingredient that exhibits remarkable pharmacological and physiological activities (Bode and Dong, 2011).

Ginger roots contain a very high level of antioxidants (Halvorsen. *et al.*, 2002) which helps to reduces oxidative stress and has anti- inflammatory effects.(Topic. *et al.*, 2002). Other potential benefits of ginger are it helps to reduces cholesterol, increases lipid metabolism thereby helping to reduce risk of cardio-vascular diseases and diabetes.It also helps to reduce age related oxidative stress markers, decrease pain, swelling and inflammation(Young *et al.*, 2005) as well as osteoarthritis and rheumatism. It can be consumed as fresh, dried, pickled, powdered, candied form and is advocated by AYUSH to boost immunity.

5. Turmeric (*Curcuma longa*):

Turmeric since time immemorial has been proclaimed as one of the prominent medicinal spice of India and often referred to as the Golden Spice.Curcumin is the most important bio active compound present in turmeric which is considered as a potent chain breaker antioxidant like vitamin E (Priyadarsini, 2003).

The essential oils extracted from turmeric exhibits anti fungal properties and is active against respiratory viruses such as those causing flu. The oil helps to remove sputum, provides relief from cough, prevents asthma and aids in better digestibility.Other benefits of turmeric includes lowering of bad cholesterol and elevation of good cholesterol, better wound healing effect, anti-inflammatory effect, anti- cancer properties , anti-anxiety effect etc. Curcumin

improves insulin sensitivity, reduces high blood pressure, oxidative stress and such disease (Hewlingsand Kalman, 2017). Benefits of curcumin are best achieved when it is combined with agents as piperine which increases its bioavailability significantly.

Golden Milk Recipe:

To 150 ml warm milk add half teaspoon of turmeric (haldi) mix well and drink once or twice a day -advocated by AYUSH to boost immunity.

6. Black pepper (*Piper nigrum*):

Spices like black pepper have been an integral part of human diets and used extensively in traditional medicine for ages. The bioactive components present in it are of considerable significance as they protect us from various ailments and exert therapeutic effects. The seeds were considered to be as black gold for its usage as a commodity in ancient times.

The key alkaloid component of black pepper is Piperine which helps in better nutrient absorption of curcumin, selenium, vitamin B_{12}, beta-carotene as well as other compounds (Dudhatra *et al.,* 2012), assists in cognitive brain function .Antioxidant properties help to scavenge free radicals, prevents progression of tumor growth, anti microbial potential, gastro protective activity, possesses anti inflammatory, anti depressant activities too (Butt *et al.*, 2013). Normally black pepper is powdered and sprinkled over food, salads or curries. It can be also added to herbal tea with other ingredients like dry ginger, basil and cinnamon as suggested by AYUSH to boost immunity.

Conclusion:

At timeswhen we are facing global crisis, it is important that we take charge of our health seriously. Immunity cannot be built in a day or two but with inclusion of a well balanced diet, regular exercise and adequate sleep we can become strong enough to face our daily challenges. Due to our faulty diet and physical inactivity we are becoming prone to a host of lifestyle diseases like obesity, diabetes mellitus, cardio-vascular diseases, high blood pressure etc therefore a little bit of awareness regarding benefits of medicinal plants would prove beneficial as it can answer many of our primary health care concerns easily. Indiscriminate use of antibiotics is fatal to human health as it causes antibiotic resistance and the situation is alarming in India. Thus use of medicinal plants need to be promoted among general population to stop the menace of antibiotic resistance and promote overall health and wellness.

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EVALUATION OF TOTAL PHENOLIC CONTENT AND *IN VITRO* ANTIOXIDANT ACTIVITY IN CALLUS, SEED AND LEAF EXTRACT OF *TRIGONELLA FOENUM-GRAECUM* L.

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Abstract:

Trigonella foenum-graecum L. due to presence of consortium of phytochemicals is used as spice, food flavoring agent and in medicines worldwide. In present study total phenolic content and *in vitro* antioxidant activity of cotyledon derived callus extract of *Trigonella foenum-graecum L.* was assayed out and subsequently compared with respective seed and leaf extract. Callus was induced from cotyledon explants in MS medium supplemented with different concentration combinations of auxins (2, 4-D/NAA) and cytokinins (kinetin/BAP). The total phenolic content determined by Folin- Ciocalteu reagent was 2.145, 1.534 and 1.372 gallic acid equivalents mg/g in methanolic callus, seed and leaf extract respectively. *In vitro* antioxidant activity was evaluated by DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging method. Higher antioxidant activity was reported in methanolic callus extract of fenugreek (87±1.12%) followed by methanolic seed extract (85±1.55%). Although biological activity of callus in term of total phenolic content and antioxidant activity not depicting a significant difference from that of seed extract but certain drawbacks like limited supply, seasonal and regional phytochemical variations associated with plant could be overcome by *in vitro* grown callus which could be a potential and continuous source of raw material for extraction and formulation of therapeutics to be used as antioxidants in clinical applications and food preservation.

Key words: Phenolic content, antioxidant activity, callus, 2,4-D, NAA, BAP, gallic acid.

Introduction:

Trigonella foenum graceum L. commonly known as fenugreek belonging to family Fabaceae, is a small annual herb used as spice and green leafy vegetable around the globe. Fenugreek due to occurrence of vast array of phytochemicals in different parts has been widely explored for its nutritional and therapeutical properties (Basch, 2003). Traditionally both seed and leaf extract of fenugreek have been widely used in treatment of various ailments such as diabetes, cough, congestion, flatulence, high blood pressure, anemia, inflammation and arthritis (Acharya and Basu, 2006). The seeds extract appeared to contain various metabolites including polysaccharide, galactomannan different saponins such as diosgenin, yamogenin, mucilage, lipids, flavonoids, apigenin, luteolin, quercetin and alkaloids such as choline and trigonelline (Billaud, 2001). Phytochemical screening conducted by Cowan (1999) exhibited that fenugreek leaves contain saponins, ascorbic acid and β-carotene. These compounds owing to various pharmacological properties such as antibacterial, anti-inflammatory, antioxidant and anticarcinogenic become key compounds of research (Middleton, 2000). However, phenolic and flavonoids are the main compounds reported in different plant parts of fenugreek responsible for its antioxidants capability (Kaur and Kapoor, 2002).

There is always search for an unconventional possible source of high value bioactive metabolites which could be easily met by *in vitro* grown plant cell cultures (Alfermann, 1995). Callus suspension cultures grown under controlled environment conditions can produce considerable amount of bioactive metabolites. Various factors can be manipulated to enhance the production of secondary metabolites *in vitro* grown callus cultures (Kries, 2019). Duangporn and Siripong (2009) and Kalidas (2010) evaluated the effect of growth hormones auxins and cytokinins on phyllanthusol and vincristine production by callus cultures of *Phyllanthus acidus* and *Catharanthus roseus* respectively. Hence, this is a fascinating field of research to explore the phenolic content and antioxidant activity in *in vitro* grown plant cell cultures as there are sufficient findings suggesting the direct correlation between antioxidant activity and phenolic content (Cook and Samman, 1996).

Material and Methods:

Plant material:

Healthy Seeds and fresh leaves of *Trigonella foenum-graecum* L. were purchased from local market of Matunga, Mumbai.

Seeds surface sterilization and germination:

Seeds were washed thoroughly in distilled water containing 2 or 3 drops of 1%Teepol solution for 10-15 minute. Seeds were transferred to lamina air flow and surface sterilized by 70% alcohol for 30 seconds followed by rinsing in autoclaved distilled water for 2 to 3 times and soaked in 2% sodium hypochlorite solution for 10 minutes. The sterilant was decanted and seeds were washed 3-4 times with autoclaved distilled water to remove all traces of sterilant. After surface sterilization seeds were inoculated onto MS basal medium devised by Murashige and Skoog (1962) and incubated at 25±2ºC under 16hrs light and 8 hrs dark period for 7 days.

Callus Induction:

Cotyledons were excised from germinating seeds and used as explants for callus induction. Sterilized cotyledon explants pieces (2-3 mm) were inoculated onto surface of MS medium containing different combinations of growth regulators [1.0 to 2.0 mg/l of different auxin (2, 4-D or NAA) + 0.5 to 2.0 mg/l of cytokinins (Kinetin or BAP)]. The culture tubes were incubated for 4 weeks in 16 hrs light and 8 hrs dark at 25±2°C, and callus was sub cultured at four week intervals for continuous supply of callus.

Preparation of callus, seed and leaf extract:

2gm each of dry callus, seeds and leaves was grinded in mortar and then transferred to an Erlenmeyer flask containing 20 ml of methanol (96 %) for overnight. After filtration the methanolic solution was evaporated in a rotary vacuum evaporator at 40°C. The weighed dry matters were reconstituted in 3 ml of methanol.

Chemicals and Reagents:

Folin-ciocalteu reagent, Sodium carbonate, DPPH solution, gallic acid and methanol of analytical grade were purchased from Sigma Aldrich India.

Determination of Total phenolic content (TPC):

Total phenolic content was estimated by Folin-Ciocalteau'reagent method devised by Singleton *et al.* (1999). Folin-Ciocalteau'reagent is a mixture of phosphomolbdate and phosphotangstane which get reduced by phenolic compounds to give a blue colored compound.

The reaction mixture consisting of 1 ml of extract (1mg/ml), 5 ml of 10% Folin-Ciocalteu's reagent and 5 ml of 7.5 % Sodium carbonate (Na2Co3) solution. Samples were incubated at 40ºC for 30 minutes. Gallic acid was taken as standard to prepare the standard curve. The absorbance for test and standard solution was determined against the reagent blank (without extract) at 760 nm with an ultraviolet (UV)/visible spectrophotometer. For each extract

samples were taken in triplicates and results were expressed by taking mean of all three replications.

The TPC was determined from extrapolation of calibration curve of gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Determination of antioxidant activity:

DPPH (1, 1-Diophenly-2-Picrylhydrazyl) is a stable free radical with red color (absorbed are 570 nm). If free radicals have been scavenged, DPPH generates its color to yellow. This assay uses this character to show free radical scavenging activity.

The DPPH scavenging activity was evaluated according to method devised by Sanchez-Moreno *et al.*, (1998). DPPH stock solution (0.1mM) was prepared by dissolving 40 mg DPPH in 100 ml of methanol and stored at -20°C for further use. The working solution was obtained by diluting DPPH solution with methanol to reach an absorbance of about 0.98±0.02 at 517 nm using spectrophotometer. Various concentrations (10 - 500 µg/ml) of plant extracts were prepared from stock solution (1mg/ml in 96% methanol). Each reaction mixture containing 0.5 ml of plant extract and 2.5 ml of DPPH solution was incubated in the dark for 30 min at room temperature. Ascorbic acid was used as positive control. The decrease in absorbance was measured at 517 nm against a blank without plant extract using spectrophotometer. The DPPH free radical activity was calculated using the following formula:

$$\% \text{ of DPPH inhibition activity} = \frac{Ac - As}{Ac} \times 100$$

Result and Discussion:

Callus induction:

The result revealed a high variation in callus induction ability demonstrated by different hormones under present study. MS medium supplemented with 1.0 mg/l 2, 4-D and 2.0 mg/l BAP exhibited highest callus induction ability (Fig. 1 and 2). It is interesting to note that media solely containing auxins failed to induce callus from cotyledon explants.

Figure 1: 7 day old callus

Figure 2: 14 day old callus

Total phenolic content:

The total phenolic content determined by Folin- Ciocalteu reagent was 2.145, 1.534 and 1.372 gallic acid equivalents mg/g in methanolic callus, seed and leaf extract respectively (Table 1). There are considerable variations in TPC in different studies conducted on Fenugreek. Muhson amd Mashkor (2014) obtained 25.90 mg GAE/gExt in acetone extract of seeds of fenugreek. Rahmani *et al.* (2018) found considerable divergence in the total phenolic content among four varieties of fenugreek collected from Algeria. Amount of secondary metabolites depends on number of factors such as plant variety, genetic makeup, growth conditions, age of material collected for research and solvent used for extraction. In this study considerable difference in the phenolic content of cotyledon derived callus and leaf extract of *Trigonella foenum graceum* L. indicates that production of secondary metabolites is affected by growth hormones and controlled environmental conditions used for *in vitro grown* cultures.

Table 1: Total phenolic content and DPPH radical inhibition activity of callus, seed and leaf extract of *Trigonella foenum-graecum* L.

Extract	Total Phenolic Content (mg GAE/gExt)	% of DPPH inhibition at different concentrations (µg/ml)				
		10	50	100	200	500
Callus extract	2.145±1.33	46±0.04	65±0.17	75±1.34	80±1.36	87±1.12
Seed extract	1.534±1.19	45±0.02	65±0.14	73±1.12	79±1.23	85±1.55
Leaf extract	1.372±1.10	30±1.12	45±0.04	51±1.13	57±1.45	61±1.69

(Values represent Mean±SD, n=3)

Antioxidant activity by DPPH inhibition method:

Antioxidant activity of methanolic extract of callus, seed and leaf was evaluated by DPPH method showed highest antioxidant activity in cotyledon derived callus extract when compared with seed and leaf extracts (Figure 3). Although there was insignificant difference between % DPPH inhibition activity shown by callus extract ranging values from 46±0.04 to 87±1.12 and seed extracts (45±0.02 to 85±1.55) but contrast was noteworthy when compared with that of leaf extract which showed lowest DPPH % inhibition values from 30±1.12 to 61±1.69. Further the % inhibition activity was dosing dependent, higher the concentration, more

the extract potent to donate hydrogen to DPPH free radical (figure 4). To great interest there was a positive correlation between total phenolic content and antioxidant activity of callus, seed and leaf extract. Similar correlation was derived in studies done by Djeridane *et al.*, (2006) and Jerez *et al.*, (2007) while working with some Algerian medicinal plants and *Pinus* species respectively.

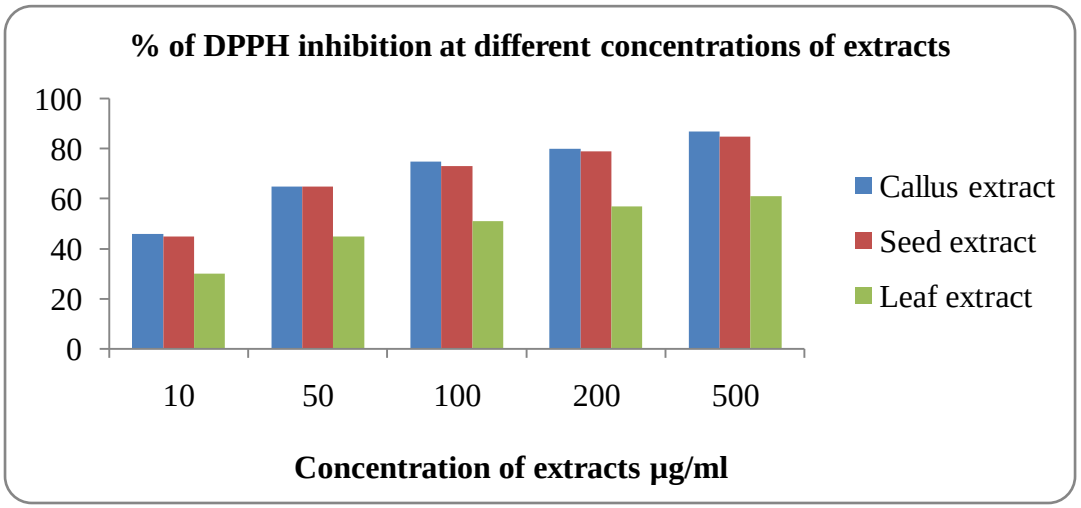

Figure 3: DPPH radical inhibition activity of callus, seed and leaf extract of *Trigonella foenum-graecum* L.

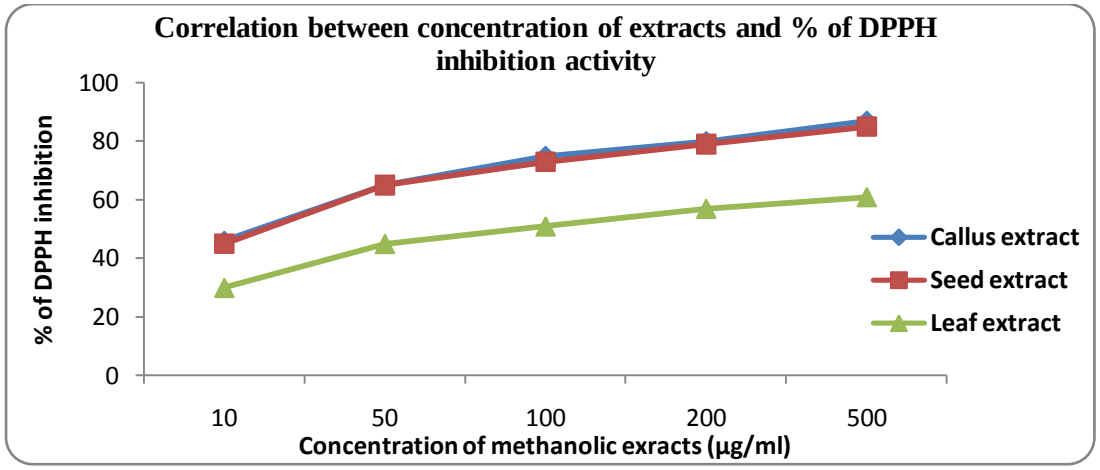

Figure 4: Correlation between concentration of extracts and % of DPPH inhibition activity

TPC and % of DPPH inhibition values of callus confer callus as potential source of active principles playing role in its biological activity. Further callus as continuous source of uncontaminated raw material coupled with uncomplicated down streaming bioprocess can be used on commercial scale for formulation of therapeutics. Further, using abiotic or biotic elicitors the active constituents can be scaled up in *in vitro* grown callus.

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BIOMONITORING OF AIR POLLUTION USING POLLEN GRAINS OF TREE SPECIES IN MYSORE CITY

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Abstract:

Pollen grains are affected by different types air pollutants as a consequence of repeated acquaintance. Pollution is assessed on the source of viability of pollen, interruption in the germination process and decrease in pollen tube length. The present investigation was conducted to examine the pollen characteristics of *Polyalthia longifolia* Hook. f., *Samanea saman* Merrill, and *Swietenia mahogany* Jacq. and Thomas as bio-indicators of vehicular air pollution in Mysore city. Effect of various type air pollutants on pollen germination and pollen viability of the tree species growing at the traffic intersection-Fountain circle was evaluated. Significant reduction was observed in the viability of pollen, delay in the onset of germination and pollen tube length compared to the pollen samples from the control area Mahadevapura situated 20kms away from the city. The present study explained the probability of pollen as bio-monitor of urban air pollution which has assumed alarming proportions.

Introduction:

Biomonitoring is the use of plant responses to explore the changes in the environment. It detects or predicts certain plant species are sensitive to a particular pollutant or to a combination of pollutants. Plant organs are highly vulnerable to air pollutants than man, animals, creatures and materials. Air pollution results in extensive loss of agriculture, economy and environment. Air pollution effect on plants related studies helps us to implement preventive measures towards pollution control (Khoshoo and Ahmed, 1981). Plants pollen grains are very delicate and respond quickly to the air pollutants. Hence, effect of pollutants on morphological, changes in the pollen grains is evaluated on pollen grains of avenue trees in the historical city of Mysore assumes significance. It is well understood from studies that Pollen has become a biomonitoring tool of

atmospheric pollution. Pollen grains the male reproductive organ are relatively more exposed to air pollutants than any other organs of plants. Biomonitoring with pollen can be certainly accomplished with simple tool and procedure.

The effect of air pollutants on pollen germination and pollen tube growth of a roadside tree species was investigated in terms of *Polyalthia longifolia* Hook. f., *Samanea saman* Merrill, *and Swietenia mahogany* Jacq.and *growing* at Fountain circle one of the busiest traffic intersections in the city of Mysore, Karnataka. The trees growing at Mahadevapura, 20 kms away from city with negligible traffic served as control. The city of Mysore has witnessed a phenomenal increase in traffic population from 6333 in 1970 about 8.15 lakh in 2020, increased emission from the vehicles has adversely affected the roadside vegetation invariably.

Materials and Methods:

Ambient air quality monitoring data at Fountain circle, Mysore was obtained from Pollution Control Board, Mysore (KSPCB). Monitoring was done at Fountain circle for major pollutants namely SPM, SO_2 and NOx using High Volume Air Sampler.

***In-Vitro* Germination Studies:**

Pollen samples from the tree species were collected both at control (Mahadevapura) and polluted areas (Fountain circle) during flowering season. Fluorochromatic Reaction (FCR) Test was done to assess the viability of pollen of the tree species following the method of Heslop-Harrison (1970). Pollen was cultured using Brewbaker and Kwack's medium (1963). To culture the pollen, hanging drop culture method was followed (Shivanna and Rangaswamy, 1992).

Per cent pollen germination = number of pollen grains germinated / total number of pollen grains in the field × 100

The length of the pollen tubes in ten microscopic fields was measured with an ocular micrometer.

Mean pollen tube length = Total length of all pollen tubes (in units of ocular micrometer) / Total number of pollen tubes measured from all fields. The values were expressed in µm.

Results:

Ambient air quality monitoring:

Air quality monitoring data for pollutants SPM, SO_2 and NOx at Fountain circle is presented in the Table 1. The data was obtained from Karnataka State Pollution Control Board (KSPCB) Mysore. The data showed that SPM, SO_2 and NOx concentrations were within the permissible limits prescribed by CPCB, New Delhi.

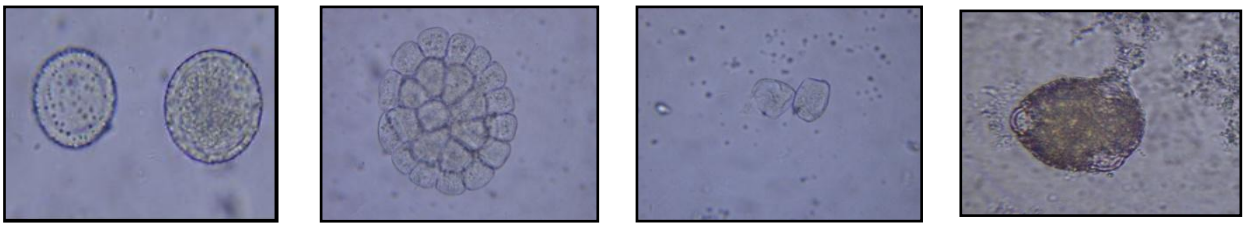
Table 1: Ambient air quality monitoring data for different pollutants for Mysore City

Pollutants	April	May	June
SPM (µg/m^3)	105.5	103.85	89.45
SO_2 (µg/m^3)	11.05	12.2	12.0
NOx (µg/m^3)	23.75	25.7	25.1

Morphology of pollen grains:

Pollen grains of all the three avenue trees are depicted in the Fig. 1. All the pollen grains are circular, monad except *S. saman* where the pollen grain is polyad with 32 grains, with the polyad size of 85.15µm. The size of the individual pollen varies from 20-30 µm.

Photomicrographs of pollen grains:



a **b** **c** **d**

Figure 1: *a. Polyalthia longifolia;* b & c. *Samanea saman*; *d. Swietenia mahogany*

***In-Vitro* germination studies:**

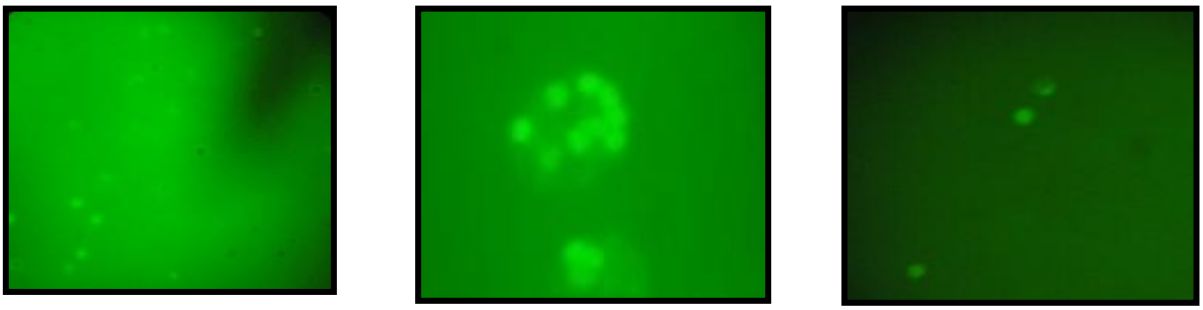
For i*n-vitro* germination studies pollen samples of the tree species from control and polluted area (Fountain circle) were collected and used for testing the pollen viability, per cent pollen germination and pollen tube length.

The Fluorescein Diacetate (FDA) test was used to test the pollen viability in the present study. The viability results of pollen from control and polluted areas are represented in the Table 2. Pollen that fluoresces brightly when seen under fluorescent microscope was considered viable (Fig.2). The study reveals that the pollen from polluted area has showed considerable decrease in the pollen viability in the tree species studied. The result of per cent germination of the tree species from control and polluted area is depicted in Fig.3. The mean length of pollen tube in the tree species of polluted and control area is shown in Fig.3. Photomicrographs of germinating pollen grains is depicted in Fig.4 There was a significant reduction in the length of pollen tube in pollen of polluted area as compared to control area pollen in the tree species Table 3a and b.

Table 2: Effect of vehicular pollution on per cent pollen viability of different avenue trees

	Control (%)	Polluted (%)
Polyalthia longifolia	62	42
Samanea saman	70	50
Swietenia mahogany	90	60

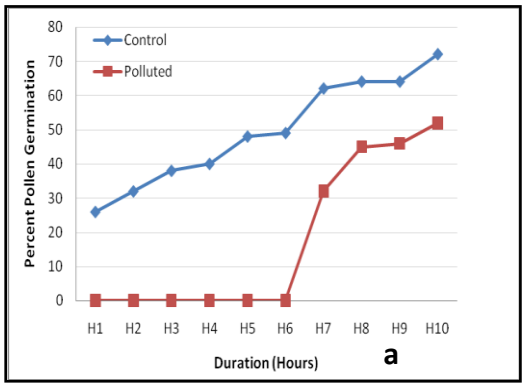
Photomicrographs of pollen stained with FDA:

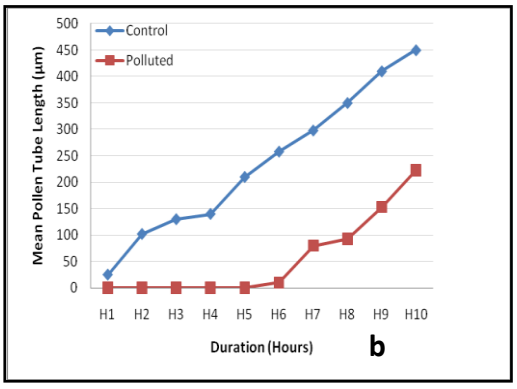


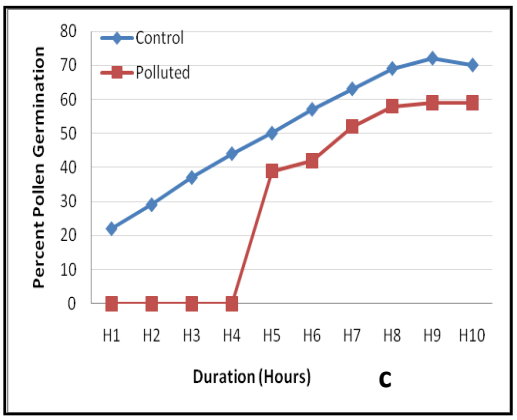
a **b** **c**

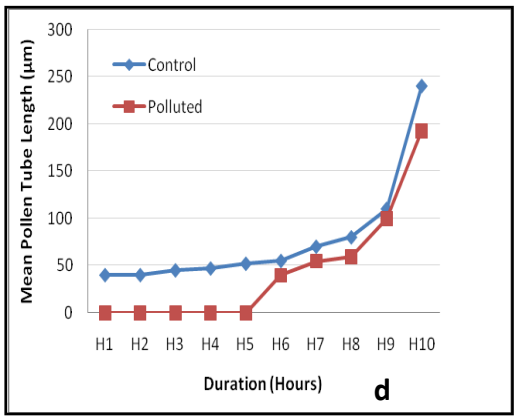
Figure 2: *a. Polyalthia longifolia*; b. *Samanea saman; c. Swietenia mahogany*

Percent pollen germination and mean pollen tube length of different avenue trees in control and polluted area:









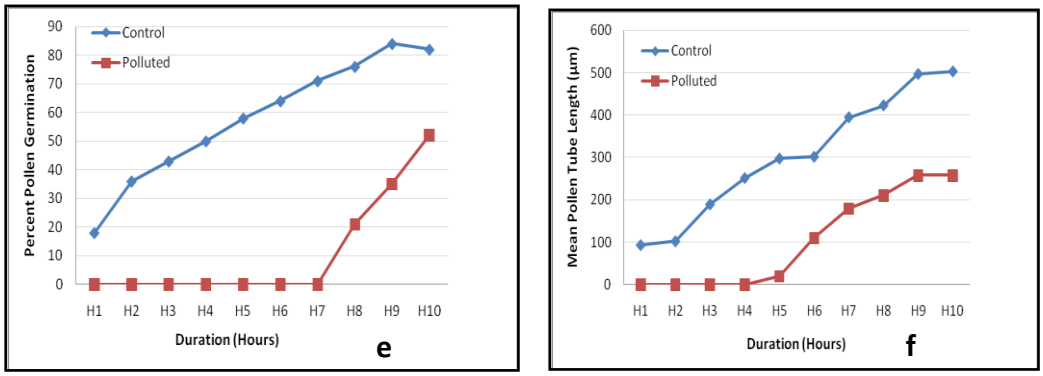
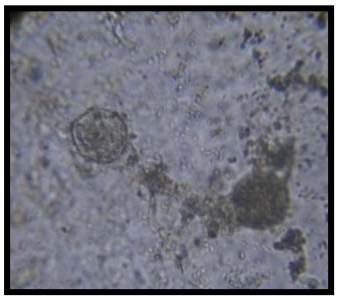
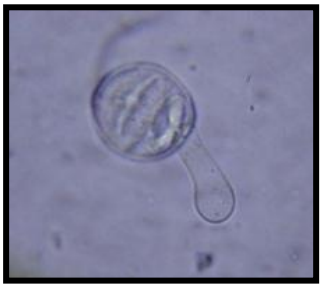


Figure 3: a & b. *Polyalthia longifolia*; *c & d Samanea saman*; e & f. *Swietenia mahogany*

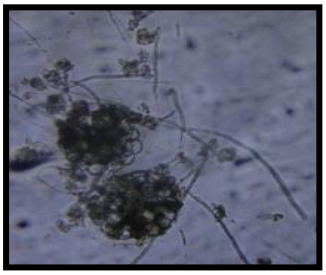
Photomicrographs of germinating pollen grains:



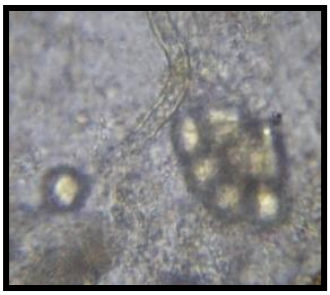
a



b

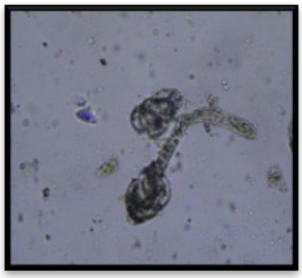


c



d

Figure 4:
a & b. *Polyalthia longifolia;*
***c &d Samanea saman*;**
e. *Swietenia mahogany*



e

Table. 3a. Correlation between pollutants and percent pollen germination of different plant species

	SO_2	NO_x	SPM
P. longifolia	-.633*	-.954**	-.907**
S. saman	-.706*	-.813**	-.639*
S. mahogany	-.800**	-.950**	-.760*

Table.3b. Correlation between pollutants and mean pollen tube length of different plant species

	SO_2	NO_x	SPM
P. longifolia	-.517	-.941**	-.890**
S. saman	-.799**	-.990**	-.826**
S. mahogany	-.834**	-.535	-.822**

** Correlation is significant at the 0.01 level (2 tailed)

*Correlation is significant at the 0.05 level (2 tailed)

Conclusion:

The present investigation has explored the possibility of using pollen as bioindicator of air pollution. The impact of air pollutants on plants leaf are examined by the many researchers. However a few works are done on the use of pollen to evaluate atmospheric pollution. Pollens are very sensitive to air pollutants. They are used as bioindicator of air pollution consequently (Varshney and Varshney, 1981). Fedotov *et al.* (1983) have also observed SO_2 induced reduction in pollen viability, size and shape of pine pollen grains.

Pollen used as bioindicator does not indicate levels of pollutants, but it measures their biological impact. It certainly provides information on the potential adverse effects of pollutants on living organisms. This direct assessment of risk by bioindicator methods is relevant as compared to the physico- chemical methods. The plant bioindication methods are not a substitute to physico - chemical methods for air pollution studies. The present study emphasis on that use of pollen as bioindicator of air pollution constitutes complementary method as they provide essential information on biological impact.

Acknowledgement:

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INTEGRATED CROP POLLINATION: DIVERSITY CONSERVATION STRATEGY OF BEE POLLINATOR TAXA TOWARDS SUSTAINABLE AGRICULTURE

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Abstract:

Sustainable advance in agriculture aims to get rid of hunger through attaining headway production and yield from agriculture produce. Pollination plays an effective starring role in the production of food items by diversified pollinators. Among wide-ranging pollinators, bees are the most accredited pollinators. Integrated Crop Pollination, an innovative intervention to sustain food production which comprises of the use of managed pollinator species in combination with farm management practices that support, augment and safeguard pollinator diversity to deliver reliable and effective pollination of crops. In diverse bee pollinator taxa, interspecific interactions may modify the behaviour and upsurge the pollination efficiency of individual species which may be due to complementary effect, behavioural interaction, functional synergy or functional complementarity. This will endure pollinators diversity, which can functionally complement each other, not only leading to complete pollination effectively in crops also promising conservation of bee taxa in their diversified ecosystem. It seeks to combine tactics that are appropriate for the handling of wild and managed bee species, and enhancing the cropped area for these pollinators through the farm and habitat management. Thus conserving wild habitat and altering selected farm management strategies could lead to the sustainable yield of crops.

Keywords: Conservation, Pollinators, Integrated crop pollination, Sustainable agriculture.

Introduction:

Pollination is transferring pollen from the anther to the stigma. Cross-pollination leads to complete pollination and fertilization and ultimately increases product quality and quantity. There is a significant contribution of pollination between biotic and abiotic agents that facilitate pollination. Eighty per cent of crops in India depend on insect pollination. Bees, flies, wasps, butterflies, beetles, birds and bats form the common pollinators of those crops. Among different pollinators, bees are highly pollinated, contributing seventy-three per cent of total animal pollination (Thakur, 2012).

Bees - credited as pollinators:

It is estimated that bees make up one-third of the human diet for pollination. They pollinate eighty-six per cent of the world's one thousand five hundred crop species. Their participation in food production ranges from fifteen to thirty per cent (Kremen *et al.*, 2002). Bees pollinate seventy-five per cent of crops worldwide (Klien *et al.*, 2007). With Thakur, (2012) sixty-three items (ie; 70%) and thirty-nine pollinators of the eighty-two species and bees that were assigned to ninety per cent of the world's food supply were plant species. Important known pollinators are present in those plant species (48%). Eighty per cent of crops in India are pollinated by bees.

Bee pollinators - An Overview:

The bees belong to the genus Apoidea, which includes major families such as Apidae, Andrenidae, Colletidae, Halictidae, Melittidae, Anthophoridae, and Megachilidae. Honey bees of monkeys and exhibit eukaryotic temperament. All other bees that nest in wild habitats are generally considered to be wild bees. Wild bees are more solitary than bumblebees. Nomia, Nomada, Megachile and Xylocopa are the main wild bee species found throughout the country. Managed pollinators include commonly domesticated honey bee species which are Indian bee (*Apiscerana*) and Italian bee (*Apis mellifera*). Managed wild bees include Alfa alfa leafcutter bee (*Mehachilerotundata*) for alfaalfa seed production, *Osmia* spp. for pollination in apple, almond etc., and *Bombus* spp. for pollinating greenhouse tomatoes (Issacs *et al.*, 2017). Even though rock bee (*Apisdorsata*) and little bee (*A. florea*) belongs to genus Apis, they are not domesticated and commonly seen in wild habitat. Carpenter bee (*Xylocopa* spp.) alkali bee (*Nomia* spp.) Halictids (*Halictus* spp.) Collectids (*Collectes* spp.) etc are other common wild bees. Nesting habitat can be open nesting, cavity-nesting or ground nesting. Open nesting bees are *A. dorsata* and *A. florea*. They construct single combs which are exposed to the atmosphere under

branches of trees, undisturbed building etc. Megachile, Osmia, Anthophora, Lithurgus and Xylocopa are the cavity-nesting bee genera which nest in holes of bamboo, crevices of dead woods etc. Ground nesting bee genera are Bombus, Andrena, Nomia, Colletes, Halictus and Mellitta which construct the nest in soil.

Wild bees as candidate pollinators:

Although bees are highly pollinated, they have been reported to be unfit for this in some crops (Bidinger and Rajot, 2015). In contrast, wild bees perform better as candidate crops for certain crops. Thakur (2012) reported the alfaalfa leafcutter bee (*Megachilerotundetta*) as enhanced pollination for bees in alfalfa seed. The tripping process of releasing the staminal column from the nail petals is efficiently cultivated by the leaf bee and increases seed yield to 350 kg/ha. Stanghellini *et al.* (1998) reported a linear relationship between the frequency of epilepsy in the watermelon (*Citrulluslanatus*) and seed sets of bumblebee (*Bombusimpatients*). This is the importance of native or wild bees as complementary agents for pollination.

Lacunae in resorting to single pollinator group:

As cropland grows, farms gradually separate from pollinators, leading to a decline in pollination-dependent crop productivity. Pollination remedies are not always appropriate or too expensive to include pollinators. Besides, such managed pollinators are susceptible to pests and diseases. Although wild bees have been reported to be good pollinators and resist pests and diseases, their isolation and difficulty in harbouring this single pollinator group. Integrating both managed pollinators and wild bees into the current ecosystem is a good alternative strategy for sustainable yields. This general concept is called integrated crop pollination.

Integrated crop pollination (ICP):

It is proposed to incorporate native pollinators into croplands as a complement to crop pollination. This alternative strategy is called integrated crop pollution. Integrated crop pollination can be defined as the use of managed pollinator species to support, improve and conserve agricultural management practices to provide reliable and affordable pollination of crops (Issacs *et al.*, 2017). It seeks the management of each pollinator, along with the integration of pollinators, and various management strategies. Pollination management and habitat management for wild bees can be implemented simultaneously with this framework. An important point beyond the core concepts of ICP frameworks is that pollinator habitats and farm

management practices designed to support wild or managed bees can provide additional environmental benefits. ICP strategies for raising wild bees can also support natural enemies, especially if horticulture is designed to be a focus of attention.

Integration of pollinators in farms:

From an ICP perspective, bee pollinators can be classified as pollinators, alternative management pollinators, and wild bees. These should be incorporated into the farmland justly to effect a sustainable yield. The pollinator should be introduced to the crop area based on the pollinating capacity of the crop and the pollinator's ability to pollinate.

I. Managed pollinators:

Managed pollinators usually include indigenous bee species (*Apiscerana*) and Italian bees (*Apismellifera*). Depending on the density of bees in the cropland (number of beehives per hectare), beehives are introduced to grow ten per cent. The stock density of *A. mellifera* colonies (the number of beehives per hectare) was determined by the Food and Agriculture Organization (FAO) for proper pollination of crops. Sugar solutions should be provided during the dying period when activity is low. During the developmental period, the maximum population is a single hive, and therefore the colonies must be divided to avoid flocks. In the newly divided colony, an equal proportion of honey, pollen and brood must be ensured.

II a) The general welfare of pollinators:

Wild bees can be well integrated with cropland by following common welfare activities that prioritize agricultural and habitat management.

Farm management:

Agricultural management practices include crop production and crop protection practices, which must be incorporated reasonably to support pollinating populations. During the harvest season, these techniques are manipulated to support, grow, and protect the bee population. Crop production includes ploughing, irrigation and nutrient management practices that affect wild bee populations. The effects of ploughing and irrigation mainly affect the habitat of bee bite nests. Crop protection practices are related to the use of pesticides. It affects bees by multiple means of exposure, and the combination exerts greater effects than individual exposures. Bee venom pathways have been reported by Issacs *et al.* (2017). Poisoning is done by direct contact with the flowers. When pesticides are accidentally sprayed in windy conditions or a target crop, they may travel to non-targeted sites because it is close to the target crop.

Crop protection should be ensured during crop bloom with limited or timely use of pesticides. For the protection of bees from pesticide poisoning, time, formulation, pesticide combination and pesticide use should be considered. Chemical treatments are also dangerous in the area around the apiary. Dust aggregates are more dangerous than sprays because they can be transported to neighbouring areas. Wet and dispersant powders have long-lasting residual effects. Well-grounded formulas are safe for bees. Oil emulsion is more dangerous to bees and should be avoided. It is always safe to avoid sprays during an active distance and therefore to a somewhat harmless spray in the evening. The proper dose of pesticide should be used. Hives can be transported to other areas or restricted before application (Mishra, 1995).

Habitat management:

Habitat loss directly affects forest pollination by reducing forest resources and nesting on agricultural land, leading to the deterioration of pollination services for many crops. Forests, meadows, large-scale flower crops, the establishment of different areas, etc. are different habitats in which wild bees live. Conservation of such natural habitats may add to the local bee species. Forestlands serve as nesting sites for bees, such as rock bees (*A. dorsata*). Krishnan and others participated. (2012) found that fifty eight per cent of flower visitors to *A. dorsata*overpopulated, coffee (*Coffeaconephora*) population.

II b) The provision of foragingsites:

In addition to regular welfare activities, outdoor access can greatly attract bee pollinators. Flower boundaries that bloom before and after harvest are a precondition for maintaining diverse bee populations. Apart from these flower strips, large scale crops also serve as good pastures.

III c) Establishment of nest sites:

Establishment of nesting sites adjacent to the area of cuttings may result in the nesting of wild bees. Cavity nesting and ground nest beekeeping methods have been proposed (Vaughan and Black, 2007). About 30 per cent of our 4,000 native bee species are solitary wood-nests, which nest inside hollow wood or pithy trunks. The diameter holes of different holes support different sized bee species. Even paper straws or hollow stem bundles serve the same purpose. Seventy per cent of our native bee species dig their nests underground. Naked patches provide good support for ground-nest bees. A stable soil pile, at least two feet high, and at least thirty-five per cent of sand can attract them. Protect sloped or well-drained field sites to nest these bees.

Benefits of integrating pollinators:

In different pollinator societies, interspecific interactions can modify behaviour and enhance the pollinating effects of individual species. Integrating bees and wild bees in crop fields can result in efficient and sustainable pollination of crops, be it due to complementary effects, behavioural interactions, functional synergy or functional complementarity.

Conclusion:

The comprehensive crop hypothesis is a unified theme under which various strategies can be developed to support the pollination of crops and coordinate to ensure the reliable and sustainable yield of crops. It seeks to combine strategies for the use of wild and managed bee species and these pollinators seek to increase crop area through agricultural and habitat management. It manages pollinators and pollinator diversity leads to complete and permanent pollination with each other. Thus conserving wild habitat and altering selected farm management techniques could lead to the sustainable yield of crops.

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RP-HPLC ANALYSIS OF DELPHINIDIN CONTENT IN FLOWER COLOR MUTANTS OF *DELPHINIUM MALABARICUM* (HUTH) MUNZ.

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Abstract:

The flower color of *Delphinium* cultivars depends on the pigmentation with anthocyanins produced and their accumulation affecting the appearance. We first examinedthe flower color mutants of *D. malabaricum* obtained from ethyl methane sulfonate (EMS), sodium azide (SA), and gamma irradiation treatment with an idea that the analysis of anthocyanins is necessary in order to understand flower color variations in the mutants. In this view, we analyzed the content of delphinidin in flower color mutants of *Delphinium* by reversed phase high performance liquid chromatography (RP-HPLC) and discussed the relationship between delphinidin content and the flower color. The flower color mutants which ranged in color from blue to pale pink revealed a marked difference in the delphinidin content, the quantity of delphinidin differed in the respective mutants as compared with the parent cultivar. Color intensity was positively correlated with delphinidin content, the concentration of delphinidin varied from 0.130 mg/g to 8.207 mg/g of sample in pale pink and dark blue color mutants respectively with delphinidin content 6.668 mg/g in the traditional blue cultivar. Thus we provide evidence indicating that these differences in delphinidin content are caused may be by a genetic change in the biosynthesis of anthocyanins, which explains the phenotypic variance in the flower color. The major commercial benefit of the application of this technology has so far been the improvement of novel flower colors through mutation breeding. The results obtained in the present study may be helpful in systematic breeding for flower color alterations in *D. malabaricum.*

Key words: *Delphinium malabaricum*, Mutation, Flower color, Anthocyanins, Delphinidin, RP-HPLC.

Introduction:

The genus *Delphinium* is one of the most important genera of the family Ranunculaceae, which represents a group of very attractiveplants, commonly referredas Larkspurs. The range of colors and shapes of their flowers bestows this genus a very fascinating ornamental potential. In India, the genus *Delphinium* is represented by approximately 24 species (Rau, 1993), mainly confined tothe Himalayan regions. *Delphinium malabaricum* is the only species, restricted to Western Ghats of Maharashtra (Pai *et al.*, 2007). The plant bears very attractive and beautiful flowers of violet-blue color, which are of great ornamental value. Flower color is one of the most strikingtrait in ornamental plants, contributing to the major value in the floricultural market andanthocyanins are the most common flower pigments responsible for the range of colors from yellow to orange to red to purple. The studies describing floral color and anthocyanins of several major ornamental plants have been reported (Bloor and Falshaw, 2000; Iwashina *et al.*, 2001; Uddin Jamal *et al.*, 2002; Zhang *et al.*, 2007, Li *et al.*, 2008; Yang *et al.*, 2009; He *et al.*, 2011), but, no information is available for the species *Delphinium malabaricum*. In this context the main objectives of our study concerned to the improvement of *D. malabaricum*are novelty and uniqueness. For the accomplishment of these objectives, we have used the mutagenesis technique for obtaining different flower color mutants and we have evaluated therelationship between flower color and pigment composition, in particular the presence of anthocyanidin – delphinidin in flower color mutants of *D. malabaricum*.

Materials and Methods:

Induction of mutation:

The mutation was induced in *Delphinium malabaricum*by using chemical mutagens like ethyl methane sulfonate (EMS), sodium azide (SA) and physical mutagen like gamma rays. Total of 300 seeds were used for each concentration/dose of the treatment and control.

Mutagen treatment:

EMS and SA treatment:

For chemical mutagen treatment seeds were presoakedfor 12 hours in distilled water, blotted dry and treated with a freshly prepared solution of ethyl methane sulphonate (EMS) and sodium azide (SA) at different concentrations (0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30%) for 6 hours at room temperature (23±2^{o}C) with recurrent shaking. Immediately after the treatment, the seeds were washed thoroughly with distilled water for half-hour to leach out residual chemicals.

Gamma rays treatment:

Dry and healthy seeds of *D. malabaricum* were irradiated from a ^{60}CO source at Bhabha Atomic Research Centre (BARC), Mumbai with a dose of 5, 10, 15, 20, 25 and 30kR and with rate 2.8 Krad per minute and untreated seeds were used as control.

Isolation of mutants:

After mutagenic treatment the seeds were sownin the experimental plots within the Botanical Garden, Department of Botany, Shivaji University, Kolhapur (MS) India. M1 plants were harvested and grown in successive seasons and developed $M_2$and M_3 generations. M_2 generation was carefully screened for various flower color and morphological mutations and the mutants scored were harvested separately and raised in the field to obtain M_3 generation. In M_3 generation flower color mutations were individually marked in the field and mutants that were visually distinguished as having inherited and maintained that mutated color was selected and analyzed for delphinidin content. The colors of the flowers were assigned based on the code from the Color Chart of the Royal Horticultural Society (RHS; London).

RP-HPLC analysis of anthocyanidin – Delphinidin:

Anthocyanidins were extracted with aqueous 70% CH_3CN containing 3% trifluoroacetic acid (TFA) at 5^oC (Honda *et al.*, 1999). The extract was filtered through a membrane filter (0.22µm) and analyzed by reversed phase high-performance liquid chromatography (RP-HPLC). The chromatographic system consists of Waters 2690 Alliance HPLC separation module equipped with Waters 2487 dual λ absorbance detector (UV/VIS) and Waters 996 photodiode array (PDA) detector (Waters corporation Milford, MA, USA).Chromatographic separation of anthocyanidinswas achieved on a C-18 reversed-phase column (Princeton SPHER, 5µ, 250mm). The anthocyanidins were separated by gradient elution using solvent A(acetonitrile) and solvent B (10% acetic acid+5% acetonitrile+1% phosphoric acid in water) with the following gradient program:

Initially, 5% A and 95% B then from 0min to 10min - 5 to 15% A and 95 to 85% B; from 10min to 15min 15 % to 5% A and 85 to 95% B. A total run time is of 15 min. Elution was carried out at room temperature with a flow rate of1ml min^{-1} and detection at 510nm. The stock solution of compound Delphinidin chloride was prepared. The volume of the reference compound and samples injected was 20µl. The program was run up to 15 minutes. The spectral data were collected and analyzed.

Results and Discussion:

Isolation of flower color mutants:

Flower color is one among the foremost attractive characteristics in ornamental plants, contributing to the major value in the floricultural market. In nature, various patterns concerningthe flower color is often easily observed. However, most of these phenotypic changes are not transmittable and thus novel varieties with commercial value cannot be obtained. Furthermore, alteration in flower pigmentation is anobservable attribute to study the expression and regulation of floral genes in plant molecular biology. Thus, examination and manipulation of flower color is not only important in basic research areas, but it also features a great benefit in biotechnological applications (To and Wang, 2006). In this regard, *D. malabaricum* was analyzed for flower color variation. The examination of *D. malabaricum* and its mutants revealed significant variation in the color of the flower with promising ornamental features. Flower color mutants varied for their sepal/petal color from blue to pale pink with several intermediate colorations, whereas the control possessed blue flower color (Fig. 1a). Wide variations in the flower color from traditional blue were exhibited as bluish-white, pinkish purple, sky blue, blue with purple shading, purple, dark blue, light purple, light pink, sky blue with pink shade and light violet in different mutant lines of *D. malabaricum* (Fig. 1). The mutant phenotypes varied from blue to pale pink and several hues of blue. The mutant with a pale pink flower color (Fig. 1m) is one of the most attractive mutants amongst the other flower color mutants obtained from different mutagenic treatments. The intensity of the pale pink color of flowers during the course of development did not vary and it also did not vary within a plant. In progeny testing also, the plants had pale pink flowers, indicating that expression of the mutant trait was not influenced by the environment and was propagated true to type. This mutant is a new flower color mutant for genetic research in *Delphinium*. High enough variation in the color of flowers of *D. malabaricum*, showed a wide genetic diversity.

RP-HPLC analysis of anthocyanidin – Delphinidin:

Anthocyanidins are the major set of structural pigments that give flowers their unique colors. The main anthocyanidin pigment, which occurs in *Delphinium*, is delphinidin, as identified by Willstatter and Meig (1915). In this regard the RP–HPLC analysis of *D. malabaricum* and its nineteen mutant lines identified with dark blue, sky blue with pink shades, very light purple and pale pink colors (Fig. 1) were analyzed for anthocyanidin content, to clarify the contribution of delphinidin to the flower color change in the mutantsof *Delphiniummalabaricum*. The RP- HPLC chromatograms of the flower extracts of the traditional blue color and different flower color mutants were obtained. In the RP-HPLC profile, a number

of peaks were observed to be present with identical absorption maxima. The RP-HPLC chromatograms of anthocyaninsobtained from the traditional blue color florets and mutant florets in the visible spectral region of 510 nm were compared to the known reference delphinidin (Fig. 2).

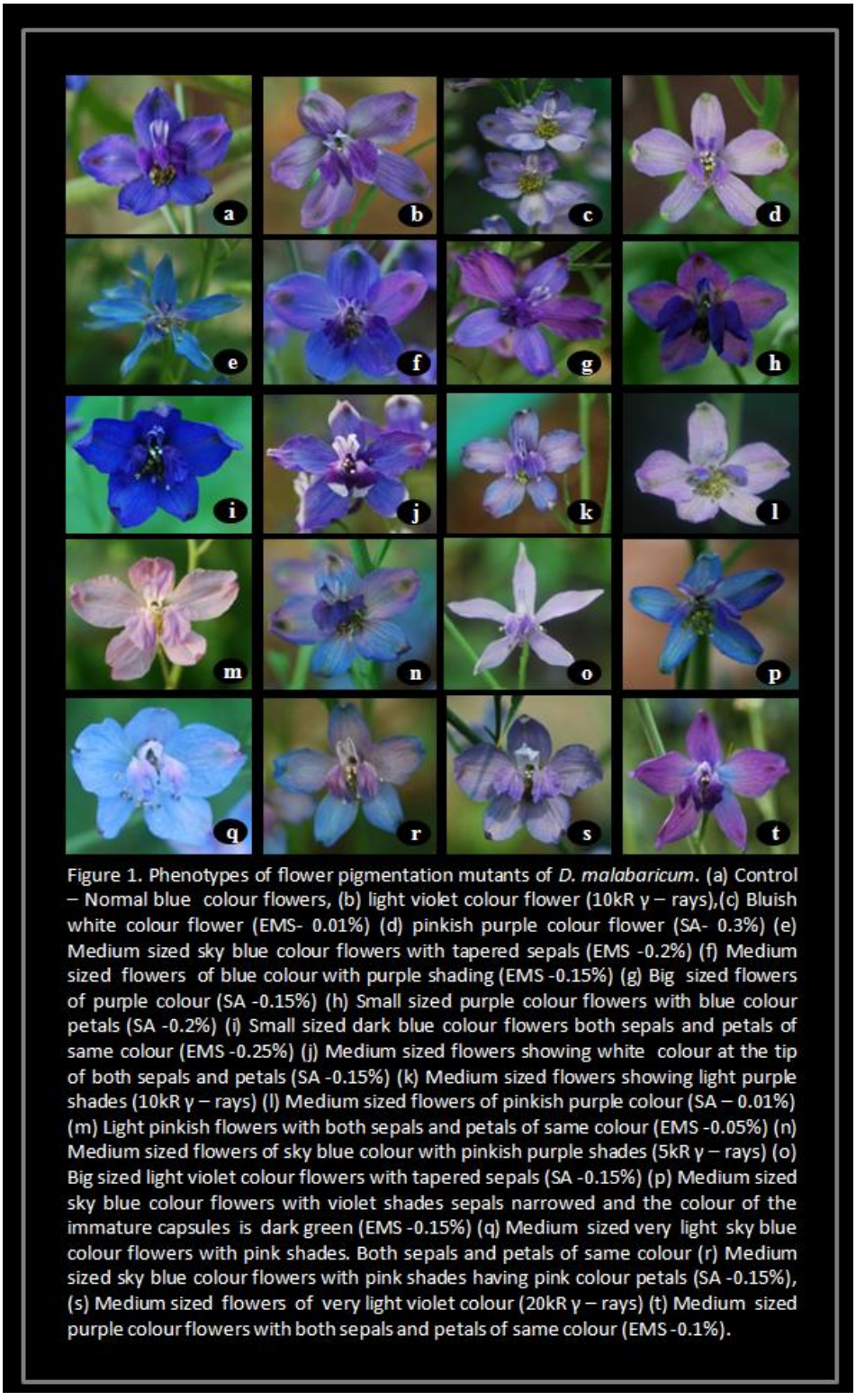


Figure 1. Phenotypes of flower pigmentation mutants of *D. malabaricum*. (a) Control – Normal blue colour flowers, (b) light violet colour flower (10kR γ – rays),(c) Bluish white colour flower (EMS- 0.01%) (d) pinkish purple colour flower (SA- 0.3%) (e) Medium sized sky blue colour flowers with tapered sepals (EMS -0.2%) (f) Medium sized flowers of blue colour with purple shading (EMS -0.15%) (g) Big sized flowers of purple colour (SA -0.15%) (h) Small sized purple colour flowers with blue colour petals (SA -0.2%) (i) Small sized dark blue colour flowers both sepals and petals of same colour (EMS -0.25%) (j) Medium sized flowers showing white colour at the tip of both sepals and petals (SA -0.15%) (k) Medium sized flowers showing light purple shades (10kR γ – rays) (l) Medium sized flowers of pinkish purple colour (SA – 0.01%) (m) Light pinkish flowers with both sepals and petals of same colour (EMS -0.05%) (n) Medium sized flowers of sky blue colour with pinkish purple shades (5kR γ – rays) (o) Big sized light violet colour flowers with tapered sepals (SA -0.15%) (p) Medium sized sky blue colour flowers with violet shades sepals narrowed and the colour of the immature capsules is dark green (EMS -0.15%) (q) Medium sized very light sky blue colour flowers with pink shades. Both sepals and petals of same colour (r) Medium sized sky blue colour flowers with pink shades having pink colour petals (SA -0.15%), (s) Medium sized flowers of very light violet colour (20kR γ – rays) (t) Medium sized purple colour flowers with both sepals and petals of same colour (EMS -0.1%).

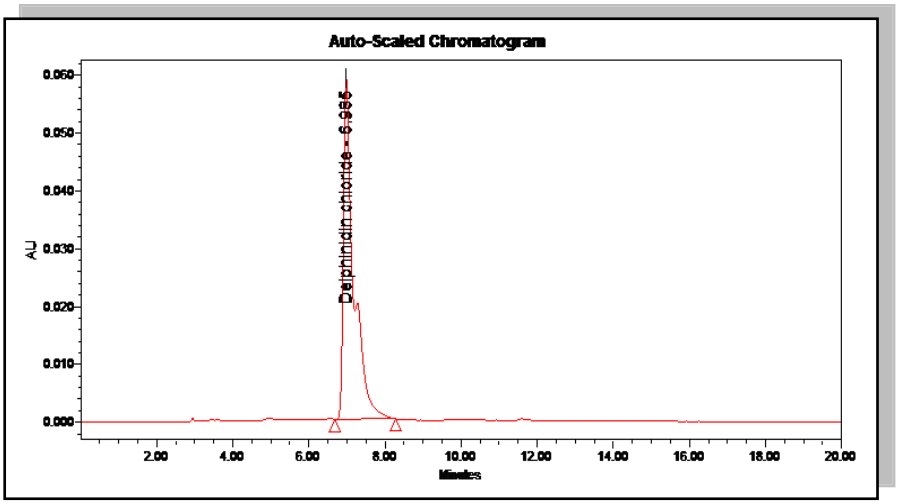


Figure 2: RP- HPLC chromatogram of reference compound

The chromatograms of different anthocyanin profiles obtained for each cultivar are illustrated in Fig. 3 and 4. The chromatographic analysis has shown that in all samples, delphinidin was present but the quantity of the compound varies (Table 1). The concentration of the delphinidin was observed to be highest in the dark blue mutant (8.207mg/g of sample) and was lowest in the pale pink mutant (0.130 mg/g of sample). A close correlation between the occurrence of delphinidin and color of the flowers was found in *Delphinium* and its mutants. The lightness of the flower has a negative correlation and the level of blueness has a positive correlation with the quantity of delphinidin (Table 1). The flower color and pigment profile vary widely among the different mutants of *D. malabaricum* analyzed for pigment content. Flower color is predominantly blue in normal with the variation of different colors among the mutants. Blue flower color was due to the predominance of delphinidin. The delphinidin content in the flowers of *Delphinium* and its mutants analyzed by using a known standard of delphinidin explains that apart from delphinidin there were other anthocyanidins also, but were unidentified. As estimated, multiple anthocyanidins were found in the different flower color types (Katsumoto *et al.*, 2007). The variations observed were may be due to the presence of several other anthocyanidin compounds. However, the change in the flower color of the *D. malabaricum*is explained by the composition and quantity of the delphinidin. The higher the concentration of delphinidin was, the bluer the flower color became. Blue colored mutants were also distinguished by pale blue and dark blue mutants, though they had continuous variation. Pale blue mutants, had less total delphinidin content than control (parent plant), while dark blue mutants had more total delphinidin content than control (Table 1).

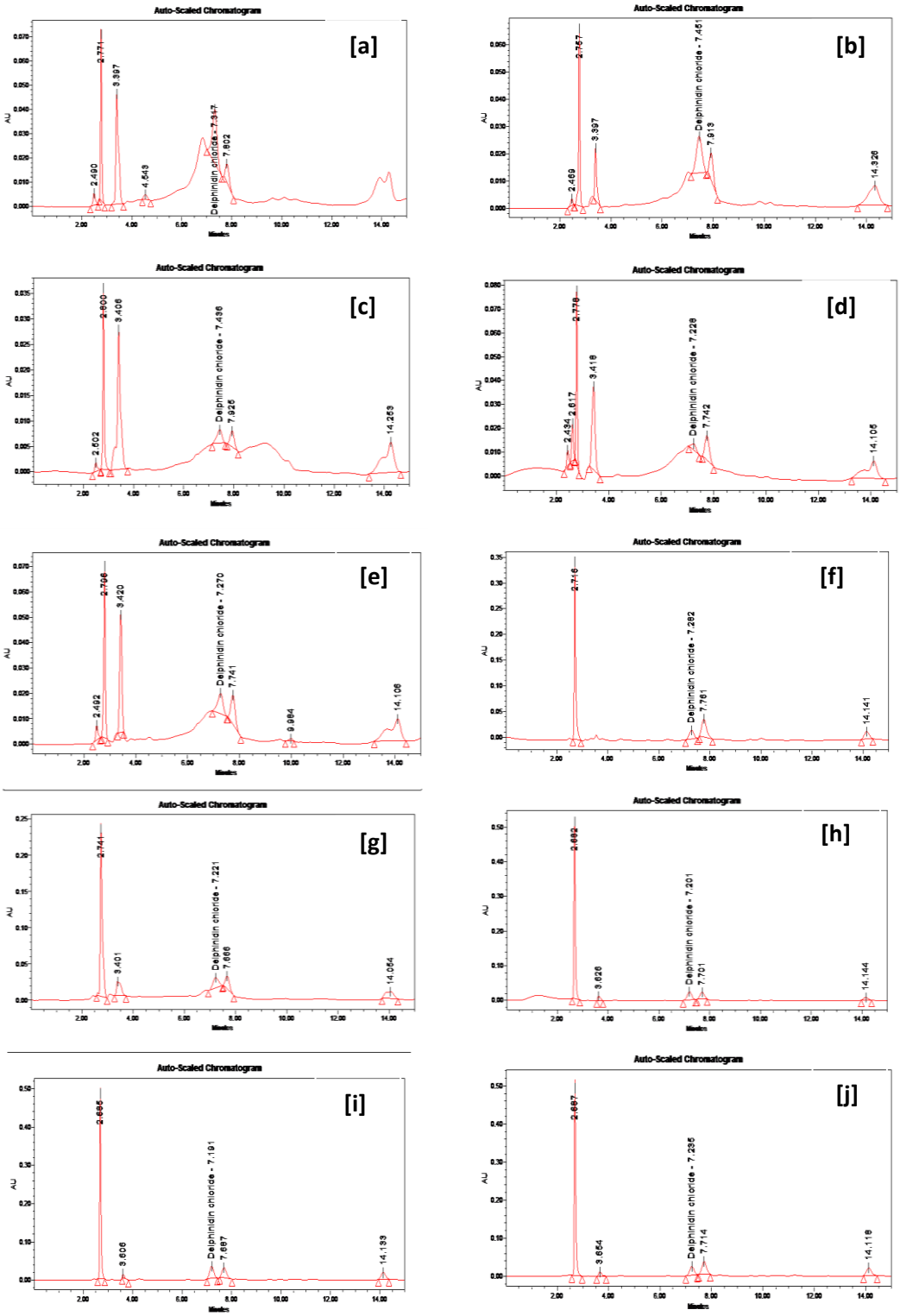


Figure 3: Reverse-phase HPLC chromatograms of anthocyanin profiles of *Delphinium malabaricum* and its mutants: a: Control, b - j: mutants; b: violet, c: pale violet, d: pale purple, e: sky blue, f: bluish purple, g: purple, h: purple violet, i: dark blue, j: blue with white shade

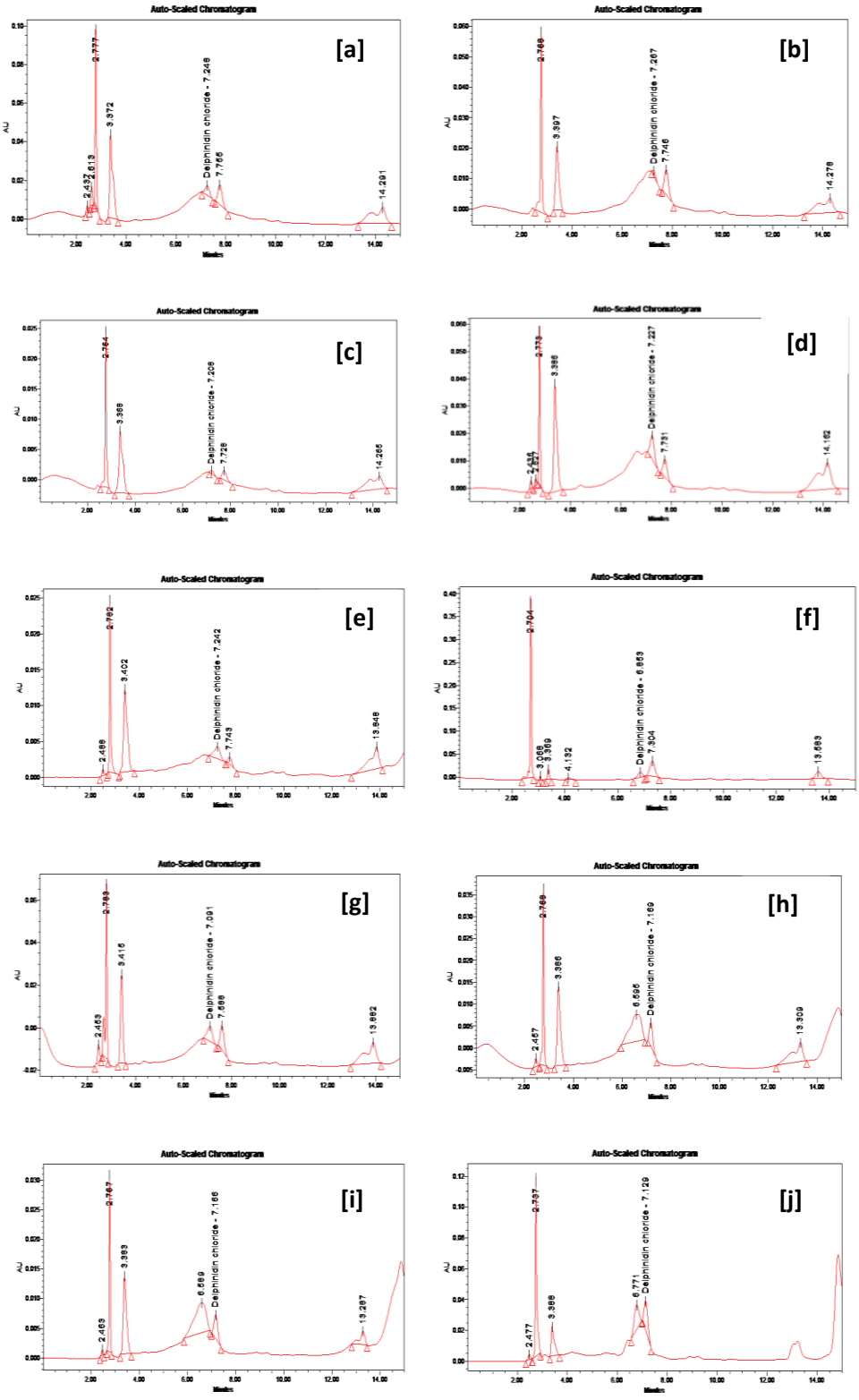
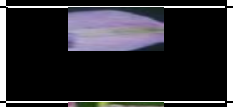
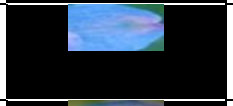
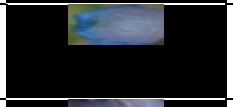
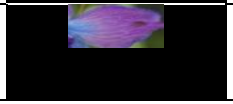


Figure 4: Reverse-phase HPLC chromatograms of anthocyanin profiles of *Delphinium malabaricum* mutants: a: pale violet, b: pinkish purple, c: pale pink, d: sky blue with pink shades, e: very light purple, f: pale blue, g: sky blue with pink shade, h: light blue with pink shade, i: violet blue, j: purple.

Table 1: RP-HPLC quantification of anthocyanin-Delphinidin in *D. malabaricum*and its mutants

Sample	Mutant No.	Mutagen	Dose/Conc. of Mutagen	Flower color	Colour Variation	RHS Color code	HPLC Retention time (min)	Concentration (mg/g)1
1.	**Control**	**-**	**-**		Normal blue colour	95B	7.317	6.668
2.	**M1**	**γ–rays**	**10kR**		light violet colour	83D	7.451	4.713
3.	**M2**	**EMS**	**0.01%**		Bluish white colour	92B	7.436	0.867
4.	**M3**	**SA**	**0.3%**		Pinkish purple colour	77C	7.228	0.734
5.	**M4**	**EMS**	**0.2%**		Sky blue colour	107B	7.270	2.635
6.	**M5**	**EMS**	**0.15%**		Blue colour with purple shading	83C	7.282	4.715
7.	**M6**	**SA**	**0.15%**		Purple colour	77A	7.221	4.884
8.	**M7**	**SA**	**0.2%**		Purple colour with blue shading	82A	7.201	6.690
9.	**M8**	**EMS**	**0.25%**		Dark blue colour	105A	7.191	8.207
10.	**M9**	**SA**	**0.15%**		Sepals and petals with white colour tip	99B	7.235	6.201

11.	**M10**	**γ–rays**	**10kR**		light purple shades	85B	7.248	1.376
12.	**M11**	**SA**	**0.01%**		Pinkish purple colour	84C	7.267	0.303
13.	**M12**	**EMS**	**0.05%**		Light pink colour	75D	7.208	0.130
14.	**M13**	**γ–rays**	**5kR**		sky blue colour with pink shades	89D	7.227	2.243
15.	**M14**	**SA**	**0.15%**		Light violet colour	76C	7.242	0.677
16.	**M15**	**EMS**	**0.15%**		Sky blue colour with violet shades	100A	6.853	3.038
17.	**M16**	**EMS**	**0.2%**		Very light sky blue colour with pink shade	106B	7.091	2.387
18.	**M17**	**SA**	**0.15%**		Sky blue colour with pink shade	92 C	7.169	1.175
19.	**M18**	**γ–rays**	**20kR**		Very light violet colour	91B	7.166	0.989
20.	**M19**	**EMS**	**0.1%**		Purple colour flowers	78B	7.129	4.399

[1]Results are expressed as milligram of fraction equivalent (Delphinidin chloride) per gram of sample.

M: Mutant, EMS: Ethyl methane sulphonate, SA: Sodium azide, γ–rays: Gamma rays.

The process of pigmentation shows that blue-colored mutants had floral pigment concentrations similar to the parent plant, it was determined that blue-colored mutants were quantitative, not qualitative mutants, i.e. pigment production was promoted in dark blue mutants and restrained in pale blue mutants. The change of the flower color in the mutants obtained that way involves the quantitative modifications of the pigments. The violet and purple group observed a considerable decrease in the content of delphinidin in the mutants obtained, while in the dark blue colored' mutant there occurred an increase in the content of delphinidin, while in 'Pale pink' – a decrease. One can assume that irradiation and chemical mutagens in 'light violet' or 'light purple' and 'pale pink' mutants resulted during apartial inactivation of the genes participating in the biosynthesis of this pigment. What seems interesting, however, is an increase in the content of delphinidin in 'Dark blue' color mutant as compared with the traditional blue cultivar. In many cases the mutation in a single gene causes an accumulation of intermediary compounds, which results in a change in the flower or seedcolor (Onozaki *et al.,* 1999; Selinger and Chandler, 1999; Kobayashi *et al.,* 2001).

The development of new colors of inflorescence due to mutation seems to be related with the destruction of the new genetic material (Lema-Ruminska and Zalewska, 2004). It can be assumed that in parent cultivars covered by the present study the genes of biosynthesis of respective pigments may be blocked. Blocking genes the so-called inhibitors, if they occur in a dominant form effectively block paths of pigments biosynthesis. Destruction of these genes by the application of, e.g. radiation or chemical mutagen shows a possibility of developing a given pigment in the mutant. The increase in total pigment in blue mutants may be related to the changes in the pigment production gene expression after mutagenic treatment. Although a decrease in color pigment often occurs with mutagenic treatment, an increase in color pigment is very unique. Therefore, the use of mutagens was effective for the acquirement of mutants in flower color induced by decreasing or increasing color pigment and the mutants were beneficial for the horticultural industry. Such changes within the color of the flowers etc. unlike other morphological abnormalities may sometimes be genetical in nature. Lawrence and Strugess (1957) have advanced a hypothesis explaining the evolution of flower color in *Streptocarpus* through the successive mutation of genes, they are becoming dominant and finally epistatic to their predecessors. If this hypothesis is correct, the variations in the flower colors of certain mutated plants may be explained on the basis of mutation of the gene or genes controlling the expression of pigment, as a result of the mutagen treatment. However, some of the mutagen induced color variations are supposed to be chimaeral in nature, e.g., the red color induced in carnation (*Dianthus caryophyllus*) by the X- rays (Sagawa and Mehlquist 1956), while still others are believed to be arising as a results of mutations or deletions, e.g. in *Antirrhinum*

*majus*through chronic gamma irradiation (Sparrow and Pond 1956). A similar change of color in flowers has also been reported by other workers in *Chrysanthemum grandiflorum* (Lema-Ruminska and Zalewska, 2005; Lee *et al.*, 2008), in *Chrysanthemum caryophyllus* and *Cyclamen* species (Nakayama *et al.*, 2012) and in *Torenia* hybrid (Miyazaki *et al.*, 2006).

Genetic engineering is effective in changing a target character, but mutations, which can be induced efficiently, as in this study, are often effective in expanding the variation of unspecified characters, especially for plant like *Delphinium*. The present confirmation of the production of identical blue pigments in the mutants of *Delphinium* indicates that all the mutants examined possess a common biosynthetic pathway of the flower-color pigments and suggest that these mutants may have the identical dominant gene to produce blue-color pigment. It is also suggested that the identical pale pink color pigment in the mutant is produced, when the dominant gene was mutated or was deleted, either by the recessive genes having mutant specific different defects in the biosynthetic pathway or by an identical recessive gene with a particular defect.

Conclusion:

The present data confirms the applicability of the RP–HPLC method to define the changes recorded in mutants exposed to ionizing radiations and chemical mutagens and showing their distinctness as compared with the cultivar they originated from. All the mutants differed from one another as well as from the original cultivar. The information gathered in this study helps towards characterizing the *D. malabaricum* and its mutants. This may assist breeding of *D. malabaricum* oriented to flower color providing new cultivars with novel flower colors.

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GALLS ON *FICUS RACEMOSA*: A MORPHO-BIOCHEMICAL PERSPECTIVE

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Abstract:

Galls are the typical outgrowth of plant due to Insect, Bacteria, Fungi, Parasites and Mites which provide nourishment, shelter and protection to the inducer and its progeny. Galls study was done in *Ficus racemosa* belong to family Moraceae, a common plant in India. Galls Inducing wasp was identified up to species level and morpho-biochemical study was carried out in gall induced leaf and normal leaf of host plant. We found that galls in Ficus leaf was caused by *Pauropsylla depressa*, a hemipteran insect. The galls sample was collected from Badlapur to Dombivali zone. 35 galls were dissected after measuring the size and observed the stages of growth of the insect in them. Egg, 1st instar, later stages larvae were observed in collected sample. In the present study also noted that size of galls depends on number of vacuoles in it. Qualitative study of carbohydrate and protein was conducted by standard procedure. Present study observed significant difference in morpho biochemical study conducted in wasp inducing gall in Ficus.

Keyword: *Ficus racemosa*, Galls, *Pauropsylladepressa*, Moraceae, Hemiptera,

Introduction:

Ficus racemosa tree is having traditional and medicinal importance and is a large, deciduous evergreen plant coming in family Moraceae. They are commonly known as fig tree as "gular" in north India and "*atti"*in south India. It occupies a wide variety of ecological niches. Galls are the typical outgrowth of plant due to Insect, Bacteria, Fungi, Parasites and Mites which

provide nourishment, shelter and protection to the inducer and its progeny. The production of galls is considered as the most complex interaction between the plant and insect. The fascinating diversity of galls-inducing insect and their galls has attracted the attention since the time of Greek philosophers.

Herbal drugs are curing a variety of human diseases without side effect and not developing resistance towards pathogens. Area wise Biodiversity of galls were conducted by Sharlene and Valeria (2018). But in this zone no study was reported in galls, Ours is the basic study to know the gall in ficus and causative agent with biochemical aspects in Dombivali to Badlapure zone. According to Petrovska (2012) 80% of the world population use herbal medicine in some aspects of primary health care and tendency is growing to "Go Natural". Galls are known as "Karkatshringi" in Sanskrit are one of the appendages of plant formed due to the invasion of insect -psyllids. Karkatshringi is used in indigenous systems of medicine (Ayurveda, Unani and Siddha) as a remedy in cough, asthma, fever, respiration and liver disorders (Santha *et al.*, 1991). Karkatshringi also represents usage in the treatment of children's ear infections, suppress *haemorrhage* from gums and used to suppress nosebleeding, pulmonary infections, diarrhoea and vomiting (Sukh, 1997). Although most of the plants used in the traditional medicine have been identified and their applications are well-documented, the anticancer, antiviral, antibacterial property of them is yet to be verified.The present basic study aims to increase the knowledge of galls in this zone and we observed ultrastructural morphology and biochemical changes in galled leaves and normal leaves of host plants.

Materials and Methods:

Collection of leaves:

Gall affected leaves and normal leaves of same host plant were collected at morning time (8am-9am) frequently in three months from Badlapur to Dombivali zone, Thane, Maharashtra.

Observation of galls:

Sizes of galls were noted and were dissected to observe under microscope. First two instar larvae galled leaves which are green in colour pooled and named green galls and third and fourth instar stages larvae, which are red in colour pooled as red galls. After taking measurement, they were homogenized using water and used for biochemical studies.

Figure 1: Ficus tree with galled leaves and normal leaves

Result and Discussion:

Causative agents of galls were identified as *Paurospylla depressa*, a hemipteran insect. Host plants normal leaves and gall leaves show difference in ultrastructure that in galled leaf stomata are not seen. Morphological study revealed more than one larva was present in single chamber. Empty chambers are also observed in gall tissue which has to elucidate. The galls size varied according to the number of larvae, number of chamber and size of larvae (Table 1). In galled leaf epidermal cells are relatively narrow and more elongated with the reduction in stomata (Table 2) as Huang *et al.* (2015) reported less photosynthesis activity in galled leaves of *Litsea acuminata* with a smaller number of stomata.

Table 1: Morphological details of Larval stages insect *Paurospylla depressa*

Size (cm)	Number of Chambers	Number of Larvae	Stages of larvae
0.3cm -0.9cm	1 -3	1, 2 or 3	First instar stage
1.0cm	3	2	Second instar
1.0cm-1.2 cm	4	3	Third instar larva stage
1.5cm	3	2	Fourth instar larvae
1.5cm	4	3	Larvae with Antennae

Table 2: Differences observed in Normal and galled leafs

Tests	Normal leaf	Leaf with galls
stomata	Present	Absent
carbohydrate	Present	Present
protein	Present	Absent

Protein were absent in galled leaves (Table 2) and sugarcontent was much higher in galled leaf and in two colour galls express high content of sugar with little difference in percentage. (Fig. 4).

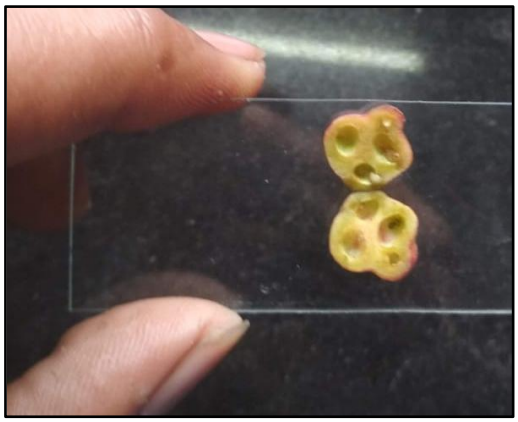

Figure 2: Three chambered, first instar larvae of *Pauropsylla depressa* seen in one

Figure 3: Microscopic observation of fourth instar larvae in galls

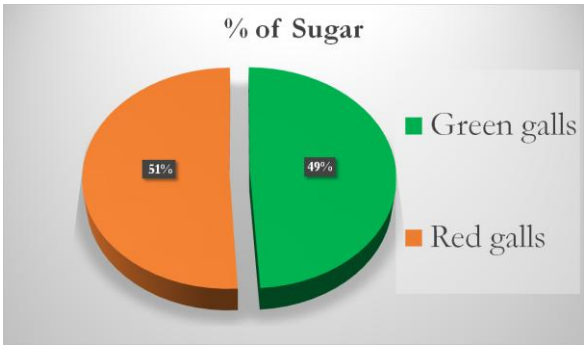

Figure 4: Percentage of Sugar in galled leaf measured by colorimetry

Our study shows similiarity with Huang *et al.* (2015) as reported high sugar content in galled leaves and no protein in galled leaves and as Shim *et al.* (2003) reported in aqueous extract from the gall of *Rhuschinensis* (AEGRC) inhibited alpha-glucosidase activity, an enzyme responsible for digestion of carbohydrate to monosaccharides in the process of intestinal absorption.

Conclusion:

Present study is the basic study for knowing galls in *Ficus racemosa* by *Paurospylla depressa* in Dombivali to Badlapur Zone, Thane, Maharashtra. More sugar content in gall extract indicates that high sugar is food for growing larvae and helps them to change instar stage. In our study we have not receive protein content in gall extract, which has to further confirm. Importance of galls in ethnomedicine as reported by Shanta *et al.* (1991), or as antidiabetic drugs like (Shim *et al.,* 2003). Galls in leaves reported over expression of auxin and cytokinin hormones which protects the plants for further growing and also insect. We found that after infection with galls also the tree is growing and flowering. Much chances are there the gall extract contain some antimicrobial, antioxidant compounds which can be used as medicine against viral, bacterial or helminths causing diseases as reported as anticancer by Ravi Shankar *et al.* (1996). More studies have to be conducted to know the galls of Ficus to know its pharmaceutical importance for human being and in animal husbandry for the betterment of society.

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REMEDIATION OF TEXTILE DYES (DIRECT RED AND ACID ORANGE) BY FRESHWATER CYANOBACTERIA

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Abstract:

Textile dyes represent a large group of organic substances that impose undesirable effects on the environment. An emerging ecological concern is the effluents from the textile industry and the dyeing-related processes that were not conveniently treated before they are introduced into the natural environment reaching reservoirs and water bodies. However, depending on the dye type and the application, the final stage of the dye can significantly contribute to the release of waste of several chemical substances with variable composition. Cyanobacteria have a wide and varied impact on natural ecosystems and can act causing beneficial or noxious effects to the health of humans and animals. Compared with some physical, chemical treatment the biological treatment has high significance due to its cost effectiveness and eco-friendliness (Thajuddin, 2005; Singh *et al.*, 1969). Among this the marine Cyanobacteria have a unique function system in the removal of colours from textiles. The present study details with the decolourisation of textile dyes (Direct red and Acid Orange) by freshwater Cyanobacteria. Maximum decolourisation takes place in case of direct red dye compared to acid orange dye at different concentration and conditions. Microscopic and viability studies were performed along with the effect of respective dyes on the pigments of Cyanobacteria was studied. Decolourisation rate of both the dyes increased upon treatment with starch and sodium chloride. Hence the given study showed that freshwater Cyanobacteria is an effective, economical alternative for the degradation of the respective textile dyes.

Introduction:

The textile dyeing industry, responsible for dyeing various types of fibre, stands out among all the industries causing high-pollution. Dyeing process involves washing in baths to remove excesses of the original or hydrolysed dyes not fixed to the fibre are the last operation of

all the steps whatever dye may be chosen. The use of the activated sludge treatment in the effluent treatment plants, leads to the addition of the dyes in the water bodies. Thus it is incapable in removing the toxicity and colouring of some types of dye. The dye reduction can occur in two steps in an aquatic environment: 1) The application of reducing agents to the newly-dyed fibers to remove the excess unbound dye, which could lead to "bleeding" of the fabrics during washing, and 2) in order to make the effluent colorless and conform with the legislation the use of reducing agents is done in the bleaching process,. This procedure leads to the formation of some mutagenic compounds.The human and environment health and its well-being is severely affected due to the liberation of untreated textile effluents into the environment. The incomplete fixation of the dyes during the textile fiber dyeing step is the major source of dye loss.These effluents are complex mixtures consisting of many pollutants, associated pesticides and heavy metals. Effluents containing a high organic load and biochemical oxygen demand, low dissolved oxygen concentrations, strong color and low biodegradability end up in the water bodies affecting the photosynthesis and oxygenation processes of the water body, for example by blocking the passage of sunlight through the water.A very important role in decomposition and ultimate mineralization of biopolymers and xenobiotics like dyes is played by the microorganisms (Lie *et al*., 1998). Reviews are available which have described in detail the use of microorganisms (bacteria, fungi) for dye decolorization as available from many literature (Banat *et al.,* 1996, O,Neil *et al.,*1999, Coughlin *et al.,* 1999, McMullan *et al.,* 2001, Stolz 2001, Bhatt, 2005, Asad *et al.,* 2007, Pandey *et al.,*2007, Khalid *et al.,* 2008).

Biological treatment methods are cheap and offer the best alternative for physicochemical processes due to their cost effectiveness, production of less sludge and environmental benignity (Chen *et al*., 2003). Options for the biological treatment of textile dyeing wastewater may consist of single phase aerobic, anaerobic processes or multiphase systems combining both aerobic and anaerobic processes (Nosheen, 2010). Some species of algae were even capable of utilizing azodyes as their sole carbon and nitrogen source (Jinqi *et al*., 1992). The biodegradation of azo dyes by the algae (*Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillatoria tenuis*) has also beenassessed. Dried *Spirogyra rhizopus* have ability to decolourize acid red 274 dye by both biosorption and biocoagulation process and the removal amounts decreased while the removed concentration of AR 274 dye increased with increasing *SpirogyraRhizopus* concentration (Ozer *et al*., 2006). The potential of *Cosmarium*sp (green algae) as a viable biomaterial for biological treatment of triphenylmethane dye and malachite green was investigated (Daneshvar *et al*., 2007). Immobilized *Phormidium* sp. has good decolourization activity under thermophilic condition (Sevgi Ertugrul *et al*., 2008). In this paper research work on decolourisation of textile dyes (Direct Red and Acid Orange) by freshwater Cyanobacteria has been discussed.

Materials and Methods:

To culture Cyanobacteria water samples were collected from Kolkata and its outskirts. Water samples were taken from stagnant greenish coloured ponds. The BG11 and Cyanophycean media was prepared, autoclaved, cooled and pH maintained at 7.5. Antibiotic Cycloheximide 50µg/ml was initially added for both liquid andsolid media (Leach, 1947; Obrig, 1971). Water samples collected were taken in tarson centrifuge tubes (15ml) and centrifuged at 2500 rpm for 10mins. The process was repeated a number of times in order to get considerable amount of green pellet. The media were inoculated with the green pelletin 1L conical flasks with respective media maintaining a sterile condition and incubated under white fluorescent light (40W) for 16 hrs of light and 8hrs of darkness at roomtemperature in undisturbed condition for 14-21days.For solid media, Cyanophycean media and BG11were used. The media were solidified with 1.5% agar. Camera lucida (Hammond, 1987) drawings and staining with Cotton blue and Lactophenol was performed with the crude sample as well as the cultured sample (Chung and Bennett, 1992). Stock solution 1g/50ml was prepared for each dye (Direct Red 81 and acid Orange 7) and 10-50mg/l concentrations were prepared for each dye respectively. The experimental set up consisted of sterile BG11 medium (pH7.4), sodium acetate buffer (pH 5.6), dye concentrations prepared and inoculum (metal resistant cyanobacteria).

The control set up contained all the constituents except the inoculum. The control and the experimental set up were incubated for 8days at 16 hrs of light and 8hrs of darkness at room temperature in an undisturbed condition. Absorbance was measured at 507nm for Direct red and 482nm for Acid Orange spectrophotometrically to calculate % of decolourisation. (Blank=Medium without dye and inoculum; Control=Medium with dye but without inoculum)

The chlorophyll was measured at 665nm (Knudson *et al*., 1977). For carotenoid estimation, absorbance was measured at 470nm (Lichtenthaler method). Staining of the dye treated Cyanobacteria was performed by Cotton blue-Lactophenol. Factors such as starch (0.65g/l amd 1.3g/l) and Sodium chloride (2g/l and 4g/l) were added to the medium to study whether the rate of decolourisation was increased or decreased. To analyse the viability of the dye treated Cyanobacteria the Ethidium Bromide/ Acridine Orange staining (following the method of Mario Roderer, 6/02; Spector *et al*., 1998) was performed.

Results and Discussion:

Culture and Isolation of Cyanobacteria:

BG11 showed profuse growth compared to other media. Growth in solid Cyanophyceanmedia occurred after an incubation of more than 45 days. The cultures on solid media was maintained by repeated streak plate and spread plate methods. Antibiotic

Cycloheximide (Himedia) 50µg/ml was added to both liquid and solid media initially to inhibit the growth of eukaryotic organisms such as diatoms, green algae and fungi (Leach, 1947; Obrig, 1971) Later on the use of antibiotic was discontinued once cyanobacterial genera were obtained.(Nagle *et al.*, 2010) Upon staining with Lactophenol Cotton blue under low and high power objectives, irregular compact or loose aggregated colonies were observed under the microscope (depending on the media type). Cells in the colonies were embedded in mucilage and gelatinous sheath. Filamentous and colonial forms of Cyanobacteria were observed after 14-21 days of culture. A number of genera such as *Microcystis, Nostoc, Anabaena, Oscillatoria, Lyngbya, Gloeotrichia, Merismopedia* along with some diatoms, green algae were observed in the crude water sample. Further experiments were performed with filamentous sample.

Analysis of Decolourisation of Textile Dyes Direct Red and Acid Orange:

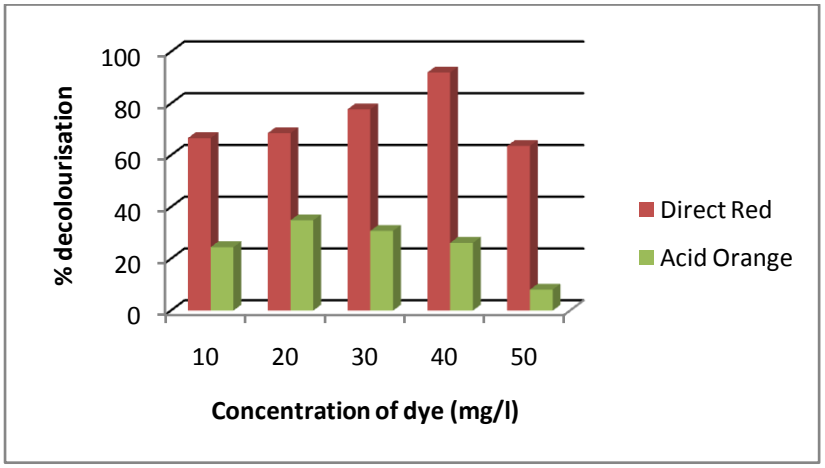


Figure 1: Decolourisation of Textile dyes by Cyanobacteria

Fig. 1 shows maximum decolourisation at 40mg/l and minimum at 20mg/l for Direct Red dye while in case of Acid Orange maximum decolourisation occurred at 20mg/l while minimum at 50mg/l.

Staining of the sample (S1- 10mg/l) and (S5-50mg/l) by Cotton blue and Lactophenol:

The Cotton blue and Lactophenol staining showed that the filaments of Cyanobacteria was intact but the mucilaginous sheath was less dense when treated at 10mg/l of both the dyes but with the increase in concentration of the dye, at 50mg/l the filaments were fully fragmented, decreased in number, along with the disappearance of the sheath after 10 days.

Effect of Direct Red and Acid Orange on Chlorophyll and Carotenoid of Cyanobacteria after 8 days:

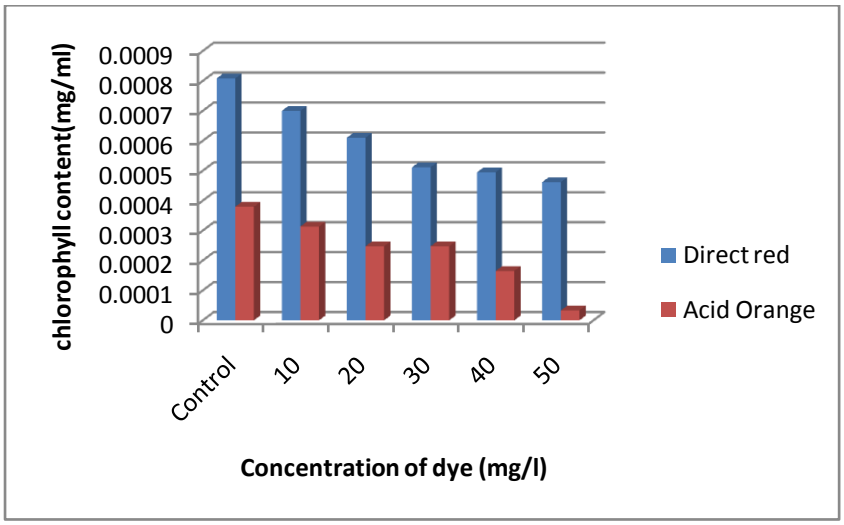


Figure 2: Effect of Textile Dyes on Chlorophyll content

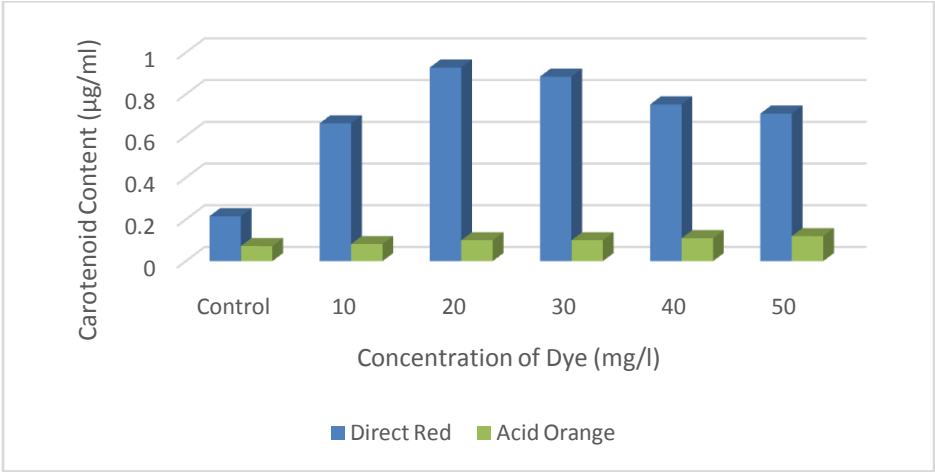


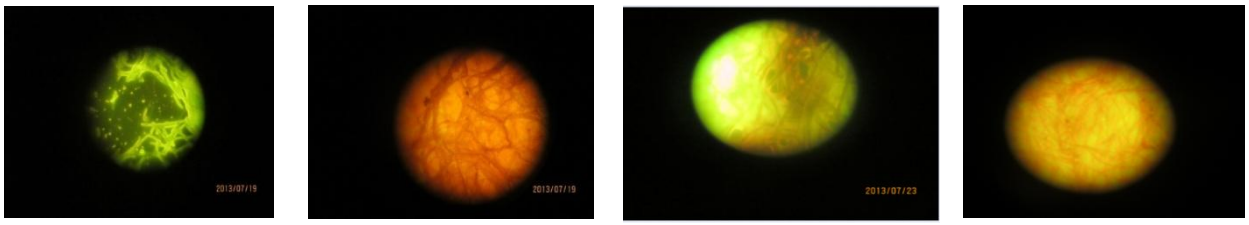
Figure 3: Effect of Textile Dyes on Carotenoid content

The bar diagram (Fig. 2) shows that there is a decrease in the chlorophyll content with the increasing concentration of both the dyes but chlorophyll was affected much more by Acid Orange compared to Direct Red dye. The bar diagram (Fig. 3) shows an increase in carotenoid when treated with Direct Red dye while it was severely affected by the Acid Orange dye. Therefore Direct red is not so toxic to aquatic life as Acid Orange.

Effect of Starch and NaCl in the decolourisation of Direct red and Acid orange:

Upon addition of starch there was an increase the decolourisation rate for both the dyes compared to that of control, maximum being at 0.65g/l of starch while addition of NaCl showed maximum decolourisation at 4g/l in case of both the textile dyes compared to that of control.

Viability test: Ethidium Bromide and Acridine Orange Staining



a **b** **c** **d**

Figure 4: a. DS1-10mg/l; b. DS5-50mg/l c. AS1-10mg/l d. AS5-50mg/l

The (Fig. 4a) refers to the live filaments of Cyanobacteria(green) upon treatment with 10mg/l direct red dye while the (Fig. 4b) refers to the dead(red) cells of Cyanobacteria treated with 50mg/l concentration of Direct Red. (Fig. 4c) also shows live cells and some dead cells of Cyanobacteria treated with 10mg/l of Acid Orange dye while at 50mg/l most of the cells were dead(red) (Fig. 4d). It can be inferred that at highest dye concentration (50mg/l) the cells were all fragmented as seen after 10 days and most of the cells die which may due to the formation of toxic compounds produced as a result of dye degradation which led to a decrease in decolourisation rate.

Thus from the above study it could be concluded that the freshwater filamentous Cyanobacteria used was able to decolourise pure textile dyes. From the reference of Priscila it has been shown that filamentous. Cyanobacteria can degrade the dye more efficiently as it can penetrate the filament which increases the contact between the organism and the compound.. Moreover the decolourisation rate of Direct Red was more than that of Acid Orange which may be due to the molecular structure of the dye, difference in adsorption to cyanobacterial cells and rapid degradation of the dye (Fekry *et al.*, 2016). Carotenoid content increased while that of chlorophyll decreased upon treatment with textile dyes. This may have played a role in the protection of the cell. Acid Orange was found to be more toxic than Direct Red dye.Thus the study showed that the isolated freshwater filamentous Cyanobacteria is an effective, economical alternative for the degradation of the textile dyes, Direct Red and Acid Orange.

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ORCHID DIVERSITY: ITS CONSERVATION AND SUSTAINABLE UTILIZATION

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Abstract:

Number of species of plants and animals present in a region is known as species diversity. In each ecosystem, maintenance of a wide diversity of species is a necessary task to preserve the web of life that sustains all living things. Orchidaceae is the largest and most diverse family of the flowering plants, consisting of more or less than 35,000 species under 800 genera. They are commercially grown worldwide as cut flower and potted plants with 8% share in floriculture trade. Due to its diverse colours, shapes, forms and long lasting blooms it is considered outstanding in the ornamentals. Whether or not interspecific hybridization is important to initiate the diversity in Orchidaceae is a controversial matter. Due to their uniqueness of shape, diversified color and exceptionally long shelf life provide a strong aesthetic value. Evident by recent increases in world floriculture trade, orchids became the second most popular cut flowers as well as potted floriculture crop with high market value. Several orchidaceous photochemical constituents are employed for a variety of therapeutic uses in traditional system of medicine. Major threats include illegal harvesting, habitat destruction and complexity in their life histories orchids are thought to be vulnerable to the effects of global environmental change. Incidentally conservation of orchids and their large scale production by modern techniques such as tissue culture have become inevitable to meet the increasing demand of the community.

Keywords: protocorm, protocorm-like-body, germplasm, cryopreservation.

Introduction:

Orchids are commercially grown worldwide and it is considered outstanding ornamental plants because of its diverse shapes, colours, forms and long lasting blooms. The family Orchidaceae includes 800 genera and 25,000 species (Ng and Hew, 2000). As one of the most species rich families of flowering plants, the Orchidaceae is well known for its immense

diversity and broad global distribution. Yet, orchids have all the earmarks of a group in active evolution – species, genera, tribes and subtribes are all difficult to delimit (Dressler and Dodson, 1960).

Among all flowering plants orchids occupy top position and marketed as cut flowers and potted plants. The immense floral variations within the species along with their long shelf lives make orchids one of the most important floricultural crops of present day (Dressler, 1993). A complicated floral make up, a specialized pollination mechanism and dependence on a suitable mycorrhizal association for seed germination are some of their adapted features. All orchids are dependent upon symbiosis with specific organisms for their survival and reproduction. Orchids have a worldwide ecological distribution both as an epiphytes and lithophytes in the same habitat (Batty *et al*, 2002). More than 70% of orchid species are epiphytic and mostly inhabit in the tropic (Atwood, 1986). The most fascinating fact that in India alone about 1331 species have been found that are distributed in 186 genera. Among them more than 85 species are endemic. The main orchid- rich belts in the country are Peninsular India, North-Eastern India, eastern and the Western Himalayas. The flowers of orchids have always fascinated botanists and horticulturists who are of various shapes, sizes and colors. Orchids have a mystique that seems to set them apart from most other flowers. They are elegant and almost unreal in their perfection. The economic importance of orchids lies mainly in their ornamental value and horticultural uses. They provide cut blooms which keep fresh for long, make pretty corsages and add to the variety of floral arrangements and potted plants (Laws, 1995). A large number of our native species have been used for long in Europe and the USA as progenitors for the production of some of the famous hybrids and now orchids are no longer just for the rich, as improved horticultural techniques have made mass produced hybrids of some genera one of the best selling pot plants in the world. In addition to their ornamental value, orchids could play an important role in herbal medicine.

History of medicinal orchids:

The medicinal use of orchids was first reported by the Chinese (Bulpitt, 2005). The Chinese legendary Emperor ‘Shen Nung’ suggested the medicinal properties of *Bletilla hyacinthine* and a *Dendrobium* species in his Materia Medica in the 28th century B.C. (Singh and Tiwari, 2007). Acharya Charak, has been crowned as the Father of medicine back 300B.C. He has described the medicinal qualities and functions of 1,00,000 herbal plants and has emphasized on the influence of diet and its activity on mind and body. While most people admire them for their good looks, others have found practical uses for them. The use of orchids for medicine can be recorded back to 3000 years ago. However its utility has declined over the years because not sufficient research has been done to determine their effectiveness and adverse effects. Orchids

are the major source of important secondary metabolites like dendrobine, nobiline, erianin etc. Several medicinally impotant orchids have therapeutic potential as antioxidant, antiangiogenic and antitumour agent. Many of the Orchid species are used in traditional medicine and a wide range of chemical compounds like flavonoids, terpenoids and several alkaloids have been isolated from several epiphytic orchids. Dried pseudo-bulbs of *Malaxis acuminate* serve as important source of 'Astavarga' utilized in the preparation of the Ayurvedic tonic, which has most promoting effect on human health and preventing disease (Govindrajan *et al.,* 2007).

Review and Literature:

A. Germination of orchid seeds: a Prologue

The germination of orchid seeds has long been recognized as difficult and uncertain of attainment. It appeared that there were some environmental factors that crippled the process of germination. Further, it was pointed out that the inherent characteristics of the seeds rendered the germination refractory (Knudson, 1922).

B. Early enquiries on germination:

The process of orchid seed germination escaped beyond detection for many years. Orchid seeds were believed as not to be viable (Bouriquet, 1947) or at least incapable of germination and they multiply by means of bud or gamma-like structures which undergo a series of metamorphoses prior to the formation of another mature plant (Arditti, 1967).

In 1840, Link presented an ambiguous graphical indication for the presence of fungi in root cells of *Goodyera* procera (Yam and Arditti, 2009). Later, universal occurrence of the endophyte within the orchid roots was firmly established after careful examination of the roots of 500 orchid species from all parts of the world and was identified as species of *Nectria* that was further verified by others (Arditti, 1967). Frank first used the term "mycorrhiza" to denote root-fungus and pointed out the possibility of a symbiotic association (Frank, 1892). MacDougal (1899a, b) suggested that the fungus might benefit the plant, but its exact role was still obscure. During 19th century, commercial demand for orchids was increased but the growers had no method for germination of the orchid seeds. When spontaneous seedlings of terrestrial orchids have been observed, they are most often found, close to roots of adult plants John Harris observed that orchid seeds when scattered at the base of the mother plant will germinate (Arditti, and Ernst, 1993). The precise requirements for germination was first revealed by Noel Bernard, who quite coincidentally, during a stroll in the forest, noticed an array of developing seedlings of *Neottia nidus-avis*, and detected that all the seedlings were infected by a fungus. Hereby, he envisaged the possible role of the fungus in orchid seed germination (Arditti, 1967).

C. Period of debate over 'Obligate Symbiosis':

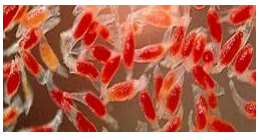
Bernard successfully isolated the fungus from the infected orchid seedlings and advocated that the germination of orchid seeds and subsequent growth of the seedlings took place only upon infection with some strains of fungi those generally found living in the orchid roots (Arditti, 1967). Bernard pioneered a method of germination known as 'symbiotic germination', in which orchid seeds and fungus were co-cultured and this was probably the first ever in vitro technique for propagation (Arditti, and Ernst, 1993). Burgeff (1909) also believed on obligate symbiosis and classified those endophytes as a separate group called Orcheomyces (Burgeff, 1909, 1959, Wynd, 1933). However, Burgeff (1909) failed to recognize the importance of his own experiment in which *Laelio-Cattleya* seeds germinated on 0.33% sucrose solution in the dark; the plants lived for 10 months, beyond which further development was impossible without the fungus either in the light or dark (Wynd, 1933). It was believed that the fungus excreted some enzymes that would digest the starch within the embryo, which in turn, would cause an increase in the cell-sap, thereby inducing the germination and the formation of protocorm. It was also pointed out that the fungus can invert sucrose and this may occur in the embryo (Bernard, 1909). The partisans of obligate symbiosis (Ramsbottom, 1922) believed symbiosis to be a pre-requisite for "normal" development of orchids. Also, they advocated that the requirement of fungal strain is stringent (Burgeff, 1909).

Materials and Methods:

Pollination and capsule collection:

At first anther cap of an orchid flower was removed with the help of a needle and then pollinium was taken out and transferred to the sticky stigmatic surface of another flower, whose pollinium was already removed. After this, the pollen receiving flower (female parent) was labeled with a tag containing the date of pollination and the name of the pollen-donor species. The cross-pollinated flower was then left as such for the development of capsule. This procedure was applied for both siblings (crossing two plants of the same species) and hybrids (crossing of two plants of different species). After several months the undehisced fruits were harvested while they were still green and immediately taken to the laboratory for the culture of seeds. The age of the fruits of each species and hybrid would be mentioned individually in later sections.

Determination of seed viability:



The viability of the seeds was tested with 1% (w/v) TTC (2, 3, and 5-triphenyl tetrazolium chloride) solution, as described by Shivanna and Johri[21] for the angiosperm pollen grains. The solution was prepared in

0.15M Tris-HCl buffer (pH 7.8) and stored in a dark bottle under refrigeration. The test was performed on cavity slides. First, a thin film of petroleum jelly was applied around the cavity of the slide and a drop of TTC solution was added inside the cavity. The seeds were then added in the solution and a cover glass was placed above. Care was taken to prevent entrapment of air bubbles inside the cavity chamber. Three replica slides are prepared for each fruit sample. For each fruit sample three replicate slides were prepared. The slides were then placed inside a pair of Petri plates lined with moistened filter paper and kept under dark condition at 30 ± 2^0C for 48 hour.

Culture establishment and incubation:

The pods were harvested from the plant and the capsules were rinsed with tap water for about 30 mins. Then the capsule was soaked in 0.1% (w/v) mercuric chloride solution for 15 mins and surface disinfection was done by rinsing the capsule in 95% (v/v) ethanol for 60 seconds. Surface sterilized capsules were dissected aseptically with a scalpel followed by swift passing of the capsules through the flame. Finally the seeds were taken out and sown on the surface of semisolid culture media. At 25 ± 2^0C and exposure of 10h photoperiod provided by Philips white fluorescent lights of 3000 lux intensity the cultures were maintained.

Results and Discussion:

Conservation Strategies of orchid germplasm:

Despite the many-fold utilities the commercial cultivation of orchids was considered as highly difficult, due to the lack of knowledge on orchid seed germination as well as their very slow rate of vegetative propagation. Therefore to meet the commercial demand for the orchids, illegal collection of the plants from their natural habitats became a common practice with many traders. As a result, a large number of orchids have become rare, threatened or endangered in the natural habitat either as an indirect or direct effect of human activities (Batty *et al.*, 2002).

Direct threat to plant survival includes climate change, pollution, habitat loss through clearing and fragmentation of natural habitat for urban development, shifting cultivation and various natural calamities. Moreover for orchid one critical factor undoubtedly is the decrease or loss of the fungi, required for seedling development and other life-history stages (Whigham *et al.*, 2006). So it has become an immediate taste to take appropriate measures to conserve such precious orchid germplasm.

Biological conservation aims to maintain the diversity of living organisms, their habitats and the interrelationships between organisms and their environment (Spellerberg and Hardes, 1992). According to IUCN red list of threatened plants, 1779 species of orchids are reported to be threatened with extinction (Walter and Gillett, 1998). The main purpose of the IUCN red list

is to catalogue and highlight those taxa that are facing a higher risk of global extinction (i.e. those listed as critically endangered, endangered and vulnerable). Such categorization is based on biological factors related to extinction risk and includes rate of decline, population size, area of geographic distribution, in India, out of 1100 species. 215 species have been declared as endangered and 14 nearly extinct, which comprise more than 20% of its total orchid flora (Hegde, 1996). There are two conventional approaches for conservation of plant genetic resources, viz., ex-situ and in-situ conservation.

In many cases, *in vitro* technologies are used as alternative strategies for conservation where safety measures of the living collections are ensured. There are 3 basic approaches of *in vitro* conservation, like *in vitro* propagation, *in vitro* storage and cryopreservation

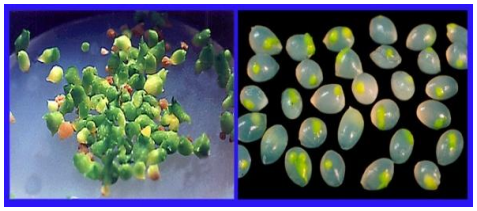
***In vitro* propagation**:

Apart from the difficulties of seed germination, vegetative propagation of orchids an extremely slow process, therefore mass scale production of orchids depends heavily on different *in vitro* methods. Such methodologies could be effectively used for conservation purposes in both direct and indirect ways. Both in vitro seed culture and different clonal propagation methods could be used for commercial production of economically important species.

***In vitro* storage**:

Seeds of many orchid species are regarded as processing orthodox storage behavior (Frank, 1892). Both these methods could be carriedout either using agar medium or through the employment of gel-encapsulation techniques. This approach of in vitro storage is useful primarily because it could be utilized to maintain the valuable germplasm in *in vitro* condition. This approach also could be efficiently implemented in the industrial arena, so that whenever required, the seedling stored at reduced growth condition could be transferred to suitable regrowth media and plantlets could be generated as per the requirement within a short duration (Ramsbottom, 1922).

Cryopreservation:



Seeds shoot meristems. Protocorms and protocorms-like bodies could be stored in liquid nitrogenat -196^{0}C under in vitro condition. During this storage period, all the metabolic processes of the cells and tissues are completely arrested and seed aging is also completely inhibited. Several successful attempts have been made to cryopreserve the cells and tissues of orchids and at present about 20 species of orchid appear as promising for germplasm storage.

Conclusion:

Substantial information is available about the folklore therapeutic useas and phytoconstituents of orchids, but biological evaluation of these plants or of their derived products has not been systematically undertaken so far. Thus, this particular area of research on orchids requires more in depth attention so that the drugs and phytochemicals may be suitably utilized on the basis of modern scientific evaluation. India is expected to boost the production of traditional drugs from the well represented medicinally important orchids. Today, the local as well as tribal populations of north eastern region of India use many orchids as folk medicines and many diseases are cured as orchids are rich in alkaloids, flavonoids, glycosides, carbohydrates, and other phytochemical which have great importance in medicinal fields. Incidentally, conservation of therapeutically important orchids and their cultivation on a large scale using modern techniques such as tissue culture are absolutely essential to meet the increasing demand of the drug industry, otherwise through their excessive use in the manufacture of traditional drugs, such orchids may get depleted.

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ENDOPHYTES OF TEA PLANTS FROM DARJEELING, WEST BENGAL

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Abstract:

A total of 14 bacterial and 6 fungal endophytes were isolated from Tea plant i.e. *Camellia sinensis* grown in famous Tea garden Happy valley, Darjeeling District, West Bengal, India. Endophytes are a group of microorganisms that grow within the tissue of higher plants and colonize them without causing any noticeable injury to the host. Both bacteria and fungi are considered as endophytes. Endophytes represent a potential hub of novel bioactive compounds such as antibiotics, anticancer, and other biological control agents. The bacterial population showed a high level of growth hormone production namely auxin and gibberellins to the level ranging from 160 to 300 µg/ml and 172 to 383 µg/ml respectively. None of the strains were found to solubilize phosphorus and fix nitrogen. The bacterial population also showed antimicrobial activity against human pathogenic strains such *as Escherichia coli, Vibrio cholera, Klebsiella sp. Pseudomonas aeruginosa, Acinetobacter baumannii* and *Burkholderia sepsia*. Most of the fungal genera showed amylolytic and proteolytic activity. Thus, the study suggests that these microbes have huge potential to synthesis of numerous novel compounds that can be explored in pharmaceutical, agricultural and other industries.

Keywords: Endophytes, Growth Hormones, Auxin, Gibberlins, antimicrobials, Tea plant

Introduction:

Endophytes are microorganisms that ubiquitously colonize the internal tissues of plants without causing any negative effects, and some endophytes are able to control plant pathogens and promote the growth of plants (Santoyo *et al.,* 2016; Kandel *et al.,* 2017; Wei *et al.,* 2018). They are considered as endosymbiont (Mukhopadhyay and Chakraborty, 2019). They enter in the plant body either through root or aerial parts (Kobayashi and Palumbo, 2000). Initially they remain localized and then spread in different tissues. During endophytes

colonization the microorganisms resides in almost every internal part of plant ranging from tissues of the underground roots to stem, leaf, flower, fruit and seed (Strobel and Daisy 2003).

Endophytic bacteria promote host plant's growth through direct mechanisms by producing plant hormones like IAA, gibberellins or indirectly by inhibiting plant pathogens (Kumar *et al.,* 2016). Natural products from endophytic bacteria have been observed to inhibit or kill a wide variety of harmful disease-causing agents including bacteria, fungi, viruses, and protozoans that affect humans and animals (Lodewyckx, 2002).

Camellia sinensis commonly known as tea is a herbaceous plant of Family Theaceae cultivated in South Asia. It plays a very important role in Indian Economy as Tea is the most widely consumed as beverage in the world. Its polyphenolic compounds have been found for medinical properties (Devi and Wahab, 2012). Among the three varieties of tea found in India Assam Tea, Darjeeling Tea and Nilgiri Tea, Darjeeling tea is the most superior variety and having great demand throughout the World. Many Plant growth promoting (PGP) endophytic acitinobacteria are reported from *Camellia sinensis* showing PGP traits like, phosphate solubilization, indole-3-acetic acid (IAA), ammonia, siderophore and chitinase production (Borah and Thakur, 2020). Some endophytes showed direct growth promoting activity in tea plants by enhancing the vegetative parameters such as dry/fresh weight of root and shoot of tea plants in nursery conditions (Borah *et al.,* 2019).

Until a viable alternative can be accessible, the emergence of resistance to antimicrobials requires the constant development of new antibiotics. The actinomycetes isolated from plant parts are reported to produce antmicrobial substances and inhibited many human pathogens (Beiranvand *et al.,* 2017). Not only human pathogens these organisms are also helpful in controlling plant pathogens (Mohamd *et al.,* 2018). Various plant pathogens attack plant tissues which can be outcompeted and inhibited by the residing endophytic fungi with aid of extracellular lytic enzymes like chitinase, protease, cellulase etc. production (Choi *et al.,* 2005) which breaks down the plant pathogen cell wall constituting chitin, modified cellulose, starch, as storage material (de Bashan *et al.,* 2005).

Our current study focuses on the isolation of endophytes from important beverage plant - *Camellia sinensis* (Tea) and exploring their potential for plant growth promoters, extracellular enzymes and antimicrobial compounds.

Materials and Methods

Sample collection:

Samples were obtained from Tea plant (*Camellia sinensis*) collected from Happy Valley Tea Estate, Darjeeling, West Bengal, India. The roots, stems and leaves of the collected plants were taken aseptically to the laboratory and kept refrigerated until use.

Isolation of Endophytes:

Surface sterilization of the root, stem and leaves was done with tap water, Tween 20, Sodium hypochlorite, 70% alcohol and sterile distilled water.

Slurry was prepared in isotonic saline solution and then plated in Luria Bertani agar and Czapek Dox agar, incubated at 37ºC and 30°C for consecutive days. Isolated colonies are sub cultured in respective slant and morphology and Gram nature were determined by standard method.

Gibberellin production assay:

Gibberellic acid was estimated by (Borrow *et al.,* 1955). The isolates were grown in Nutrient broth for 4 days then were centrifuged at and the supernatant was used for extraction of Gibberellin. pH of the supernatant was adjusted to 2.8 by 1N HCL and to 1.5ml supernatant 0.2ml of Zinc acetate solution and 0.2ml of Potassium ferrrocyanide solution was added and centrifuged, 0.5ml of supernatant was then added to 0.5 ml of 30% HCL and the mixture was then incubated at 27°C. Absorbance was measured at 250 nm in UV-Vis spectrophotometer and compared to a standard curve.

Auxin production assay:

Auxin was estimated by (Gordon *et al.,* 1951). The isolates were made to grow in IAA production media for 10 days and then centrifuged and the supernatant was used for IAA production. 1 part of the supernatant was added to 2 parts of Salkowsky reagent and incubated for 30 minutes to observe red colour. Absorbance was measured at 530 nm and compared to a standard curve for quantification.

Phosphate solubilization:

The isolates were streaked onto plates containing the Pikovskaya's agar medium and were incubated for 7 days at 28°C.

Nitrogen fixation:

In order to screen the bacterial isolates for nitrogen fixation ability, they were made to grow on slants of glucose nitrogen free mineral media. Slants were prepared of the mentioned agar and isolates were streaked onto it and incubated at 28°C for 7 days.

Antimicrobial assay:

Isolated bacterial strains were cultured in Luria bertani broth for 5 days then were centrifuged and the filtrate was used for the assay. The assay was done by agar well diffusion method on Luria Agar plates containing test pathogenic organisms namely, *Vibrio cholerae, Pseudomonas aeruginosa, Burkholderia cepacia, Acenatobacter baumanii, Klebsiella sp.* and *Escherichia coli.*

Screening of fungal isolates for Extracellular Enzyme production:

a) Cellulase Activity - The fungal isolates were assessed for cellulase activity by streaking on CMC Agar Media(K_2HPO_4-1gm, $MgSO_4$-0.5gm, NaCl-0.5gm, $FeSO_4.7H_2O$-0.01gm,$MnSO_4$-0.01gm,NH_4NO_3-0.03gm, CMC-10gm, Agar-20gm,dis.H_2O-1lit.) and incubated for 5 days. After fungal growth appearance, the plates were flooded with 0.1% Congo red solution for 15 mins with shaking and destained with 1M NaCl solution for 15 minutes. Appearance of clear zones around fungal colony indicated cellulase activity.

b) Amylase Activity - The fungal isolates were assessed for amylase activity by inoculating on Starch Agar Media (Beef Extract-3gm, Soluble Starch-10gm, Agar-20gm, dis.H_2O-1lit.) and incubated at 28°C for 24 - 48hrs . After incubation, the plates were flooded with 1%Gram's iodine solution and observed for the appearance of a clear zone of hydrolysis around the fungal growth.

Results and Discussion:

In the present study, 14 bacterial and 6 fungal isolates resulted from the root, stem, young leaf and mature leaf samples collected from tea garden of Happy Valley, Darjeeling, West Bengal which signifies a diverse amount of residing endophytes in Tea plant. Bacterial endophytes are either rod or coccus in shape and Gram positive in nature. 23 bacterial endophytes were reported from Rice plant were found mostly belong to Gram positive reaction (Mukhopadhyay and Chakraborty, 2019). The bacterial endophytic isolates were microscopically characterized as gram positive rods, which is consistent with those of *Curcuma longa* L.(Kumar *et al.*, 2016). Also, *Aspergillus sp*. are identified as endophytes in *Andrographis paniculata* (Elfita *et al.*, 2015).

Gibberellin is an important plant hormone that regulates various developmental processes, including stem elongation, germination, dormancy, flowering, flower development, and leaf and fruit senescence. All of the 14 isolates could produce appreciable amounts of Gibberellin ranging from 172-383µg/ml (Figure 1). The strains HVR1, HVLY2 and HVLM1 could produce maximum amount of gibberellic acid. Figure 1 shows the amount gibberellin production by the isolates. Ambawade and Pathade (2013) estimated Gibberellic acid production from banana plant endophyte to the levels of 0.240 mg/ml where as in rice plant it maximally produced at 250µg/ml (Mukhopadhyay and Chakraborty, 2019). Giberellic acid produced from Endophytic *Fusarium oxysporum* was reported to affect positively on morphological and physiological parameters in Tomato plant (Rohuma *et al.*, 2020).

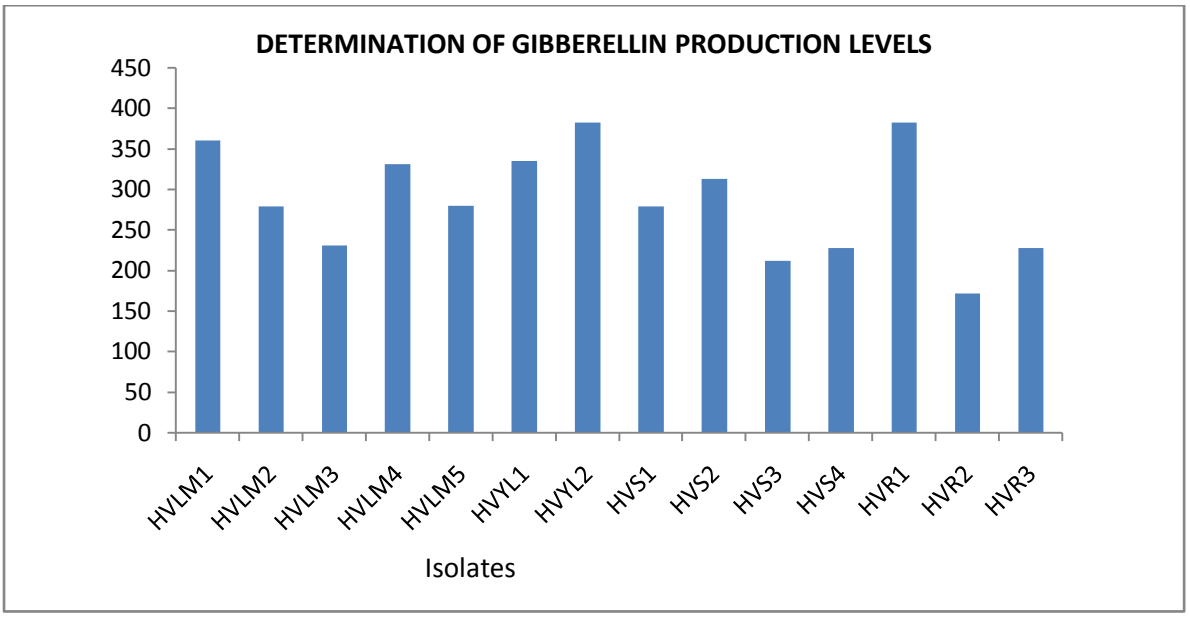

Figure 1: Production of Giberellin by Endophytc Bacerial Isolates

Table 1: Amount of Auxin produced by each isolate

Isolate	Auxin producing ability	Conc. of IAA produced (µg/ml)
HVLM1	+	300
HVLM2	+	200
HVLM3	+	300
HVLM4	+	200
HVLM5	+	300
HVLY1	+	300
HVLY2	+	160
HVS1	+	160
HVS2	+	160
HVS3	+	300
HVS4	+	200
HVR1	+	182
HVR2	+	200
HVR3	+	182

Under natural condition endophytes are reported to promote growth by IAA production (Khan *et al.*, 2020; Mukhopadhyay and Adhikari, 2020). All the isolates produced auxin at

appreciable levels of 160-300 µg/ml which was confirmed by production of red colouration of the supernatant (Table 1). The strains isolated from tea leaf could produce more auxin compared to other parts. Diverse microorganisms including bacteria (Arshad and Frankenberger, 1998; Khalid *et al.*, 2004), filamentous fungi (Kaldrof and Ludwig-Muller, 2000) and yeasts (El-Tarabily, 2004) are capable of producing physiologically active quantities of auxins and which have pronounced effects on plant growth and development.

Table 2: Antimicrobial activity of selected endophytic bacterial isolates against selected test organisms

Endophytic bacterial Isolates	Test organisms	Sensitivity	Diameter of inhibition zone (cm)
HVLM1	*Pseudomonas aeruginosa*	Resistant	-
	Klebsiella sp.	Resistant	-
	Acenetobacter baumanii	Resistant	-
	Vibrio cholerae	Sensitive	1.4
	Escherichia coli	Resistant	-
	Burkholdaria sepsia	Sensitive	1.3
HVLY1	*Pseudomonas aeruginosa*	Resistant	-
	Kleipbsiella sp.	Resistant	-
	Acenetobacter baumanii	Resistant	-
	Vibrio cholerae	Resistant	-
	Escherichia coli	Resistant	-
	Burkholdaria sepsia	Resistant	-
HVS1	*Pseudomonas aeruginosa*	Sensitive	0.6
	Kleipbsiella sp.	Resistant	-
	Acenetobacter baumanii	Resistant	-
	Vibrio cholerae	Sensitive	1
	Escherichia coli	Resistant	-
	Burkholdaria sepsia	Resistant	-
HVR1	*Pseudomonas aeruginosa*	Sensitive	0.8
	Kleipbsiella sp.	Resistant	-
	Acenetobacter baumanii	Resistant	-
	Vibrio cholerae	Resistant	-
	Escherichia coli	Resistant	-
	Burkholdaria sepsia	Sensitive	0.3

Unlike other endophytic organisms (Goldstein *et al.*, 1995; Tonooka *et al.*, 2008) these strains were not efficient in solubilizing phosphate or fixing nitrogen.

Antimicrobial potential of the endophytic bacteria isolate were evaluated against six pathogenic bacteria (*E.coli, Burkholderia sepsia, Acinetobacter baumannii, Kleibseilla sp., Vibrio cholera, Pseudomonas aeruginosa*) (Table 2).

In primary screening 3 bacterial isolates (HVLM 1, HVR 1, HVS1) were found to show antimicrobial activity against 3 pathogenic bacteria out of the 6 and appeared to have a broad spectrum of antimicrobial activity in vitro. Several endophytic isoaltes are reported to produce antimicrobial substances against different pathogens (Hussain and Mustakim, 2017; Morris, 2003; Mukhopadhyay and Adhikari, 2020).

Apart from various primary and secondary metabolites, antioxidants, anticancer agents (Gunatilaka *et al.*, 2006), the endophytic fungi also serve as potent sources of industrially important enzymes with invaluable roles in biotechnology. They extracellularly produce hydrolases like pectinase, lipase, proteinase, amylase, laccase, xylanase etc. to resist pathogen invasion and nutrient acquisition from the host (Sunitha *et al.*, 2013) which are available for mankind with industrial and biomedical potentialities (Strobel *et al.*, 2003).

Of the isolated 6 strains 4 of the isolates could produce protease indicated by the hydrolysis zone in the casein agar medium. Fungal amylases have been widely used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production (Burhan *et al.*, 2003). All the 6 fungal isolates could degrade starch by amylase production, which is shown by significant area of clear zone around fungal mycelial growth (Plate 1).

Plate 1: Fungal isolate showing amylolytic activity

Conclusion:

In conclusion, the endophytes of important beverage plant Camellia sinensis are novel and diverse. They exhibit several Plant Growth Promoting traits that results in improvement of growth and development in tea plants, serve as a resource of medicinally important compounds

against different human pathogens, help the plants in pathogen resistance and play an important role in nutrient cycling as well. Thus, the endophytes are promising sources of various bioactive compounds in agricultural, biotechnological and pharmaceutical fields.

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MORPHOLOGICAL OBSERVATION OF *CHARA SOCOTRENSIS* NORDST F. *PASHANII* (DIXIT) R.D.W. FROM SATARA DISTRICT (MAHARASHTRA)

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Abstract:

Present communication deals with morphological observation of aendemic species of *Chara*viz. *Chara socotrensis* Nordst f. *pashanii* (Dixit) R.D.W. along the foothills of Sahyadri ranges within Satara district.Comparison with the specimen described by R.D.Wood, and that originally described by S.C.Dixitis also made in this account. Present study focused only on endemic species of *Chara* occurring abundantly along the foothills of Sahyadri ranges, their correct identification and correlates their distribution, morphology with that of Indian charophyte flora. In order to update knowledge of taxonomy of the charophytes from Maharashtra this attempt has been made.

Keywords: Chara, Pashanii, Morphology, Satara

Introduction:

Charophytes are the macroscopic green algal forms which have puzzled biologists since long time. Due to their close morphological resemblance with angiosperms, many times they are confused with aquatic higher plants. Evolutionary biologists are paying special attention to these aquatic macrophytes due to their characteristic, morphology, physiology and cytological features. Thorough survey of literature on charophytes reveals that voluminous information is available with us from the world over (Pal, 1962; Wood and Imahori, 1965; Kundu, 1965). In India, the studies on charophytes have been restricted to either North or South region. However meagre information on charophytes is available from Maharashtra State (Dixit, 1935; Kamat, 1965; Jawale, 1986; Patil and Chaugule, 1992; Karande and Chaugule, 1999).

While screening different localities for algal collection it was found that Satara District harbours number of localities where luxuriant growth of charophytes is observed. Of these we

are paying special attention to ecorticate, monoecious species viz. *Charasocotrensis* Nordst f. *pashanii* (Dixit) R.D.W.

Material and Methods:

Plants were collected after the post monsoon shower from the temporary pools, puddles, ditches, permanent water reservoirs, small weirs, constructed dams, back water of dams, foothills of Sahyadri ranges around Satara and within Satara, Morphological observations were made and plants were preserved in 4% formaldehyde solution. Camera lucida drawings and micro-photographs were taken from temporary preparations.Identification was made using monographs by Wood and Imahori (1965), Zaneveld, (1940), Pal and Kundu (1960) and D. Subramanian (2002) and recent publications.

Observations:

In our survey of charophytes from Satara district *C. socotrensis* f. *pashanii* occurred at wide localities and was abundant in its occurrence around Satara and within Satara district than other species of charophytes. This species was collected from following localities:

Table 1: Survey localities for study of charophytes from Satara district

Sr. No.	Taluka of Satara district	Name of Localities
1.	Wai	Kavathe,Ozarde
2.	Jawali	Medha
3.	Koregaon	Jarandeshwar, Satararoad
4.	Mann	Pingali Lake, Dahiwadhi, Rajewadi
5.	Karad	Masur
6.	Satara	Godoli, Parali,Pateghar,Pateshwar, Degoan,Urmodi Dam, Ajinkaytara fort, Kas

***Charasocotrensis* f. *pashanii* (Dixit) R. D. W.:**

Plants monoecious, 4 – 15 cm. high, Stem slender, erect, stout, 234 - 460 µm in diameter; internodes 0.5 – 2 cm long 1 – 3 times as long as branchlets. Stipulodes present in 1 tier but rudimentary. Branchlets 10 – 12 in a whorl, 0.7 to 2 5 cm. long; 2 – 5 segments; terminal segment one celled conical, acute, the lower one to two segment short and curved.Cortex entirely absent. Bract cells present only at fertile nodes 2, 115 – 180 µm long. Bracteoles 2, shorter or nearly equal to the mature oogonium 215 – 420 µm long.Gametangia conjoined and aggregate at lowest 1 – 2 branchlet nodes, usually 2 antheridia below 1 – 2 oogonia. Oogonia 1 – 2 together.Oogonium 360 – 805 µm long, 175- 530 µm broad (incl. coronula), 400-820 µm long

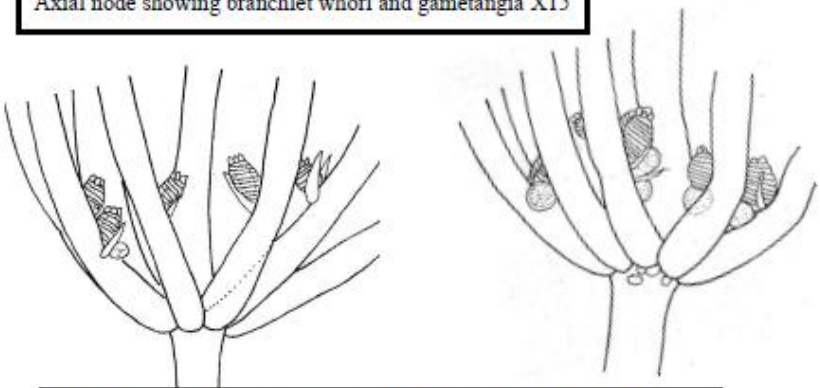
200 -580 um wide (excl. coronula) convolutions 9; coronula 60 – 150 µm high, 146 - 175 µm wide. Oospores orange to black in colour 215 -270 µm long, 210 – 270 µm wide; striae of 8 – 10 prominent ridges; fossa 58 µm across; Antheridia 205 – 265 µm in diameter; octosutate.

Results and Discussion:

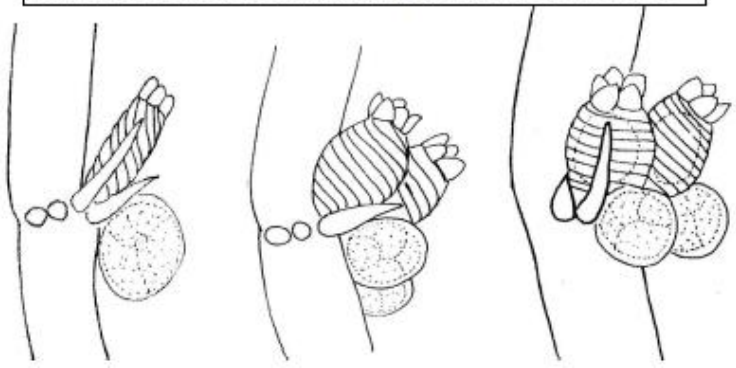
1. The plants always occurred along the margins of pools, puddles and on wet mud where the soil was rich in calcium.
2. *Chara socotrensis* f. *pashanii* is endemic taxon of the complex having very restricted distribution in the foot – hills of the Western Ghats (Chaugule and Patil, 1992). Our collection revealed that f. *pashanii* also has very restricted distribution.
3. Compared with the specimen described by R.D.Wood and that originally described by S.C.Dixit most of our specimens showed some distinguishing features like downwardly growing corticating threads running over the main axes and presence of stipulodes.
4. Our observations after screening of large number of specimens revealed that there is tendency of forming corticated threads in *Chara socotrensis* f. *pashanii*. The cortication may be said vestigial or imperfect but definitelythere is tendency of cortication in these plants.

Plate 1

Axial node showing branchlet whorl and gametangia X15



Branhlet node with conjoined gametangia, bracts and bractioles X50



Oospore X50

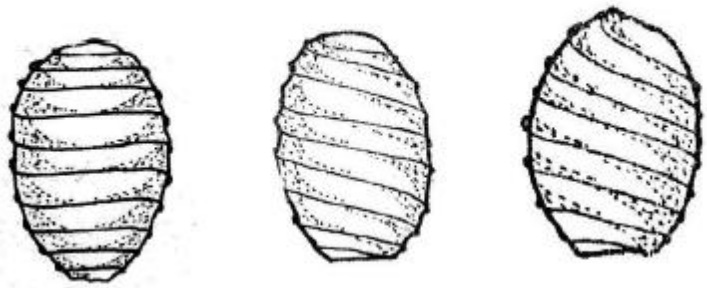
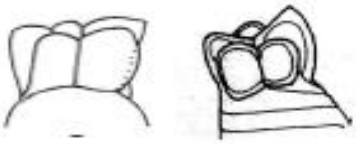


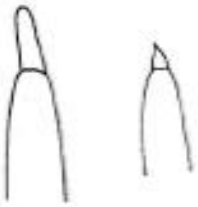
Plate 11

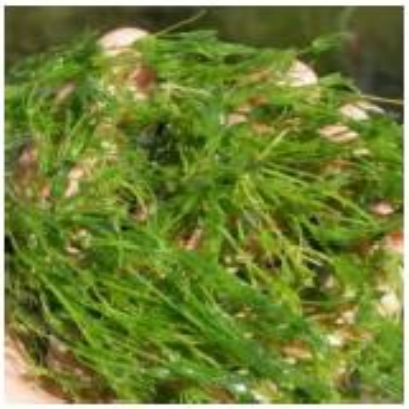
Mature Oogonium X50

Coronula with spreading cells X 100



Tip of branchlet X 50





Habit

Entire Plant

Gametangia

Comparative account of *Charasocotrensis* f. *pashanii* (Dixit) R. D. W.

Sr. No.	Character	R. D. Wood ' 65	Satara. Specimen
1.	Habit	Monoecious, c 16 cm. long	Monoecious, 4 – 15 cms long
2.	Axes (diameter)	200 -400um	Slender, stout 234 -460 µm in diameter
3.	Internodes	Elongated	0.5 - 2 cm shorter than branchlet
4.	Stipulodes	Absent	Rudimentary in 1 tier.
5.	Cortex	None	Absent
6.	Branchlets Number Length Segments	 8 -10 in a whorl 2 cm long 2 – 3	 10 -12 in a whorl 0.7 – 2.5 cm long 2 – 5
7.	Bract cells	Only at lowest nodes	2 , only at fertile nodes
8.	Bracteoles	Not mentioned	2, shorter or nearly equal to mature oogonium
9.	Gametangia	Conjoined and aggregated at 1 – 2 lowest nodes usually 2 antheridia	Conjoined, geminate at lowest 1 – 2 nodes, branchlet nodes.
10.	Oogonia Length Breadth Convolutions	2 – 3 together 525 – 615 µm 460 – 480 µm 8 – 10	1 – 2 together 360 – 805 µm 175 – 530 µm 9
11.	Coronula Height Width	 85 – 95 µm 170 – 180 µm	 73 – 100 µm 146 – 175 µm
12.	Oospore Colour	Dark brown to black	Orange to black
13.	Length Breadth Ridges Fossa Membrane	420 – 450 µm 270 – 310 µm 8 – 10 53 µm Obscurely granulate	215 – 270 µm 210 – 270 µm 8 – 10 58 µm Not seen
14.	Antheridia (diameter)	180 – 220 µm octoscutate	205 – 265 µm octoscutate

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BIODIVERSITY OF VESICULAR ARBUSCULAR MYCORRHIZAL (VAM) FUNGI IN *TECTONA GRANDIS* TREES OF FOUR SELECTED DISTRICTS OF ASSAM, INDIA

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Abstract:

Roots and rhizosphere soil samples of *Tectona grandis* trees have been collected from four different districts of Assam namely Jorhat, Golaghat, Nagaon and Karbi Anglong to study the biodiversity of vesicular arbuscular mycorrhizal (VAM) fungal colonization and spore population. From the study, it is observed that vesicular arbuscular mycorrhiza (VAM) are present in all soil and root samples of *Tectona grandis* collected from the said districts. Vesicular colonization responsible for the transfer of phosphorus and other minerals from the soil to the plants are observed in all 4 plant samples. Study shows that pH plays an important role in VAM colonization and spore population and thereby the growth of *Tectona grandis*. Highest growth of *Tectona grandis* is observed in the soil pH ranges from 5.0 to 8.0. High abundance of VAM of *Tectona grandis* is observed in sample of Karbianlong followed by Nagaon, Golaghat and Jorhat.

Keywords: *Tectona grandis*, VAM, colonization, spore population, pH

Introduction:

*Tectona grandis (*teak, Assamese name Segwan) is one of the most valuable timbers yielding plant in the world with predominant distribution in tropical or sub-tropical countries like India, Myanmar, Thailand etc. Large scale production of teak is observed in some Indian states like Assam, Bihar, West Bengal, Orissa, Andamans, Madhya Pradesh, Gugarat, Rajasthan, Andhra Pradesh etc. In Assam the highest population of teak is observed in the districts like Karbi Anglang, Nagaon, Golaghat, Jorhat, Dibrugarh, Sibasagar etc. The demand of teak in Assam is very high as it has a good commercial value. It is considered as class 1 timber used for

exterior construction, furniture etc. because of its moderate weight, appropriate strength, dimensional stability and durability, easy workability and finishing qualities and most appealing grain, texture, colour and figure. Most of the parts of teak are used in different applications. For example flowers are considered useful against a number of diseases such as biliousness, bronchitis and urinary discharges, leaves are used in indigenous medicine and their extract indicates complete inhibition of Mycobacterium tuberculosis[1]. The leaves can also be used for the preparation of dye which can be used for dyeing of silk, wool and cotton[1]. The leaves are occasionally used as plates for dining purposes; the bark is regarded as an astringent and considered useful in bronchitis[1].

Various valuable compounds have been isolated and identified from the wood, bark, root and leaves of the tree. Activated charcoal can be prepared from its saw dust. Though teak is considered to be very essential plant species having good commercial value, this plant species is becoming endangered in Assam. So to main biological diversity and considering the economic importance the plant cultivation and preservation is utmost important. From study it has been observed that Vesicular Arbuscular Mycorrhizal (VAM) fungi plays an important role in the growth and thereby productivity in terms of economic importance. Vesicular Arbuscular Mycorrhizal (VAM) is a fungus which has the ability to increase phosphorus level in soil required for the growth of cells of the plant[2]. VAM also provides great strength to teak to resist disease germs in unfavorable weather conditions. The present study is therefore targeted to take the following objectives for consideration.

Objectives:

1. Collection of root and soil samples from 4 different districts of Assam namely Jorhat, Nagaon, Golaghat and Karbi Anglang.
2. Determination pH, organic carbon, phosphorus (P) and potassium (K) of soil samples
3. Analysis of soil and root samples for identification of VAM
4. To relate the growth of VAM with respect to pH

Materials and Methods:

Collection of Roots and rhizosphere soil samples of *Tectona grandis* trees:

Collection of fine roots and rhizosphere soil samples of *Tectona grandis* trees have been collected from four different sites of four districts of Assam namely Jorhat, Golaghat, Nagaon and Karbi Anglong to study the biodiversity of vesicular arbuscular mycorrhizal (VAM) fungal colonization and spore population. These samples were collected during January-February, 2019. The collected samples were taken in Sterilized polythene bags, labeled and transported to the laboratory in an insulated container.

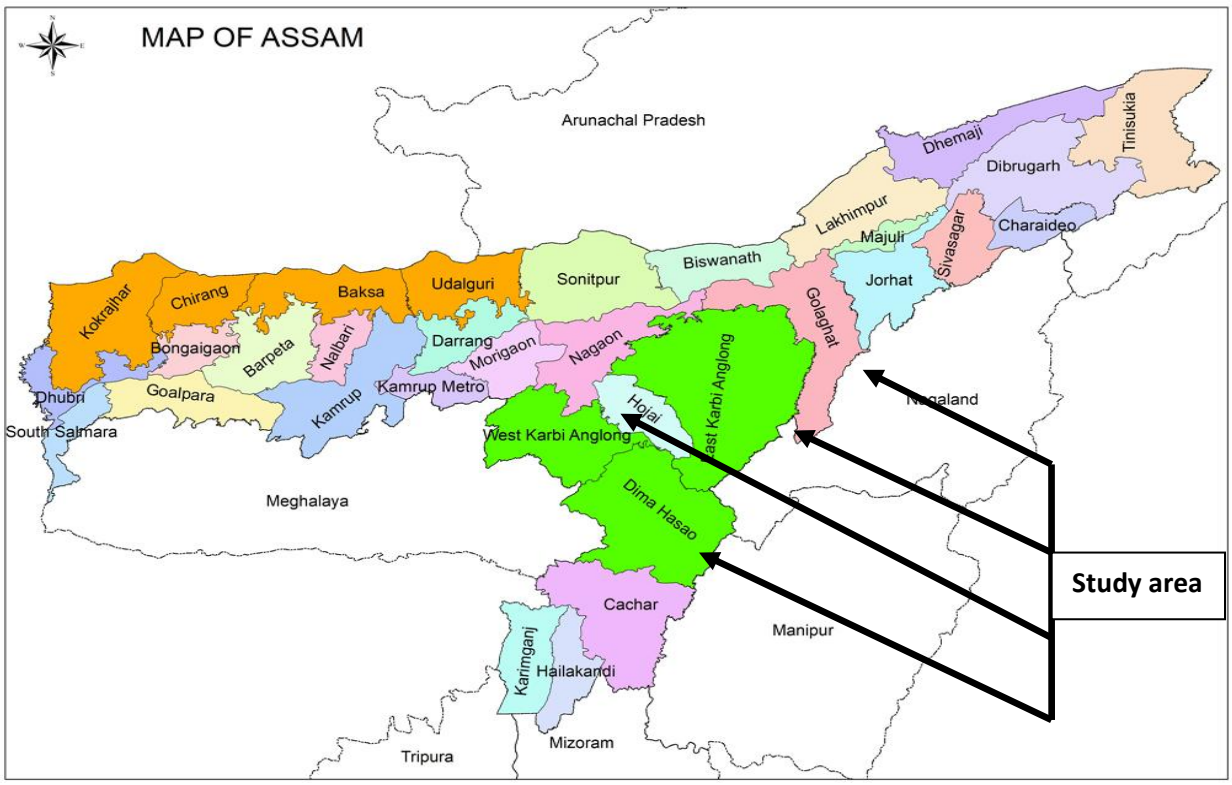


Figure 1: Location map of the study area

Rhizosphere soil samples were collected at the depth of 4-16 cm. These samples were naturally air dried for further experimental analysis. Before processing, all the samples were sieved to remove stones, coarse roots etc. The roots were preserved and later on stained for determination of percent mycorrhizal colonization.

Figure 2: Soil samples

Figure 3: Root samples

Isolation of VAM spores from rhizosphere soil mixture:

Spores were isolated from root rhizosphere soil mixture by using the wet sieving and decanting described by standard method. The coarse materials like straw, rocks etc were removed with 2 mm sieve. 100 g of air dried soil was placed into a glass container with 1000 ml tap water. The root soil mixture was vigorously mixed with a glass rod for 30 seconds. 10sec

pause enable to settle heavier particles and organic materials. Remaining soil water suspensions were slowly poured a set of 4 sieves.

Figure 4: Isolation of VAM spores from rhizosphere soil mixture

The extracts were washed away and spores were collected from the sieves into petri dish. The final product was filtered using filter paper and VAM was collected in the paper. Spores were identified by Microscope and then separated by a needle. A drop of lactophenol was spread on the centre of a clean dried slide as to hold cover slip. Spores were placed on the mount and the cover slip was placed gently by avoiding air bubbles. Thus prepared slides were labeled and then allowed to dry in a dust free chamber for 3 t0 5 days. Spores were examined by using microscope and photographs were taken.

Qualitative analysis of VAM fungi:

Wet sieving and decanting technique was applied to separate and to collect the VAM fungal spores on filter paper (Whatman No.1) which were then observed under Stereoscopic binocular. These spores were picked through needle and mounted in lactophenol on slide. Polyvinyl lactic acid can also be used as an alternative as mounting medium. All the slides with spores were observed carefully by high resolving microscope for isolation into genera and followed by species identification.

Identification of VAM spores:

VAM spores were identified using standard monographs (Hall and Fish, 1978).

Isolation of VAM from plant roots:

Fine root samples were collected and then washed in distilled water. Roots were segmented into 1 cm pieces and processed separately for determining the mycorhyzal intensity in the roots. The roots bits were boiled with 10% KOH solution for 30 minutes and washed with distilled water. The concentration of KOH depends upon the age and softness of the roots. The root bits were again boiled with 1% HCl for 5 minutes and washed with distilled water. The root bits were boiled in water with trypan blue till 10 mins for smearing. Samples were taken in petri dishes. The samples were smeared in slides and observed under microscope. 10 smeared slides of each sample is prepared for observation. Mycelium was seen in some slides. Same is with the arbascular mycorrhyza.

Determination of pH of soil sample:

The determination of pH of the soil samples were done with standard method. Digital pH meter was used for the same.

Determination of organic carbon, phosphorus and potassium:

The parameters of the soil samples collected from the four districts have been analyzed by standard methods[3].

Results and Discussion:

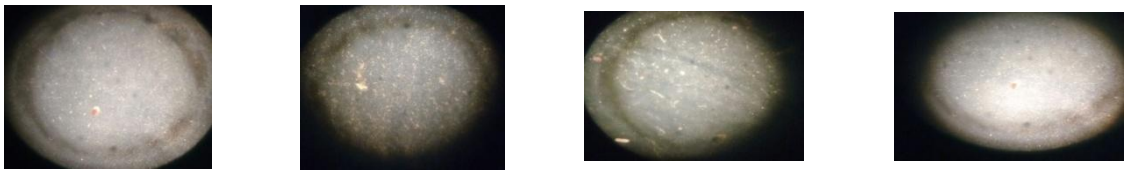
Soil texture plays an important role in the growth of tree. The water retention, total available water capacity, drainage infiltration rate and aeration etc depends on soil texture. From the study it has been observed that sandy clay loam, well-drained soil seems to be more ideal for teak growth[4]. *Tectona grandis* are very sensitive to coarse soil texture and require medium textured soil[4]. Soil pH is considered the most important factor for the growth of a specific plant. From the present study it has been observed that growth and survival of microorganism is greatly influenced by the pH of the environment. Fungi prefer an acidic environment with optimum range being 4 to 8 which means teak growth will be high at this pH range. Besides that the other parameters of soil like organic carbon, phosphorus and potassium are also responsible for the growth of teak. Study shows that VAM colonization responsible for the transfer of phosphorus and other minerals from the soil to the plants and spore population and thereby the growth of *Tectona grandis* is observed in the soils of Karbianlong, Nagaon, Golaghat and Jorhat where the pH ranges from 5.0 to 8.0. The different soil parameters like pH, organic carbon, phosphorus and potassium content of the soil samples collected from Jorhat, Nagaon, Golaghat and Karbi-Anglang have been shown in table 1.

Table 1: soil parameters like pH, organic carbon, phosphorus and potassium content of the soil samples

Sample site (District)	Soil Parameters			
	pH	Organic Carbon (%)	Phosphorus (P) Kg/ha^{-1}	Potassium (K) Kg/ha^{-1}
Jorhat	8.5	1.230	9	439
Nagaon	5.0	0.234	5	402
Golaghat	7.2	0.870	7	413
Karbi Anglong	8.0	1.090	11	420

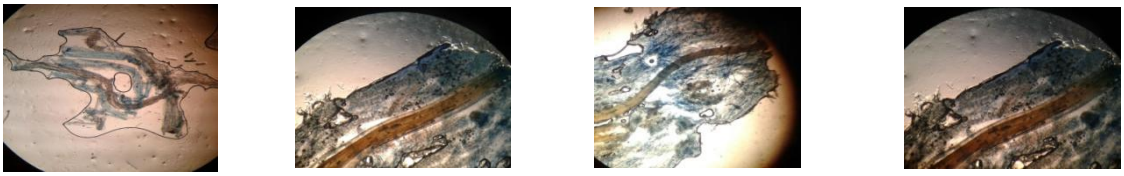
From study, it is also seen that the teak species can grow well in soils having moderate to deep solum, acidic pH, loamy texture and also having appreciable amount of organic carbon, phosphorus and potassium content.

The photograph of VAM spores isolated from soil sample of 4 sites of 4 districts Jorhat, Nagaon, Golaghat and Karbi Anglang respectively is shown in fig. 5.



Jorhat **Nagaon** **Golaghat** **Karbi Anglong**

Figure 5: Isolated VAM spores from root samples



Jorhat **Nagaon** **Golaghat** **Karbi Anglong**

Figure 6: Photograph showing isolation of VAM from teak roots

Conclusion:

Vesicular colonization responsible for the transfer of phosphorus and other minerals from the soil to the plants are observed in all 4 plant samples. Study shows that pH plays an important role in VAM colonization and spore population and thereby the growth of *Tectona grandis*. Highest growth of *Tectona grandis* is observed in the soil pH ranges from 5.0 to 8.0. High abundance of VAM of *Tectona grandis* is observed in sample of Karbianlong followed by Nagaon, Golaghat and Jorhat. Since this plant species is becoming endangered in Assam so to main biological diversity and considering the economic importance, the plant preservation is utmost important. In this regard the NGOs, Governmental organizations and concerned departments have to do work hard to preserve the endangered species.

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BIODIVERSITY ASSESSMENT OF FLORA OF DADA PATIL MAHAVIDYALAYA KARJAT, DISTRICT- AHMEDNAGAR, (MS), INDIA

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Abstract:

A preliminary survey was conducted so as to assess the flora of the college campus. The flora was identified with the help of standard keys, we recorded near Ninety -Three species. The study indicates rich diversity inside the college campus, the reason for this high diversity is proper care of the flora taken in the summer season by the Institute. The climate outside the college campus is harsh and dry. It experiences extreme climate conditions which lead to less diversity. The study area being a part of grassland habitat has fewer evergreen trees and shrubs. Whereas College campus has many cultivated evergreen species of trees. The study indicates that there is a strong correlation between the diversity of flora, perennial sturdy plants and dry, xeric environment.

Keywords: Biodiversity, Flora, Karjat, Xeric.

Introduction:

The term Biodiversity derived from two words, Biology and diversity; it includes biological diversity. Biodiversity is the variety and variation found in flora and fauna; it has the variation of living organisms present in a given ecosystem, which can be aquatic or terrestrial. Biodiversity is based on genetic, specific and ecotype diversity. Biodiversity of an area is totally depending in its climatic factors. Biodiversity is not distributed evenly on all over Earth highest diversity is found in the tropical areas (Gaston, 2013). On the Earth there are major diversity is found as a terrestrial diversity than marine diversity and contain near More than 90 percentage Worlds species diversity was found over terrestrial habitat than 90 percent of the world's species (Young, 2003). Dada Patil Mahavidyalaya Karjat, it is located seventy-five Km away from

Ahmednagar and about 5-6 km away from 'Rehkuri Sanctuari', world famous sanctuary which provides natural habitat for deer and Great Indian Bustard. The area is a grassland area which constitutes floral diversity of grasses primararily. The elevation of district is 649 meters from sea level. The area is having less rainfall, so all area is totally xeric habitat. Dada Patil Mahavidyalaya, was established in the year 1964 by Rayat Shikshan Santha to provide education to the downtrodden; socially and economically backword class and irrespective of caste and religion. Now it will become one of the best leading institutes in Asia. The present study was carried out to study biodiversity in the campus of Dada Patil Mahavidyalaya, Karjat.

The harsh climatic conditions due to law rainfall cause low species diversity in the campus. But to maintain species diversity and to introduce and show plants habitat to the students of Botany and Bachelor of Vocation students the initiative taken by college.

Material and Methods:

Visited the campus periodically and list of plants, habit and compile the data according to their plant types and types of resources of floral diversity in campus. The identification of plants is carried out by using flora of Presidency of Bombay by Theodore Cooke CLE and Flora of Kolhapur District.

Results and Discussion:

From the table 1, it is clear that, the campus is rich in variety of Angiosperm plants than Gymnosperm plants diversity. From the Table 1 and Figure1, it was found that, there are near about 94 plant types and out of these only four percentage are from division Gymnosperms and rest of the diversity is from Angiosperms.According to Figure 2 it was observed that, the diversity of flora from various 52 families and among them a greater number of generas are found from the families like Fabaceae, Apocynaceae, Araceae, Euphorbiaceae, and Moraceae. According to Agarwal, 2002 that due to diversity in climatic conditions in India wide range of diversity was found in areas. It comprises of near about 45000 species of plant types and denotes 7% of World's diversity in India. As per the Figure 3 reported that Biodiversity of Campus flora according to plant Category are distributed as in Herbs, Climbers, 36% Shrubs and 48% trees (Figure 4). Biodiversity of Campus flora according to plant resources are shown, it gives plant distributes higher in Medicinal plants (45%) and Lowest in Vegetables (1%), while others are Dyes (2%), Weeds (4%), Fruits (5%) and ornamentals are 34%.

India is the country of diverse climatic conditions, and it is rich in fauna and flora. India has two hotspots like Himalaya and Western Ghats. Extreme conditions are found in India as high and low rainfall, High and low height from sea level, extreme high and low temperature with variety of soil conditions. According to Mani (1978) India is divided into 10

phytogeographic regions and they show endemism in species. Gudadhe and Niranjane (2020) studied Biodiversity of Malkhed Reserve Forest from Amravati in which they found total 275 faunal species in the forest.

Table 1: Biodiversity in Dada Patil College, Karjat, Campus (List of Plants)

Sr. No.	Name of Plant	Family	Common Name	Plant Type
Gymnosperms				
1	*Auracauria columnaris*	Aracauriaceae	Christmas tree	Tree
2	*Cupressus* species	Cupressaceae	Italian Cupressus	Tree
3	*Cycas revoluta*	Cycadaceae	Cycus	Shrub
4	*Thuja occidentalis*	Cupressaceae	White cedar	Shrub
Angiosperms				
5	*Amaranthus Spinisus*	Amaranthacaea	Kathemath	Herb
6	*Argemone mexicana*	Papaveraceae	Pivladhothra	Herb
7	*Abruspreca torius*	Fabaceae	Gunj	Climbers
8	*Acacia corcinna*	Fabaceae	Shikekai	Tree
9	*Acora calamus*	Acoraceae	Wekhand	Herb
10	*Adalsonia digitata*	malvaceae	Gorakh chinch	Tree
11	*Adhatoda vesica*	Acanthaceae	Adulsa	Shrub
12	*Aloe vera*	Liliaceae	Kumari	Herb
13	*Asperagus racemossus*	Asperagaceae	Shatawari	Shrub
14	*Annona squamosa*	Annonaceae	Custard apple	Shrub
15	*Annona cherimola*	Annonaceae	Ramphal	Shrub
16	*Areca catechu*	Arecaea	Khair	Tree
17	*Alstonia scholaris*	Apocynacaeae	Saptaparni	Shrub
18	*Azadirecta indica*	Meliaceae	Kadulimb	Tree
19	*Bacopa monnieri*	Plantaginaceae	Nirbrahmi	Herbs
20	*Balospermum montanum*	Euphorbiaceae	Danti	Shrub
21	*Bauhunia varigata*	Fabaceae	Orchid tree/ Kanchan	Tree
22	*Bismarckia noblis*	Arecaceae	Fan palm	Herbs
23	*Bixa Orellana*	Bixaceae	Shendri	Shrub
24	*Bombax ceiba*	Bambacaceae	Katesawer	Tree
25	*Bouganvillia spectabilis*	Nyctaginaceae	Kagadiphule	Shrub
26	*Bryophyllum manginii*	Crassulaceae	Panphuti	Herbs
27	*Butea monosperma*	Fabaceae	Palas	Tree
28	*Canna indica*	Cannaceae	Kardal	Herb
29	*Cammiphorum mukul*	Burseraceae	Guggul	Tree

30	*Careyaarborea*	Lecythidaceae	Kumbha	Tree
31	*Catheranthus roseus*	Apocynaceae	Sadaphuli	Shrub
32	*Cissusquadra angularis*	Vitaceae	Kandvel	Climbers
33	*Cocos Nucifera*	Arecaceae	Coconut	Tree
34	*Cordia dichotoma*	Boraginaceae	Bhokar	Shrub
35	*Croton species*	Euphorbiaceae	Croton	Shrub
36	*Cymbopogon winterianus*	Poaceae	Jawa Citronella	Herbs
37	*Dalburgiasisso*	Fabaceae	Indian rose wood	Tree
38	*Diffenbachia sp.*	Araceae	Dumb cane	Herb
39	*Dracaena marginata*	Asparagaceae	Dragon tree	Herb
40	*Dracaena fragrence*	Asparagaceae	Dragon tree	Herb
41	*Dracaena verigata*	Asparagaceae	Dragon tree	Herb
42	*Durantaerecta*	Verbinaceae	Angles whisper	Shrub
43	*Dypsislutescens*	Arecaceae	Areca Palm	Herb
44	*Euphorbia milli*	Euphorbiaceae	Milky weed	Shrub
45	*Ficus bengamina*	Moraceae	Weeping Fig	Tree
46	*Ficus bengolensis*	Moracaea	Baniyan	Tree
47	*Ficus carica*	Moraceae	Anjeer	Shrub
48	*Ficus religiosa*	Moraceae	Pimpal	Tree
49	*Gliricidiasepium*	Fabaceae	Quick stick	Tree
50	Guazumaulmifolia	Sterculiaceae	Bhadraksh	Shrub
51	*Heviabrasiliensis*	Euphorbiaceae	Rubber	Tree
52	*Hibiscus rosachinensis*	Malvaceae	Chinarose	Shrub
53	*Jatropha species.*	Euphorbiaceae	Jatropha	Shrub
54	*Kijeliapinnata*	Bignoniaceae	Bramhanand	Tree
55	*Lantana camara*	Lamiaceae	Ghaneri	Shrub
56	*Lowsoniainnermis*	Lythraceae	Mehandi	Shrub
57	*Madhucaindica*	Sapotaceae	Mahu	Tree
58	*Mangiferaindica*	Anacardiaceae	Mango	Tree
59	*Moringa oliefera*	Morangaceae	Drum stick	Tree
60	*Millingtonia hortensis*	Bignoniaceae	Buch	Tree
61	*MurrayyaKoenigii*	Moraceae	Curry tree	Shrub
62	*Muntingia calabura*	Muntingiaceae	Singapur Cherry	Tree
63	*Nerium oleander*	Apocynaceae	Kanher	Shrub
64	*Nyctanthusarbortristis*	Oleaceae	Night flowering Jasmine	Shrub
65	*Parthenium hysterophorus*	Asteraceae	Congess grass	Herb
66	*Phylanthusniruri*	Phylanthaceae	Bhuiawala	Herb

67	*Pithecellobium duke*	Fabaceae	Blach bead Cumachile tree	Tree
68	*Plumeria Pudica*	Apocynaceae	UltaChapha	Tree
69	*Plumeria rubra*	Apocynaceae	Pandharachapha	Tree
70	*Polyalthialongifolia*	Annonaceae	False Ashok	Tree
71	*Pongamiapinnata*	Fabaceae	Karanj	Tree
72	*Pouteria sapota,*	Sapotaceae	Chikku	Shrub
73	*Pterocarpus marsupium*	Fabaceae	Bibla	Tree
74	*Raulfiniasarpentina*	Apocynaceae	Saptaparni	Shrub
75	*Ravenalamadagascariensis*	Strelitziaceae	Travellers Palm	Herb
76	*Rosa indica*	Rosaceae	Rose	Shrub
77	*Rutagraveloens*	Rutaceae	Satappa	Herb
78	*Santalum album*	Santalaceae	Chandan	Tree
79	*Sapindusmucrosi*	Sapindaceae	Ritha	Shrub
80	*Saracaindica*	Fabaceae	Sitaashok	Shrub
81	*Senna siamea*	Fabaceae	Kassod	Tree
82	*Simarubagluca*	Simaraubaceae	Laxmitaru	Tree
83	*Solanum khasianum*	Solanaceae	Ranwangi	Shrub
84	*Somecarpus anacardium*	Anacardiaceae	Biba	Tree
85	*Tamarindus indica*	Fabaceae	Tamarind	Tree
86	*Tecomella undulata*	Bignoniaceae	Raktarohida	Tree
87	*Tectonagrandis*	Lamiaceae	Teak	Tree
88	*Terminalia arjuna*	Combretaceae	Arjun	Tree
89	*Terminalia belerica*	Combretaceae	Behda	Tree
90	*Terminalia catappa*	Combretaceae	Deshibadam	Tree
91	*Tetomastrans*	Apocynaceae	Yellow bell	Shrub
92	*Treminalia chebula*	Combretaceae	Hirda	Tree
93	*Tribulus terrestris*	Zygophyllacaea	Waghati	Herb
94	*Tridex Procumbance*	Astraceae	Dagadipala	Herb

As the area under cultivation in India, is higher as our county is agricultural rich 70% of population depends upon Agriculture and agriculture related businesses. So, biodiversity conservation strategy was put backwords on the Karjat like areas. According to CBD Secretariete (2001), described Biodiversity as the differentially of varieties and variations among living organismsand of the ecological adaptations they form part of, which includes diversity within each species among terms of each species and that of ecosystems. Giltay *et al.* (2002), giving an opinion that the term Biodiversity is “relative number and abundance of genus, species and communities or ecosystem”. Due to extreme dry conditions, sensitive species are not found in campus. Due to

drought climatic conditions, the plants only survivewhich are adapted themselves to the arid climatic conditions. So, they have more shrubs and trees than climbers. Karjat area has three main seasons: the less monsoon, the cool dry winter, from October to December and the hot dry season from March till the onset of rains. Temperature of Karjat average ranges from 13˚C to a maximum of 30°C- 48°C with the less relative humidity varying from 10-15% to 60- 95%.

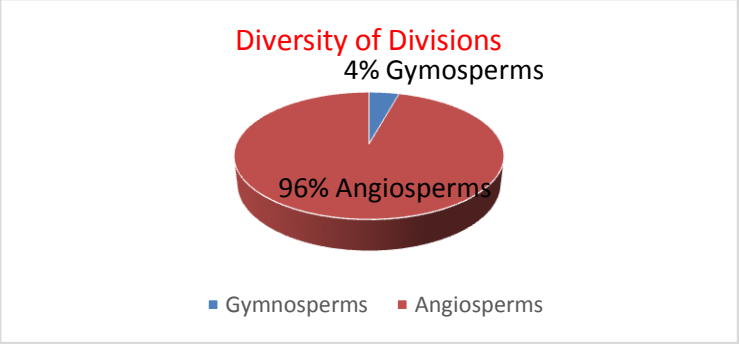

Figure 1: Diversity of Flora with the respect to families

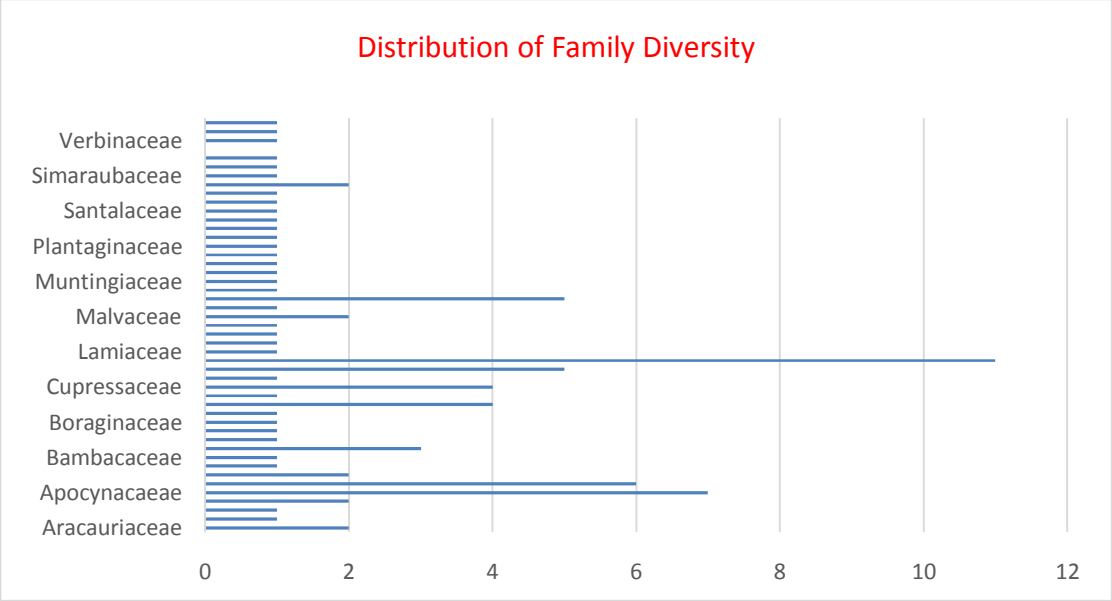

Figure 2: Biodiversity of Campus flora according to plant type

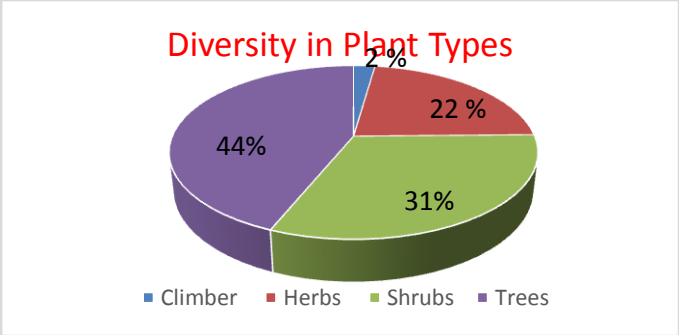

Figure 3: Biodiversity of Campus flora according to plant Category

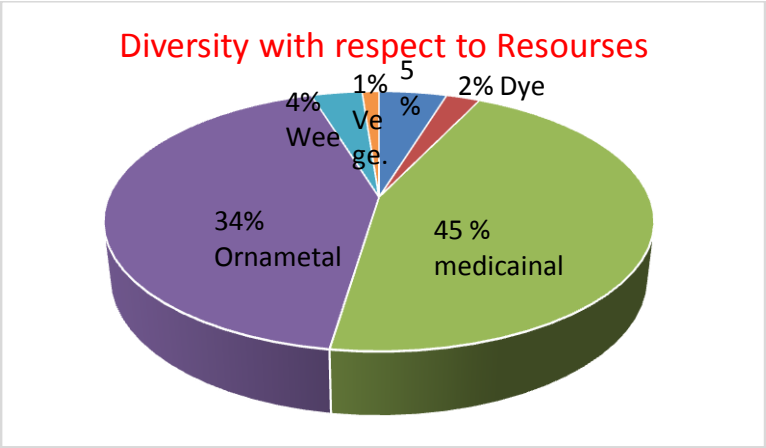

Figure 4: Biodiversity of Campus flora according to plant resources

Banares *et al.* (2003), shows evidences to this observation as Spanish flora shows about two third, of vascular plant in the vicinity due to climatic change and adaptations in their area and the observations of Moreno Saiz *et al.* (2003) also shows that the species theyare ecological state of a species being special to a particular geographical point, or area, part, region, locality, nation wise, country or restricted area or habitat type called as endemic plants.

Conclusion:

The Biodiversity in the campus of Dada Patil Mahavidyalaya Karjat shows quite diversity in families of Fabaceae, Apocynaceae, Araceae, Euphorbiaceae, Cupressaceae and Moraceae. The climate conditions of Karjat was very dry and sturdy due to less rainfall in the area and these families are adapted themselves to the dry sturdy conditions and live in starvation of water. They are woody, so, can manage stress of drought themselves as well as a large contribution of the management of college also taken an initiative to conserve biodiversity of campus particularly in extremely dry condition of summer.So, the campus area is the only place where majority of visitors are visited for the fun and to feel cool place in the Karjat District.

The present study reveals that in the college campus near about 93 Taxa are theyare distributed from various 85Genera it includes, 49 Families as per the APG-III System of classification. As plant type shows diversity like 2% climbers, 14%herbs, 35% shrubs and 48% trees most diversity of families in the campus are Fabaceae, Apocynaceae, Araceae, Euphorbiaceae etc. the flora is enriching with ornamental plants which will add beauty to the campus and it is dominated by Angiosperm plants. The flora show richness of medicinal plants which are essential to take care for the next five years to maintain diversity of flora in the campus, the college has well developed to greenhouses and a botanical garden for the conservation strategy as a college has two important courses like B. Voc. and Botany post-

graduation to study these plants in natural habitats, with proper care should be taken by the department of Botany under the management of college.

Acknowledgement:

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MEDICINAL PLANTS AND THEIR USES IN NATURAL IMMUNITY IMPROVEMENT WITH SPECIAL REFERENCE TO COVID-19

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Abstract:

Since ancient civilization peoples relied on medicinal plants for treating ailments and immune booster. Various studies revealed that medicinal plants boost immune system and stimulate the activity of cells responsible for fighting infections in the body. These natural immune boosters an important tool to develop immune system to fight against viruses, bacteria, parasites and nematodes etc. Immune system is one of the more complex systems within the human body. It helps keep healthy body system by tackling viruses, bacteria and abnormal cells in the body. Present scenario in reference to COVID-19 new concept of quarantine and self isolation people should know medicinal herbs which boost immunities.

Present paper deals study of medicinal plants, their properties, prescription and mode of application that help to boost immune system using by the people. Study conducted in small localities in Durg city. 50 families were interviewed and 22 medicinal plants found frequently used by the respondents in different forms. Ashwangadha, Kalmegh, Adusa, Satawar, Amla, Tusli, Neem, Giloe, termuric, zinger, neem etc are helpful in strengthening the immune system to fight against the viruses and bacteria.

Keywords: Medicinal Plants, Immune booster, COVID-19

Introduction:

Medicinal plants are precious gift of the nature for human beings. Since ancient civilization peoples relied on medicinal plants for treating ailments and immune booster. Immunity in human body is one of the system that helps to keep healthy body, development of strengthen to fight against infectious diseases specially caused by bacteria, viruses, parasites and toxins etc. and also helps to remove foreign bodies and malignant cells from our system. Many

of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties. Plant-derived chemicals in the form of terpenoids, steroids, phenolics, flavonoids, etc., are all manifesting worth mentioning immunomodulatory activities. Various researches exhibited that medicinal plants play an important role to develop immune system in human body. About Boosting Immunity Naturally Over 80% of the earth's population depends on plants that increase immunity and promote healing.

Currently COVID-19 has affected the people globally and killed more than 5000000. Corona virus responsible for the common cold aatack the body. Therefore, the immune system has a predictable response, it is the degree to which this response is tolerated by the body that determines mortality rates. Various studies show that many medicinal herbs help in protecting against Covid-19. Present paper highlighted those medicinal and aromatic plants that are frequently used by the people in their home remedies to cure cold and cough. Medicinal and aromatic plants their uses, mode of administration and dose that are uses by the respondents tried to incorporate in the paper. Present studies aims to give the importance of medicinal and aromatic plants which boost immunities.

Materials and Methods:

Medicinal and aromatic plants and their uses in immunity responses were determined on the basis of personnel interview from the peoples, those who are uses medicinal plants to cure cold, cough and fever. Study conducted in small localities of Durg city. 50 Families were interviewed for different age groups of the family members i.e. old age, middle age and children. At the time of interactions with peoples about medicinal and aromatic plants and their uses were recorded by common name of the medicinal plants. Information of Medicinal plants, parts of the plants, doses and mode of administration were recorded. Common name used by the peoples for medicinal plants were confirmed by botanical name. Uses of medicinal plants by the respondents were referred and confirmed from available literatures like Indian Medicinal Plants, Materia Medica etc.

Result and Discussion:

On the basis of personnel interview it is found that medicinal and aromatic plants which are frequently used by the respondent were documented as listed in table. Medicinal plants their parts used and mode of administration were documented.

Sr. No.	Common name of the medicinal Plants	Botanical Name of the medicinal plants	Family Name	Parts used and mode of administration by the respondents
1.	Adusa	*Adhatoda zeylanica* Medik.	Acantahceae	•1 tea spoon of Fresh juice of adusa leaves with honey thrice in day for 3 days is very effective for cold and cough
2.	Amaltash	*Casiia fistula* L.	Fabaceae	•Pulp of fruits chewing helpful to fight against cold and cough
3.	Anjwaian	*Trachyspermum ammi* (L.) *Sprague*	Apiaceae	•1 teaspoon powder of fruit with warm water using in cold and cough
4.	Aonla	*Emblica officinalis* Gaertn.	Euphorbiaceae	•Fresh fruits in the season taken daily before or after meals improve digestion and rich supplements for vitamins C •During the off seasons various value added products Candy, Aonla pachak, Pickels etc uses by the people, most of the respondents used powder of dry amla with warm water before sleeping
5.	Ashwagandha	*Withania somnifera* (L.) *Dunal*	Solanaceae	1 tea spoon root powder in empty stomach with warm milk or warm water daily in the morning believes improves body strength
6.	Bach	*Acorus calamus* L.	Araceae	•Powder of rhizome of bach mixed with honey twice in day is considered effective for cold and cough
7.	Baheda	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	•1 tea spoon powder of dried fruits with honey for 7 days effective for cough and cold.

8.	Black Peeper	*Piper nigrum* L.	Piperaceae	•Seeds used as species 1 gram powder cooked with ghee found fight against cold and cough
9.	Clove	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	Myrtaceae	•1 gram powder of clove boiled in half glass of water thrice in day for 7 days is effective in cold flue and fever •Chewing of clove bud helpful in improve in digestion.
10.	Garlic	Allium *sativum* L.	Amaryllidaceae	• Daily 1 or 2 garlic buds in empty stomach •Is very effective in cold and cough
11.	Giloe	*Tinospora cordifolia* (Willd.) Hook. F. & Thoms.	Menispermaceae	•Most of the respondent Fresh stems and powder of stem in water were used alternate days in the morning for improving immunity
12.	Harra	*Terminaila chebula* (Gaertn.) Retz.	Combretaceae	•1 Teaspoon powdered daily at the night before sleeping taking with warm water improve digestion and effective in cough
13.	Heang	*Ferula asafoetida* L.	Umbelliferae	•Peace of hing Daily added in curries and dals, Paste of hing and dry ginger powder with some honey twice in a day to get relief respiratory problem
14.	Kalmegh	*Andrographis paniculata* (Burm.f.) Wallich ex Nees	Acanthaceae	•Decoction of Powder of whole plant for 3 days thrice in a day is very effective in cold cough and in all type of fever.
15.	Mulaithi	*Glycyrrhiza glabra* L.	Papilonaceae	•Chewing of mulaithee stem and one tea spoon powder of stem used daily in the empty stomach in the morning fight against cold and cough
16.	Neem	*Azadirachta indica* (L.) A. Juss.	Meliaceae	•Neem leaves and giloe stem decoction thrice in day for 3 days effective in fever control

17.	Nirgundi	*Vitex nigundo*L.	Verbinaceae	•Leafy juice with honey used for 7 days to cure lungs infection.
18.	Pipalli	*Piper longum* L.	Piperaceae	•1 gram powder with tulsi leaves boiled in 1 cup of water used in the treatment of throat infection.
19.	Safed musli	*Chlorophytum tuberosum* (Roxb.) Baker	Liliaceae	•1 teaspoon powder uses daily empty stomach improves body weakness.
19.	Satawar	*Asparagus racemosus* Willd.	Liliaceae	•1 teaspoon powder were used daily in the morning improves body weakness
20.	Tulsi	*Ocimum sanctum* L.	Labiateae	•Daily 5 to 7 leaves chewing in empty stomach considered as powerful to fight against bacteria and viruses,
21.	Turmeric	*Curcuma longa* L.	Zingiberaceae	•Turmeric rhizome powder with boiled milk used to fight against cold, cough and body ache.
22.	Zinger	*Zingiber officinale* Rosc.	Zingiberaceae	•Peace of rhizome used daily with cocking vegetables, 1 spoon Juice of rhizome mixed with 1 spoon honey used in instant relief in throat infection

Study resulted that people realize the importance of medicinal plants in primary health care needs as well as to improve their immunity. It is found that more than 70% families were used above mentioned medicinal plants for the treatment of cold, cough and fever. 60% families are habit to keep and stored above medicinal plants in their houses. During the interview observed that 82% peoples were used medicinal plants during the lockdown when they got affected in various ailments. Study is similar to based on ethno botanical knowledge of the people. Study indicated that ancient knowledge about medicinal plants followed by the generation to generation for treating various ailments and to improve immune system.

Conclusion:

Biological products of plant sources have been used by the people for thousand years either in the pure forms or crude extracts to treat many diseases, to use as for immune booster. Information collected from the people can be considered the medicinal plants play an important role in improving the immune system and fighting with many pathogens like bacteria, viruses including COVID-19. This type of small studies can help to collect the traditional knowledge on medicinal plants and their uses and generate the idea to develop new drug in immunity booster preparation.

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BIODIVERSITY OF SEAWEEDS ALONG THE COASTLINE OF SINDHUDURG (MAHARASHTRA)

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Abstract:

A comparative study on distribution and abundance of seaweeds was carried out along the coastline of Sindhudurg district of Maharashtra. Sindhudurg district has a coastline of about 121 km indented by estuaries, creeks, cliffs and the beaches; are mainly rocky and sandy at some places. The rocky coastline of Sindhudurg is well suited for marine plants and shows variety of rich seaweeds forms of various taxonomic groups. Biodiversity study of the seaweeds found in the post-monsoon and pre-monsoon season at Sindhudurg coastline reveals abundance of the members of class- Chlorophyta, Phaeophyta and Rhodophyta. In this work, 38 species of seaweeds were recorded from the selected sites at Sindhudurg coastline of Maharashtra. These seaweed species are belonging to three algal classes as 16 species of Chlorophyta, 10 species of Phaeophyta and 12species of Rhodophyta. All 38 species are belonging to 22 genera and distributed in 16 families. From this work, it was revealed that there was decrease in species richness from the past decades. The reduction in the seaweed forms were observed due to the biological disturbance along the seashore. Among the 38 species of seaweeds *Bryopsis plumosa* was rare. From the observed species of seaweeds, most of the seaweeds are economically and medicinally important. In view of their importance, there is a need to conserve species of seaweeds for the long term survival and to avoid the loss of economical important seaweeds and to conserve biodiversity of Sindhudurg coastline.

Keywords: Biodiversity, Seaweeds, Sindhudurg, Coastline

Introduction:

Marine algae are popularly known as seaweeds. These are one of the most beautiful groups of photosynthetic organisms which grow under the ocean's blue water. These are

relatively simple photosynthetic plants with unicellular reproductive structure (Champman, 1971). On the basis of colour, seaweeds are grouped into three classes i.e. as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae) (Sahoo, 2010). The important criteria used to distinguish the different algal groups based on the recent biochemical, physiological and electron microscopic studies (Dhargalkar and Kavlekar, 2004).

There are about 20,000 species of marine algae of which 7,000 species belong to Chlorophyta, 15,000 species belong to Phaeophyta, 4,000 species belong to Rhodophyta and 1,100 species belong to Pyrrophyta (Hum and Wicks, 1980). Distribution of algae correlated with the content of their photosynthetic pigments, that is Chlorophyta (green algae) were presumed to predominate in shallow waters, Phaeophyta (brown algae) grows in intermediate depths and Rhodophyta (red algae) in deep water (Rabinowitch, 1945). Phycologists of the world are working on morphological, cytological, physiological aspects of seaweeds and also exploring continuously the beneficial aspects of seaweeds. Their uses in different fields such as Agriculture, Industries, Medicines, Sewage disposal, Water purification, as food and fodder, in Biological Research and even in spaceships provide a brief idea of their utility for human welfare.

7,517 km coastline of India has been investigated by marine algologist since 19th century for ecology, morphology, biodiversity, biochemistry and biological activityof marine algae. Along the Indian coastline, nearly 770 species of seaweeds have been reported from the marine environments (Sahoo *et al*, 2001). Venkataraman and Wafar (2005) studied coastal and marine biodiversity of India and reported 844 algal species. Venkataraman and Wafar (2005) reported 159 marine algae from the coastline ofMaharashtra. Untawale *et al*. (1979) reported 94 species of seaweeds from the entire seacoast of Maharashtra. Biswas and Mitra, (1943) observed marine algae from the seashores of Mumbai (Bombay) and suggested for the need of fresh studies. Deodhar (1987) carried out extensive survey on marine algal species and reported 60 different algal species of blue green, green, brown and red algae from the seashore of Mumbai.Valanju, (2007) recorded 24 marine macroalgal species from Mirya beach, Alawa Beach and Kurli-Kasopbeacheof Ratnagiri which were in belonging to 16 genera and distributed in 14 families. Dhargalkar *et al*. (2001) studied Marine macroalgal diversity along the Maharashtra coast and reported 91 species from entire coast of Maharashtra. Sakhalkar and Mishra (2014) studied biodiversity of marine benthic algae from intertidal zone of Konkan coast of Maharashtra and recorded 46 species of marine algae belonging to 34 genera. According to the report of the Government of India, Department of Ocean Development, (2012) coastline of Malvan shows 73 marine algal species.

The present study was undertaken by selecting the eight study sites from the coastline of Sindhudurg District. It is known that there is a mosaic of habitats along the Sindhudurg coastline for seaweeds such as coral reefs, estuaries, intertidal mudflats, mangroves, backwaters, sand dunes, rocky shorelines, sea grass meadows and lagoons. But detailed studies on the coastal and marine biodiversity of this area are lacking. In the view of availability and importance of seaweeds in the various field, the present work carried out along the coastline of Sindhudurg to study biodiversity of seaweeds.

Materials and methods:

Sindhudurg District is situated between North 15 ^{o}37' to 16 ^{o}40' latitudes and East 73 ^{o}19' to 74 ^{o}13' longitudes. Sindhudurg has coastline of about 127 km. indented by estuaries, creeks, cliffs, rocky shores, sandy and muddy beaches etc. The beaches are mainly rocky and sandy at some places. The rocky coastline of Sindhudurg is well suited for marine plants.

For the present investigation eight beaches were selected, namely Vijaydurg, Malai (Devgad), Devgad, Kunkeshwar, Mithbav, Kolamb, and Chivala (Malvan), Nivati (Vengurla). The study sites were visited various times, in the post-monsoon and pre-monsoon season during low tides.At the site, the samples were collected manually during low tides in post-monsoon and pre-monsoon season in polythene bag randomly and brought to the laboratory. Collected species of seaweeds were preserved in 4% formaldehyde and herbarium specimens were prepared for each species for identification and confirming their taxonomic position. Identification of species was done by using publication of Taylor (1960), Deodhar (1987), monograph Phaeophyceae of India by Mishra (1966), Krushnamuthy (1972), Phycologia Indica Vol. II by Srinivasan (1973).

Results and Discussions:

Sindhudurg coastline shows diversity ofseaweeds forms belonging to various classes.Present study was undertaken to know Seaweeds biodiversity along the Sindhudrg coastline. Seaweeds species were collected during low tides in post-monsoon and pre-monsoon season. In the present work 38 species of seaweeds were recorded from selected sites belonging to 23 genera and distributed in 16 families. These species are belonging to three algal classes as 16 species (6 genera and 16 species) of Chlorophyta, 10 (6 genera and 10 species) of Phaeophyta and 12 (11 genera and 12 species) of Rhodophyta. The diversity of green, brown and red algae at different localities of Sindhudurg coast varies significantly.

Seaweeds were collected from selected site in the post-monsoon and pre-monsoon season reveals abundance of members of Chlorophyta, Phaeophyta and Rhodophyta, which were growing in complex manner. Along the selected sites seaweeds were collected in post-monsoon and pre-monsoon season. Number wise occurrence of seaweeds along these sites as, Chivala (Malvan) coast showed largest number of seaweed species (36), followed by Devgad (33) and Vengurla (33), Malai (32), Vijaydurg (31), Kunkeshwar (30), Kolamb (29) and Mithbav (27). Chivala, Devgad, Malai, Vengurla and Vijaydurg coasts were showed maximum diversity as compared to Kunkeshwar, Kolamb and Mithbav coasts (table 1).

Table 1: List of seaweeds collected from eight sites in post and pre-monsoon season.

Sr. No.	Seaweeds	Study Sites								Post-monsoon	Pre-monsoon
		1	2	3	4	5	6	7	8		
Chlorophyta											
1	*Bryopsis plumosa*	_	_	+	_	_	_	_	_	_	+
2	*Caulerpa peltata*	+	+	+	+	_	+	+	+	_	+
3	*Caulerpa racemosa*	_	+	+	_	+	_	+	+	+	+
4	*Caulerpa sertularoides*	+	+	+	+	+	+	+	+	+	+
5	*Caulerpa scalpelliformis*	+	+	+	+	+	+	+	+	+	_
6	*Caulerpa taxifolia*	+	+	+	+	_	_	+	+	+	+
7	*Chaetomorpha antennina*	+	+	+	+	+	+	+	+	+	+
8	*Chaetomorpha media*	+	+	+	+	+	+	+	+	+	+
9	*Enteromorpha compressa*	+	+	+	+	_	_	+	+	+	_
10	*Enteromorpha intestinalis*	+	+	+	+	+	+	+	+	+	+
11	*Enteromorpha flexousa*	+	+	+	+	_	+	+	+	+	_
12	*Enteromorpha clathrata*	_	_	_	_	+	_	+	-	+	_
13	*Ulva fasciata*	+	+	+	+	+	+	+	+	+	+
14	*Ulva lactuca*	+	+	+	+	+	+	+	+	+	+
15	*Ulva reticulate*	_	_	_	+	_	+	+	_	+	+
16	*Valoniautricularis*	+	+	+	+	+	+	+	+	+	+
Phaeophyta											
17	*Dictyota dichotoma*	+	+	+	+	+	+	+	+	+	+
18	*Dictyota maxima*	+	+	+	+	_	+	+	+	+	+
19	*Ectocarpus siliculosus*	+	+	+	_	+	_	+	+	+	+

20	*Padina gymnosperma*	+	+	+	_	+	+	_	+	_	+
21	*Padina tetrastromatica*	+	+	+	+	+	+	+	+	+	+
22	*Sargassum cinereum*	+	+	+	+	_	+	+	+	+	+
23	*Sargassum ilicifolium*	+	+	+	+	+	+	+	+	+	+
24	*Sargassum tenerrimum*	+	+	+	+	+	+	+	+	+	+
25	*Spatoglossum asperum*	+	+	+	+	+	+	+	+	+	+
26	*Stoechospermum marginatum*	+	+	+	+	+	+	+	+	+	_
Rhodophyta											
27	*Acanthophora spicifera*	+	+	+	_	+	_	+	+	+	+
28	*Amphiroa anceps*	+	+	+	+	+	+	+	+	+	+
29	*Ahnfeltia plicata*	+	+	+	+	+	+	+	+	+	_
30	*Coralline berteroi*	+	+	+	+	_	+	+	+	+	+
31	*Hypnea musciformis*	+	+	+	+	+	+	+	+	+	+
32	*Gelidium pussilum*	+	+	+	+	+	+	+	+	+	_
33	*Gelediella acerosa*	_	_	_	+	_	+	+	_	+	_
34	*Gracilaria corticata*	+	+	+	+	+	+	+	+	+	+
35	*Grateloupia filicina*	+	+	+	+	_	+	+	+	_	+
36	*Grateloupia lithophila*	_	_	_	_	+	_	+	_	_	+
37	*Porphyra vietnamensis*	_	_	_	_	+	_	+	+	+	_
38	*Jania rubens*	+	+	+	+	+	+	+	+	+	+

Note: 1: Vijaydurg, 2: Malai (Devgad), 3: Devgad, 4: Kunkeshwar, 5: Mithbav, 6: Kolamb, 7: Chivala, 8: Nivati (Vengurla); Presence of species (+) and Absence of species (-).

Earlier data on the distribution, abundance, composition, standing crop, biomass and diversity along Maharashtra coast were examined and referred in the present investigation for comparison to assess the changes have occurred over the years. The seaweed diversity, species composition and abundance showed variation due to the physico-chemical parameters as well as biological factors (Valanju, 2007).

In the present study, maximum numbers of seaweeds were occurred on rocky and hard substratum than sandy and muddy substrata. The rocky beaches were showed luxuriant growth of

seaweeds along these study sites. Dhargalkar and Komarpant (2003) carried out work on 'Impact of sewage on the distribution, abundance and community of rock intertidal macroalgae.

Seaweeds were collected from different sites of Sindhudurg coastline during post-monsoon and pre-monsoon season (table 1). In the beginning of post monsoon season in October many seaweeds like Chlorophyceae, Phaeophyceae and Rhodophyceae were growing in complex manner. In October month *Chaetomorpha antennina* and *Ulva fasciata* were growing luxuriantly forming green belt and at the end of this month *Chaetomorpha antennina* showed minimum growth or totally absent. Species like *Chaetomorpha antennina, Ulva fasciata, Ulva lactuca, Caulerpa racemosa, Caulerpa texifolia, Enteromorpha intestinalis* and *Stoechospermum marginatum* were abundant in post- monsoon season.

*Bryopsis plumosa, Caulerpa peltata, Padina gymnosperm a*and *Grateloupia filicina* observed frequently in pre-monsoon season. Most of the species like, *Caulerpa racemosa, Caulerpa sertularioides, Caulerpa taxifolia, Chaetomorpha antennina, Chaetomorpha media, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, Valoniautricularis, Dictyota sps., Padina tetrastromatica, Sargassum sps., Acanthophora spicifera, Amphiroaanceps, Corallinaberteroi, Hypnea musciformis, Gracilaria corticata* and *Janiarubens* in appreciable number during both post and pre monsoon seasons.

In the post-monsoon season seaweeds growth was luxuriant along the selected site.In summers months (March, April), seaweeds were varnishes and almost negligible. Most of the species start growing from October onwards. Some species like *Ulva* grow luxuriantly during monsoon only. The species like *Janiarubens* and *Gracilaria corticata* occur throughout the year. It shows decreasing trends in the number of macroalgae in the middle of pre-monsoon season.

It was interesting to notice that the *Bryopsis plumosa* was rare and appeared only once in the whole survey.It shows decrease in species richness from the past decades. Reduction in the seaweed forms were observed due to the biological disturbance along the seashore. This trend was also reported by Dhargalkar *et al.* (2001), when they were reported 46 macroalgal species from entire Ratnagiri coast. Few studies revealed cumulative impact of pollution, siltation and habitat fragmentation on macroalgal diversity. Quasim and Wafar (1979) have recorded total 72 seaweeds from Ratnagiri, Malvan and Reddi along the west coast Maharashtra and in the present study, more than half numbers of the species were reported along Malvan and Kunkeshwar.

Conclusion:

The occurrence and distribution of seaweeds species varied with the location due to variations in substratum. Rocky coastal site shows maximum distribution of seaweeds than sandy

and muddy coasts. In general, Sindhudurg coastline has a complex variety of seaweeds. Diversity of seaweeds at this site showed that members of Chlorophyta were dominant followed by Rhodophyta which follow a tropical distributional pattern of seaweeds in this region. Number of survey has been carried out at selected site to study distribution, abundance and variation. So it is necessary to have long term monitoring programme at selected site to avoid loss of economical important seaweeds and to conserve biodiversity of Sindhudurg coastline.

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ARBUSCULAR MYCORRHIZAL BIO FERTILIZER: ITSPRODUCTION AND UTILIZATION FOR SUSTAINABLE AGRICULTURE OF MICROPROPAGATED BANANA PLANTLETS

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Abstract:

Arbuscular Mycorrhizal Fungi (AMF) are obligate symbionts, mainly belonging to the Phylum *Glomeromycota* and has significant importance in biofertilizer production and in sustainable agriculture. AMF colonize the roots of majority of land plant species, and is forming a symbiotic association with 80 % of the crop plants. They potentially enhance nutrient uptake, pest resistance, water relations and soil aggregation. They also enable plants to survive against abiotic stresses such as salinity and drought. Although commercial AMF products are available in the market, they have limitations like non-adaptability and their viability in different geographic and climatic conditions, also they are too expensiveto use. The present research paper focuses on AMF production and direct inoculum application of AMF propagulesfor assessing the post-transplant performance of micro propagated Banana plantlets. Indigenous AMF biofertilizer from four AMF species weresuccessfully producedand analysis of spores in the biofertilizer showed 35 - 50 cm^{-3}spores respectively for application to the micro propagated Banana plantlets. The Banana plantlets inoculated with AMF showed increase inplant height, leaf area by approximately 45% and 56% respectively and dry weight of shoots by 43-75 %, when compared with the control micro-propagated plantlets without AMF inoculation. Plantlets inoculated with *Glomus* species were superiorthan*Acaulospora* and *Gigaspora* species in most of the evaluated growth parameters.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Bio-fertilizer, Sustainable agriculture, micro-propagated, Banana Plantlets

Introduction:

AMF are obligate symbionts, mainly belonging to the phylum Glomeromycota (Schüßler *et al.,* 2001*;* Cavalier – Smith, 1998) containing nearly 150 species all over the world which are in symbiotic association with about 80 % of land plants species and majority of crop plants. They provide the host with nutrients and water in exchange of photo synthetic products (Smith and Read, 2008). AMF hypha are thinner than roots of plants and are able to penetrate the soil easily reaching nutrients which are inaccessible by plant root system (Smithand Smith, 2008*;* Allen, 2011). Thus, AMF can alleviate the limitation in plant growth caused by an inadequate nutrients supply (Nouri *et al.,* 2014). Mycorrhizal inoculation enhances nodulation in legumes (Carling *et al.,* 1978) thereby not only increasing the availability of phosphorus, Sulphur, and micro nutrients with higher nitrogen fixation in soil. This seems to be the cheapest way to enrich soil with nitrogen. AMF symbiosis also promotes host plants to absorb K, Zn, Cu, Ca, and other mineral elementsin the soil (Hodge and Storer, 2014; Berruti *et al.,* 2016). Arbuscular mycorrhizal fungi play an important role in water relations of plants. AMF associations modify root morphology, root anatomy or indirectly by hormonal and structural changes in host plants and improves the unsaturated hydraulic conductivity of the plant roots, thus contributing towards better uptake of the water. AMF helps the plants in betterabsorption of water by the roots. Nowadays, more attention is being given to the effect of AMF on plants water use efficiency under drought stress. Studies have shown thatAMF promotes water uptake and utilization by plant roots, improves water metabolism and enhance drought resistance (Yang *et al.,* 2014; Zhang *et al.,* 2016).A recent wave of organic farming have led the farmers to rely on Bio-fertilizers for plant nutrition maintaining and managing soil health and increasing agricultural produce (Gosling *et al.,* 2006). In soil, various kinds and number of microorganisms show their importance but among them Arbuscular Mycorrhizal Fungi (AMF) have significant importance in Biofertilizer production.

Banana, *Musa* spp. is the fourth most important food commodity after rice, wheat and maize (Bushra *et al.,* 2012). It is great source of proteins and carbohydrates as well as Vitamins A, C, E, K,B complexand has great socio-economic significance in India (Molina, 2005).Banana production depends on the ability of plant root system, its growth and development for sufficient water and nutrients uptake. Soil constraints such as water scarcity, soil acidity, mechanical dependence and the activity of soil borne pests and pathogens can reduce plant uptake of essential nutrient substances. AMF increases the plant nutrition, improves growth, increase drought tolerance, pathogen resistance and makes plant fit against adverse environment (Nelly *et al.,* 2013). With this recognition, AMF biofertilizers is used for the recent study.Successful

inoculation of AMF at the beginning of the acclimatization period (Granger *et al.,* 1983; Brazanti *et al.,* 1992) or even during *in vitro*cultivation (Mathur and Vyas, 1995) has been demonstrated. However, micro propagated plants when inoculated with AMF during initial growth *ex vitro* contribute to higher colonization rates through positive mycorrhizal symbiosis effect on activity of the root meristem (Berta *et al.*1995). Fortuna *et al.* (1992) suggested the use of infective and efficient species of AMF which enhances rapid increase in plant growth.Micro propagation techniques has its own advantages as a rapid production of plantlets from callus and successful approaches; however it has low survival rates and poor growth while shifting to field conditions. These are some of the common problems which need to be addressed.AMF inoculation to the roots of micropropagated banana plays a significant role on theirpost-transplant performance. In this research paper, four AMF species were used as AMF treatments directly into the soil establishing their natural level and its effectwas evaluatedon growth of Banana plantlets.

Materials and Methods:

Micro propagated banana plantlets (*Musa* spp. Grande Naine) were obtained from tissue culture Laboratory, College of Agriculture, Naigaon, District Nanded. The plantlets with formed roots and shoot *invitro* using MS medium (Murashige and Skoog, 1961) were transferred to plastic bags (500 ml capacity) filled with fumigated substratum: soil, sand and organic matter (1:1:1) as per their protocol and soil pH 5.1. After acclimatization in green house for a period of 6 to 8 weeks, the plantlets were used as experimental material (ex vitro pre acclimated and maintained under 70% shading 20 to 25^0C and relative humidity higher than 80%).The plantlets measuring 10 to 11 cm height were selected for the study.

Production of AMF Biofertilizers and inoculum preparation:

The spores of *Acaulospora laevis, Glomus aggregatum, Glomus etunicatum and Gigaspora decipiens* were isolated from the rhizosphere of the Banana plantations from banana fields in Malegaon village area. The isolates were cultivated in a greenhouse for 3months in 3 liter containers filled with a mixture of soil, sand and vermiculite (2:1:1) and planted with sorghum and maize as host plants. The plants were irrigated with nutrient solution (Clark, 1982) on alternate days and with distilled water once in a week.

Experimental design and data collection:

Three months after inoculation with AMF propagules having nearly 45 spores per gram of substrate, various parameters such as number of spores, percent root colonization, shoot and roots fresh and dry weight,plant height and leaf area were measured.Spores were extracted from

soil by water and centrifugation using sucrose was followed according to Jenkins 1964. Rootswere stained with 0.05% trypan blue (Phillips and Haymann 1970) and percent colonization was estimated by the gridline – intersect method Giovannetti and Mosse, 1980. Plant height was measured directly and leaf area was determined by Li-3100 leaf area meter (LI-CorInc. Lincon, Neb, USA).

The following sets of experiments were established

1) Without AMF inoculation.
2) Inoculated with *Acaulospora laevis.*
3) Inoculated with *Glomus aggregatum.*
4) Inoculated with *Glomus etunicatum.*
5) Inoculated with *Gigaspora decipiens.*

The experiments were carried out with five replicates.The data analysis was carried out to know degree of variance by one way ANOVAStatistics test.The data on percent root colonization and number of spores were arcsine transformed as the square root of (x/100) and (x+2.0) and statistical differences among means was evaluated by post hoc test with ANOVA Statistics.

Result and Discussion:

AMF biofertilizer and inoculum preparation was successfully produced using the four above mentioned AMF species (Fig. 1, 2, 3) were identified on the basis of spore morphology characteristics such as size, structure, colour, hyphal growth etc. according to Moreira, 2007; Stürmer, 2012. The spore count was initially done by microscopic count and MPN bioassay method before application as AMF inoculants.

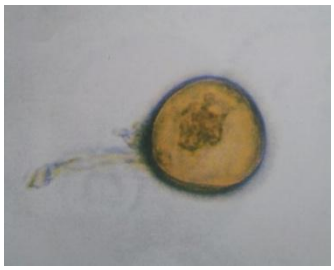

Figure 1: ***Glomus aggregatum***

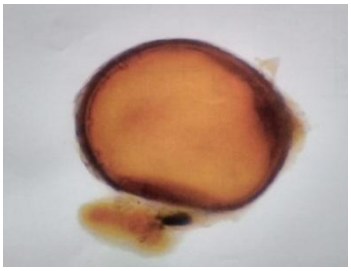

Figure 2: ***Gigaspora decipiens***

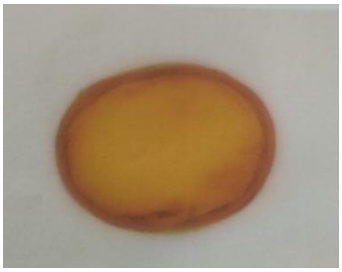

Figure 3: ***Acaulospora laevis***

After three months of AMF inoculation, the plant growth parameters were analyzed. It showed significantdifferences in plant height, leaf area, fresh and dry matter of shoots and fresh

weight of the roots without AMF and with AMF inoculants were recorded (Table 1). The plant height and leaf area of AMF inoculated plantlets were approximately 45% and 56% respectively than non-inoculated plants. Dry weight of shoots showed increase by 43-75 % in mycorrhizal inoculated plantlets. Banana plantlets inoculated with *Glomus aggregatum* showed increase in the fresh weight of shoots and roots overnon-inoculated plants. The percent colonization of roots and spore number were not significantly different among the AMF inoculated plantlets with *Glomus* species. However, *Acaulospora* inoculated plantlets showed relatively less root percent colonization and spore number when compared with *Glomus* inoculated plantlets. This might be due to soil chemical characteristics as most regions of Nanded have clayey soil texture(Varela and Trejo, 2001).

Table 1: Means of growth parameters and mycorrhizal colonization of Banana plantlets non-inoculated and inoculated with AMF and number of spores after 3 months are depicted here, Values followed by the same alphabet are not significantly different ($p<0.05$). DW and FW denote Dry and Fresh Weights

AMF Inoculant	**Plant Height**	**Leaf area**	**Roots**		**Shoots**		**No. of Spores**	**root colonization**
Treatments	**(cm)**	**(cm^2)**	**FW (g)**	**DW (g)**	**FW (g)**	**DW (g)**	**100 gm^{-1}**	%
Non inoculated	10.925b	61.401b	7.649b	1.238b	2.408b	0.396b	0b	0b
Acaulospora laevis	13.125ab	91.783ab	8.455b	0.9804ab	3.62ab	0.682a	42a	35a
Glomus aggregatum	15.888a	96.16a	12.716a	1.493a	5.985a	0.699a	78a	72a
Glomus etunicatum	15a	91.624ab	10.9ab	1.7a	3.896ab	0.623a	68a	65a
Gigaspora decipiens	13.4ab	90.834ab	10.29ab	1.402a	3.584ab	0.567ab	32a	30a

Plant height, leaf area, fresh and dry matter of shoots of inoculated banana plantlets showed significantly higher values than non-inoculated plantlets. These results are in accordance

to the obtained results by Adriano*et al.,* 2011; Rouphael*et al.,* 2015.Growth promotion of micro propagated banana with mycorrhizal inoculation was reported by Jaizme-Vega *et al.,* 1997 and Pinochet *et al.,* 1996. Increase in plant growth parameters was due to improved nutrient uptake brought about by the symbiotic association of plants with AMF (Jeffries *et al.,* 2003). Among various species of Glomeromycota, *Glomus* species are most effective in promoting growth of banana plantlets during the acclimatization as well as post transplants into the fields as demonstrated by Yano Melo *et al.,* 1999; Koffi and Declerek, 2015.Colonization of plant roots by AMF increases crop productivity through improved access to nutrientsand water and suppression of pest and diseases (Jefwa *et al.,* 2010, 2012)

Conclusion:

AMF inoculant application is an excellent growth promoter and best treatment in promoting the growth of micro propagated banana plantlets. As AMF showed remarkable increase in Plant growth parameters: plant height, leaf area, and dry weight of shoots and roots of banana plantlets inoculated with AMF, it can be used as a potential agent for sustainable agriculture in the Marathwada region.

Future Prospectives:

Many reports have demonstrated the effectiveness of AMF in drought and salinity (Augé, 2001), as well as a bio control agent against nematodes due to suppressive effects of the fungi on their reproduction (Elsen e*t al.,* 2003), this study is per sued further on this line, as major areas in Marathwada region is now facing water scarcity and nematode infestations in banana plantation.Despite its enormous potential, the application of AMF has not been fully adopted by farmers so far.

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ETHNOBOTANICAL DOCUMENTATION OF MEDICINAL PLANTS IN KAPPATAGUDDA FOREST OF GADAG DISTRICT IN KARNATAKA STATE, INDIA

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Abstract:

Ethnobotanical survey focuses on understanding the relation between humans and plants and this endeavour involves multiple disciplines. The survey was conducted during 2019-20 in and around the *Kappatagudda* Forestof Gadag district, Karnataka. Data was collected through interaction with forest protection committee, Nati-vaidyas and traditional users. A total of 55 medicinal plants belonging to 31 families was used for preparation of herbal medicine. Fabaceae was reported as the dominant family with 7 species followed by Lamiaceae with 6 species and Combretaceae with 4 species. Local people and traditional healers of study area used different plants or plant parts as medicines for the treatment of 25 human ailments. The commonly used plant part was leaves (49%) followed by fruits (20%), seeds (14%), root (5%) andwhole plant, bark, flower, rhizome, latex and clove with 2% each. Most of the remedies were taken in the form of chewing, followed by decoction, oil, powder, paste, juice and latex. Herbal formulations were administrated orally followed by the dermal route of administration. *Kappatagudda* forest is rich in its medicinal plants, especially used to cure different human ailments, the findings suggest that documentation of indigenous knowledge on traditional medicines for future research and potential development of new drugs.

Keywords: Indigenous knowledge, traditional medicine, herbal treasure, Kappat hills

Introduction:

Ethnobotany is the study of the interaction between plants and people with a particular emphasis on traditional tribal cultures (Singh, 2016). Medicinal plants play a central role as trade commodities which meet the demand of distant market. According to world health organization,

approximately 80% indigenous populations in developing countries depend on traditional medicine for their primary health care by the use of medicinal plants (Kumar *et al*., 2016). Medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population. The highpopularity of medicinal plant in rural area is due to the high cost of allopathic drugs and side effects (Mozart *et al*., 2014).

Indian Vedas and Upanishads are the evidence of traditional system of medicine and also bridging with modern medicine. Traditional medicine systems are well flourished in our country and spreading fragrance in other countries too. This system of medicine is passed from generation to generation and used to treat various health ailments of human and animal. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine during the last few decades, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world (Rekha and Panneerselvam, 2013)

A historical perspective on the use of medicinal plants for the treatment and cure of disease indicates that traditional medicinal practices have been associated with humanity time immemorial. These traditional practices involve therapeutic methods using conventional medicines that have been handed down orally through generations. In view of ethnic segregation over several years, these practices are unique to each community and group, which have survived. Knowledge of ancient traditional practices is now limited to a few closed communities, especially the remote tribal/marginalized population for whom it becomes a part of their cultural practices (Chander *et al.*, 2014). Hence the present work was undertaken to study the ethnobotanical documentation of medicinal plants of Kappath hills, Gadag district, Karnataka.

Materials and Methods:

Study Area:

The study area *Kappatagudda* Forestof Gadag district is located in the central part of Karnataka state and lies between 75° 16 to 76° 03″ E longitude and 14° 56 to 15° 53″ N latitude.The *Kappatagudda* Forestrangeare considered to be clothed with very good vegetation and have been declared as a Wildlife Sanctuary in 2019 covering an area of 17,872 hectares.

Ethnobotanical Data Collection:

Ethnobotanical survey was carried in and out of *Kappatagudda* Forestof Gadag district during 2019 and 2020. Information was gathered through interactions with the rural people,

including members of forest protection committees of*Kappatagudda* Forest.Also, interacted with traditional users and Nati Vaidya's about the common diseases and usage of available medicinal plants for the preparation of various formulations for the treatment and control of diseases by local people.The techniques employed for data collection were field walks with local people and observation with traditional healers (Jima and Megersa, 2018)

Data analysis:

The ethnobotanical data has been tabulated systematically as name of the medicinal plants, common name, family, part used and growth forms of the collected plants. Detailed information about method of preparation (i.e. decoction, paste, infusion, powder and juice) and mode of administration (i.e. oral, dermal and nasal), form of usage with fresh or dried mixture of other plants or ingredients were documented (Mahwasane *et al*., 2013).

Results and Discussion:

Data collected through ethnobotanical survey included a total of 55 medicinal plant species belonging to 31 families used for the treatment of human ailments (Table 1).Whereas Harihar and Kotresha (2012) reported wild medicinal plants of 27 species belonging to 25 genera and 17 families in and around Kappat hills.Among reported 31 families, the most significant was Fabaceae with 7 species, Lamiaceae with 6 species, followed by Combretaceae with 4 species and Apocyanaceae, Anacardiaceae, Rutaceae with 3 species each, 2 species each from Phyllanthaceae, Euphorbiaceae, Aschlepiadaceae, Asteraceae and Moraceae and one species of Vitaceae, Myrtaceae, Poaceae, Amaranthaceae, Zygophyllaceae, Aristolochiaceae, Mimosaceae, Acanthaceae, Liliaceae, Menispermaceae, Nyctaginaceae, Solanacea, Asparagaceae, Piperaceae, Annonaceae, Rubiaceae, Amaryllidaceae, Zingeberaceae, Bixaceae and Meliaceae were documented (Figure1). Traditional healers on the use of herbal medicine in Doddakavalande Hobli, Nanjangud taluk of Mysore District reported Fabaceae as most dominating family for treatment of human ailments (Nagalakshmi and Rashmi, 2020).

The analysis of growth forms among 55 medicinal species showed that 23 species were trees, followed by shrubs (12), herbs (10), climbers (6) creeper (2), and one each from twiner and rhizome which used for the formulation of medicines to treat human ailments (Figure2). The resident folk practitioners of Ethiopia used tree species mainly for the herbal formulation as they are available all through the seasons (Bekalo *et al*., 2009).

Table 1: Ethnobotanical investigationsin*Kappatagudda* Forestof Gadag district

Therapeutic indication and associated plants	**Family**	**Common name**	**Habit**	**Parts used and Ethnomedicinal preparation**
Diabetes				
Gmnema sylvestre (Retz.) R. Br.	Apocyanaceae	Ajashringi	Climber	Raw leaves consumed orally
Syzizium cumini (L.) Skeels.	Myrtaceae	Java palm	Tree	Fresh fruits consumed orally
Phyllanthus emblica L.	Phyllanthaceae	Indian gooseberry	Tree	Fresh fruits consumed orally
Tamrindus indica L.	Fabaceae	Tamrind	Tree	Seeds are dried and powdered, mixed with water or honey consumed orally
Mangifera indica L.	Anacardiaceae	Mango	Tree	
Cough				
Adathoda zeylanica Nees	Acanthaceae	Malbar nut	Shrub	Leaves are boiled mixed with jaggery and prepared Kashaya consumed orally twice a day
Oscimum sanctum L.	Lamiaceae	Holy basil	Herb	Leaf extract Mixed with salt or honey consumed orally
Curcuma longa L.	Zingiberaceae	Turmeric	Rhizome	Rhizome dried and powdered, mixed with milk, consumed orally in empty stomach
Heart disease				
Tinospora cardifolia (Wild.) Hook.f and Thomson.	Menispermaceae	Guduchi	Climber	Aqueous extract of leaf consumed orally
Acacia catechu (L.) Willd.	Fabaceae	Catechu	Tree	Bark dried and powdered consumed orally in any liquid
Toothache				
Phyllanthus amarus Schumach and Thonn.	Phyllanthaceae	Stone breaker	Herb	Leaves are dried and powdered, applied to strengthen the teeth
Terminalia chebula Retz.	Combretaceae	Black myrobalan	Tree	Seeds are dried and powdered, applied on the ache

Burn and pimple				
Aloe barbedens (L.) Correa	Asphodelaceae	Aloe vera	Herb	Pulp crushed to remove gel, applied dermal
Malaria				
Piper betle L.	Piperaceae	Betel	Climber	1 betel leaf, 1 papaya leaf, 15-20 tulsi leaves, 8-10 pepper seeds and garlic cloves are boiled with jaggery consumed orally
Oscimum sanctum L.	Lamiaceae	Holy basil	Herb	
Allium sativum L.	Amaryllidaceae	Garlic	Herb	
Snake bite				
Aristolochia indica L.	Aristolochiaceae	Indian birthwort	Creeper	Whole plant ground on stone and made into paste, applied on nails of hands and legs, can be consumed 2 tablespoons orally
Dog or Scorpion bite				
Achyranthes aspera L.	Amaranthaceae	Chaff-flower	Herb	200 grams fresh leaves consumed for 41 days in empty stomach
Indigestion				
Balanites aegyptica L.	Zygophyllaceae	Egyptian balsam	Tree	Fruit stored in salt, consumed orally
Boils				
Ricinus communis L.	Euphorbiaceae	Castor	Shrub	Heat leaves and kept on boil twice a day
Menstrual cramps				
Acacia ferruginea DC.	Mimosaceae	Rusty acacia	Tree	Leaf extract mixed with *Cicer arietinum* and slaked lime Consumed orally for first 3 days of menstrual cycle for 3 months
Roundworm infection				
Ricinus communis L.	Euphorbiaceae	Castor	Shrub	Fruit oil consumed Orally

Tiredness				
Cochlospermum religiosum (L.) Alst.	Bixaceae	Buttercup	Tree	Flowers extract with *Cuminum cyminum* and jaggery, consumed orally twice a day
Acidity				
Citrus limon L.	Rutaceae	Lemon	Shrub	Fruit juice with salt and baking soda consumed orally
Weakness				
Withania somenifera (L.) Dunal	Solanaceae	Ashwagandha	Shrub	Roots are dried and powdered, mixed with milk consumed orally
*Terminalia bellarica (*Gaertn.) Roxb.	Combretaceae	Beleric	Tree	Fruits dried and powdered, mixed with milk consumed orally
Jaundice				
Balanites aegyptica L.	Zygophyllaceae	Egyptian balsam	Tree	Fruit mixed with salt and made into tablet consumed orally
Phyllanthus amarus Schumach and Thonn.	Phyllanthaceae	Stone breaker	Herb	Dried and powdered Leaves mixed with honey or water consumed orally for 1-2 weeks
Neural ailment				
Cissus quadrangularis L.	Vitaceae	Devil's backbone	Climber	Leaves are roasted and ground to make paste consumed orally
Piles				
Semicarous anacardium L.f.	Anacardiaceae	Varnish tree	Tree	Seeds boiled in water with toor dal, filtrated with pinch of salt and consumed orally for 3-4 days
Foot corn				
Semicarpus anacardium L.f.	Anacardiaceae	Varnish tree	Tree	Seed Oil applied tropical
Calotropis gigantea (L.) R. Br.	Asclepiadacea	Giant milkweed	Shrub	Latex applied tropical

Kidney stone				
Boerhaavia diffusa L. nom. cons.	Nyctaginaceae	Hegweed	Herb	Dried and powdered Leaves extracted with banana stem, consumedorally
Hair loss				
Eclipta prostrata (L.) L.	Asteraceae	False daisy	Herb	Leaves are dried and powdered, boiled in oil and applied on head
Bronchial asthma				
Azardiacta indica A. Juss.	Meliaceae	Neem	Tree	21 ripen fruits are consumed with 1 drop of goat milk for 21 days
Male fertility				
Ficus glomerate Roxb.	Moraceae	Cluster fig tree	Tree	Fruits are mixed with sugar consumed orally
Fever				
Phyllanthus amarus Schumach and Thonn.	Phyllanthaceae	Stone breaker	Herb	Leaves are dried and powdered, boiled in water and consumed orally
Tonsils				
Boerhaavia diffusa L. nom. cons.	Nyctaginaceae	Hegweed	Shrub	Leaves are dried and powdered mixed with water or honey consumed oral
Eclipta prostrata (L.) L.	Asteraceae	False daisy	Herb	

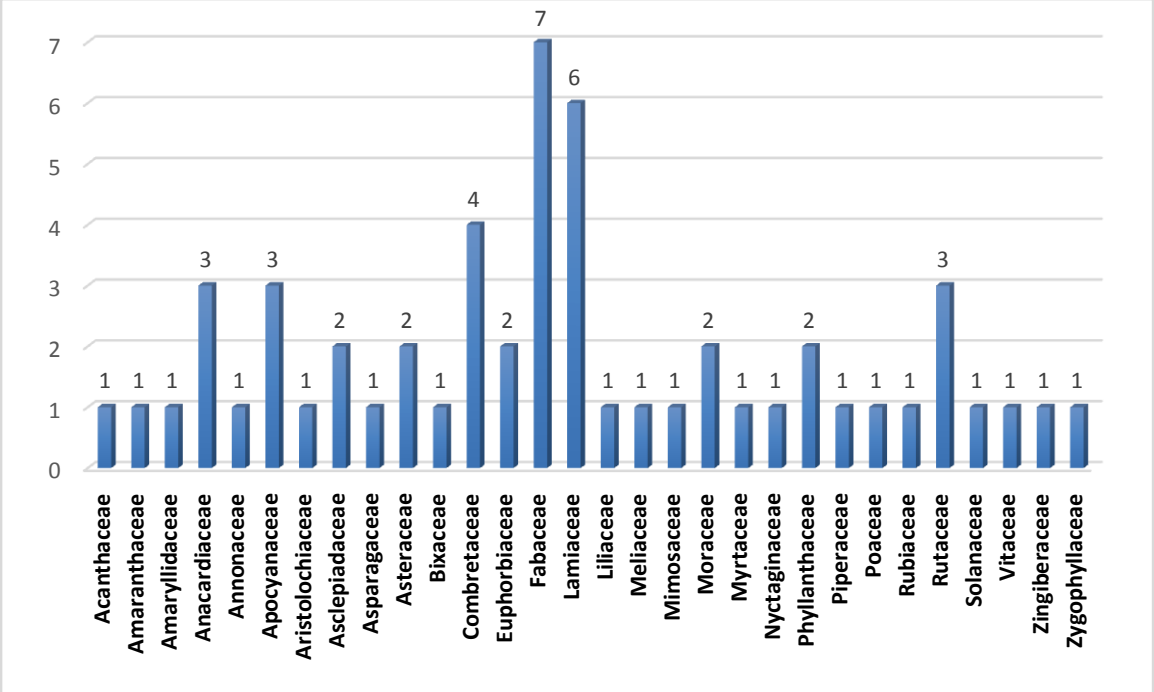

Figure 1: Families of medicinal plants used in treatment of various diseases

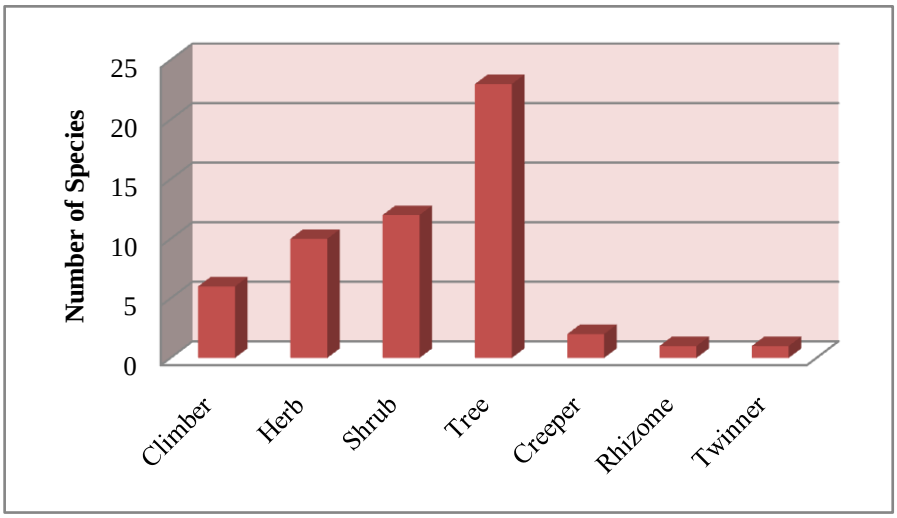

Figure 2: Growth forms of medicinal plants

Local practionaries of *study areas* have various remedies to treat 25 diseases from the collected medicinal plants. The collected information from traditional healers, local people and nativaidya's have been systematically documented as name of the disease, ingredients, part used, method of preparation and mode of administration.However, dosage depends mostly on the intensity of the disease and the age of the person concerned (Das and Choudhury, 2012).

By analysing the present ethnobotanical data, it was observed that local people and traditional healers used different plants or plant parts as medicines for the treatment of several human ailments. The commonly used plant part was leaves (49%) followed by fruits (20%), seeds (14%), bark, whole plant, root, flower, rhizome, latex and clove with 2% (Figure3). More than one plant part was used to cure human ailment. Leaves was used commonly as remedy as it was available in every season throughout the year. Fruits and flowers were used less, due to short time of availability. Traditional healers collect the fruits, flowers and seeds during the seasons, dried and stored for further formulation of medicine.

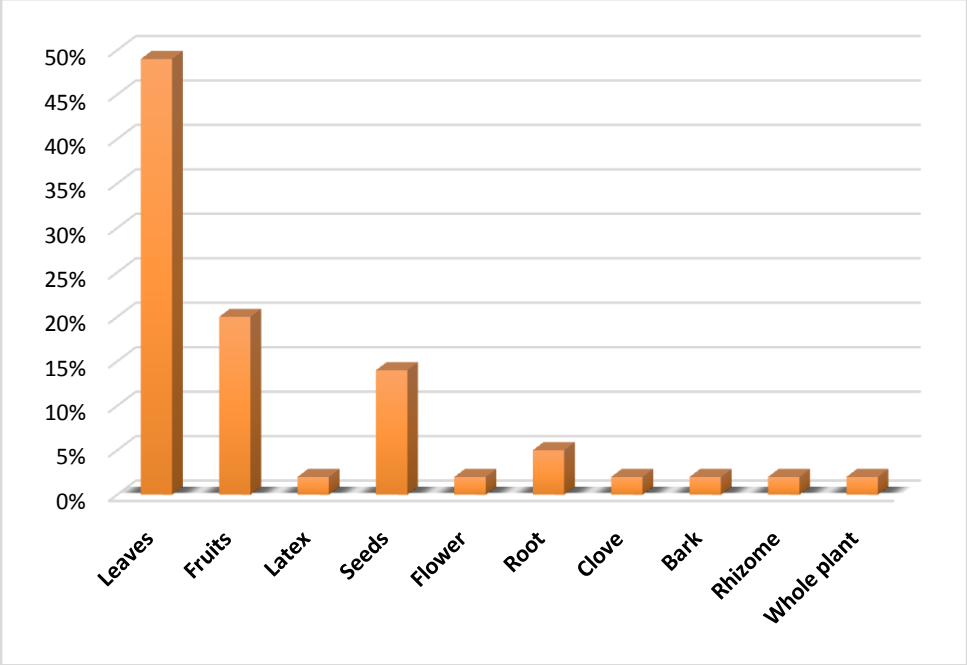

Figure 3: Parts used for preparation of medicines for human ailments

The inventoried medicinal plants were used by the population of study area in their routine practices to treat range of common ailments and disorders. Twenty-five different ailments have been documented. Most of the remedies was taken in the form of syrup (6%) followed by powder (29%), paste (14%), decoction (16%), chewing (19%), latex (3%) and oil (11%) (Figure 4).

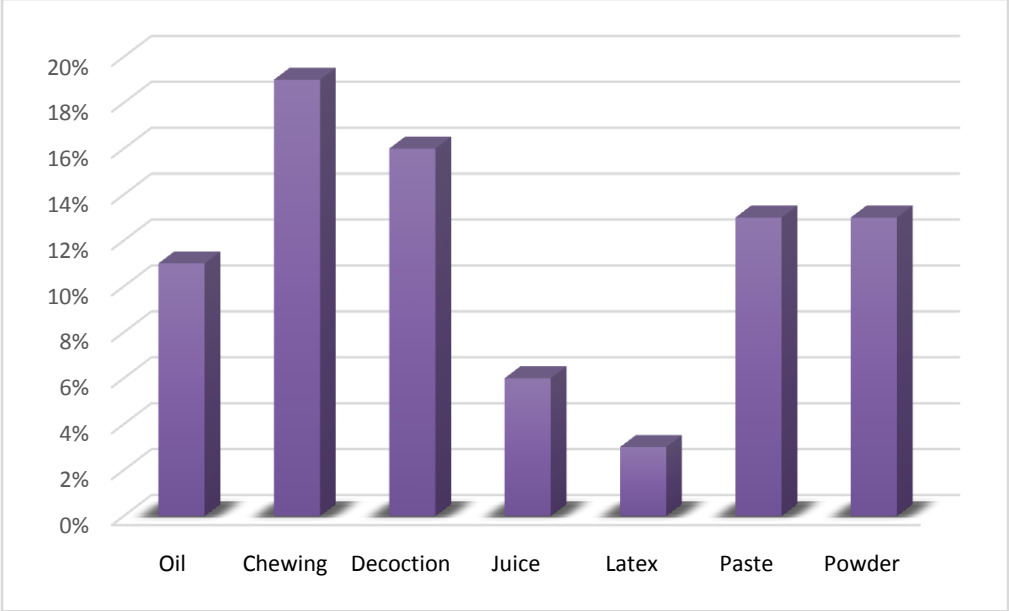

Figure 4: Method of preparation of herbal remedies for human ailments

Depending on the type of ailment, most of the medication was taken orallyin Diabetes, Indigestion, Menstrual cramps, Bronchial asthma, Malaria, Piles, Roundworm infection, Jaundice, Acidity, Kidney stone followed by tropical or dermal to cure Dog bite, Snake bite, Toothache, Hair loss, Burn and pimples, Boils. Traditional healers apply medicine externally for skin diseases. Folk medicine in South-Western Serbia, Zlatibor district was intended mainly as a mode of primary health care in healing of minor illnesses by oral administration of medicines (Savikin *et al.*, 2013).

Conclusion:

The ethnobotanical survey indicated that, the study area has plenty of medicinal plants to treat various human ailments. Economically backward and local people of study area depend on the traditional medicine due to low cost and it is a part of their culture. Certain species of medicinal plants are being exploited due to lack of awareness and knowledge about the available medicinal plants. Traditional plants are easily affordable than conventional medicine and is cost effective. The documentation may help to draw attention to the valuation of the biological diversity of the study area for the potential of being used in pharmaceutical drug development.

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UTILIZATION OF *EURYA ACUMINATA* DC. IN TRADITIONAL RECIPES BY THE HMAR TRIBE OF MANIPUR, NORTHEAST INDIA

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Abstract:

The study aimed to document the popular used of *Eurya acuminata* DC. in traditional recipes by the Hmar tribe of Manipur. Data was collected for a period of two years. Study revealed that among the different parts of the plant selected, fresh matured leaves showed highest preference (60%). Eight different traditional preparations were recorded. Of which, sizo sa changal hmepawk exhibited highest rate of preference (70%). It was also observed that traditional preparation of *Eurya acuminata* DC. is compulsory in all the social and religious feast.

Keywords: *Eurya acuminata* DC. traditional, Hmar, Manipur

Introduction:

Northeast India harbors diverse ethnic communities exhibiting great reservoirs of traditional knowledge system. Being the core of one of the biodiversity hot spots of India, diverse flora and fauna are found within the region. The rich ethnic communities of Northeast India have immense traditional knowledge on the utilization of forest and plant parts especially as food products in multi varied ways of applications (Sundryal *et al.*, 1998). Traditional knowledge is transmitted orally from generation to generation (Convention on Biological Diversity, 2006), then from ancestral to descendant cultures (Lagoudakis *et al.*, 2014). It is culture oriented and often represents the identity and culture of the indigenous tribe. It also plays a pivotal role within the food security and health of the overwhelming majority of individuals since past. However, the values of traditional concepts are deteriorating as local

ecosystems are degraded, rapid deforestation and urbanization; and the integration of traditional communities into broader society.

In Manipur, survey and documentation of wild edible and medicinal plants have been done, however, work on utilization of plants in traditional recipe is in the bottom level especially among the Hmar tribe. Consistent with 2001 census, there have been 42,993 Hmars concentrated around Pherzawl and Churachandpur district in Manipur. They utilized these traditional recipes in social and religious feastings and at homes. Therefore, the study was conducted to conserve and document the utilization of century old traditional recipes. This study also could contribute local communities towards sustainable development of their local resources, and also Government policies to improve the growth and devleopment, and to improve its potential which is currently undervalued.

Materials and Methods:

Study site:

Manipur has four types of forests: Tropical semi-evergreen, dry temperate forest, sub-tropical pine, and tropical moist deciduous. The study was carried out among several villages of the Hmar tribe that lies within the state (23°27' to 25°41' N latitude and between 93°61' and 94°48' E longitude) of Manipur.

Data collection:

Data was collected for a period of two years i.e., 2018 to 2019 in villages of Hmar tribe. Data collection on the mode of utilizing *Eurya acuminata* DC. as traditional recipes was done by household surveys, semi-structured interviews and informal discussions with the experienced and elderly people. Datas were collected mainly based on (i) parts of the plants used and (ii) people's consumption preferences.

Results and Discussions:

Fig. 1 depicts the different parts of the plant / conditions utilized for preparing traditional recipes during the study period viz., fresh matured leaves, dried matured leaves, young leaves and fruit. Out of these, the fresh matured leaves constituted the highest rate of utilzation (60 %). The freshness of the leaves attributed the studied people to prefer more than the others. This is followed by dried matured leaves (20 %). (Dried matured l eaves- it is the freshly plucked leaves are dried in the sun for few days and stored in containers for future used). This is reportedly used only in the absence of fresh leaves.

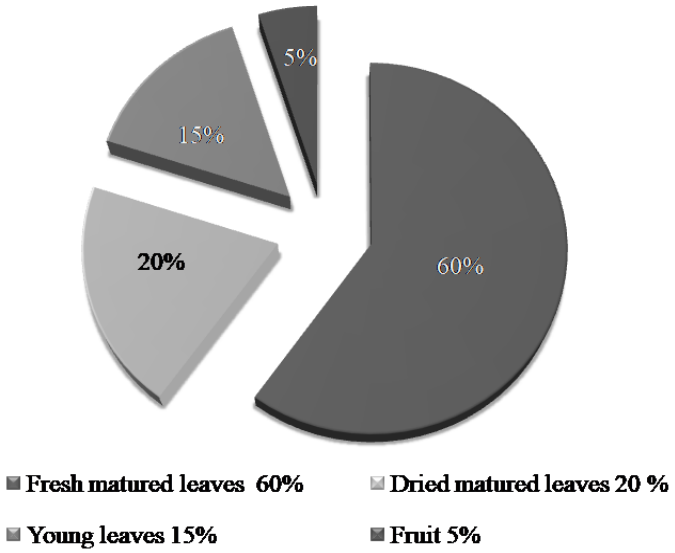

Figure 1: Parts of the plant utilized during the study periods.

Although, the dried matured leaves do not lose its original taste but, the rate of utilization is not as high as the fresh matured leaves because, some people could not get the fresh leaves but, only in the form of dried leaves. Its advantage is that it can be kept for months and year without losing its values. This may also attribute to the wide consumption of dried matured leaves.

Table 1 depicts the traditional recipes and preparations of *Eurya acuminata* DC. during the study period. Three broad major preparations were grouped: (i) porridge- simple preparation with boiled rice and less amount of chillies (ii) spicy- the addition of different spices in the preparation and (iii) fermented- the hot dish involving the addition of fermented pork, fish, soyabeans or sesame seeds. Altogether, eight traditional preparations were recorded during the study period. The study revealed that porridge is mostly prepared followed by spicy and the least was observed in the preparation involving fermented meat (Figure 1).

From these observations, it is known that *Eurya acuminata* DC. is highly valued and widely prepared in different preparations. Figure 2 shows that P1, i.e.,sizo changal hmepawk is the most preferred among all the preparations which is followed by P3, i.e., chartang, whereas, the least was observed in P4 i.e., sizo ra. Although they are cooked at home or in one's own preferences yet, they are mostly cooked in feastings of religious and social gatherings. The high preference rate also depends upon the availability of the recipes like meat and sizo leaves. The

study site, where majority of the population exhibited non- vegeterian diet, preparation is made easier.

Table 1: Traditional recipes out of *Eurya acuminata* DC. during the study period

Sr. No.	Traditional recipes	Parts of the plant used	Preparations
	Porridge		
1	Sizo sa changal hmepawk	Dried or fresh matured leaves	It is the most popular traditional sizo preparation. A kind of porridge prepared by mixing sizo leaves with boiled rice, small amount of chillies and salt. Any kind of meat is added. Sodium bicarbonate ($NaHCO_3$) is added to dissolve the leaves thereby producing yellowish green colour.
2	Sizo si hmepawk	Dried or fresh matured leaves	Sizo leaves are cooked with si (*Sesamum indicum*), rice and small amount of chillies as porridge. Sodium bicarbonate and salt is added
Spicy			
3	Chartang	Dried or fresh matured leaves	It constitutes a prominent dish of the study tribe. It is a combination of sizo leaves, meat, salt, chillies, turmeric and garlic. Spices of any type may be added according to one's own preference.
4	Sizo ra	Fruit	This is the addition of the fruit mostly in chartang. The taste of the fruit is also often compared with the taste of meat.
With fermented meat			
5	Sizo bal bekanthu changal	Dried or fresh matured leaves	The corms of bal (bal: *Colocasia esculenta*) is cut into small chunks and cooked with leaves of sizo, salt and chillies alongwith bekanthu (fermented soyabeans: *Glycine max*). Sodium bicarbonate is added for seasoning.
6	Sizo thlaihna sithu changal	Dried or fresh matured leaves	This is a combination mixture of sizo leaves with varieties of vegetables and fermented sesame seeds. Chillies, sodium bicarbonate and salt is added.
7	Sizo changal hme	Dried or fresh matured leaves	The leaves of sizo is cooked with varieties of vegetables and chillies. Fermented fish or pork is added with sodium bicarbonate and salt.
8	Sizo zik dawng hmarchadeng (Chutney)	Young leaves/shoots	The young leaves of sizo is steamed cooked and is mixed with chillies, salt and fermented fish. This constitutes sizo chutney.

Note: Sizo is the local name for *Eurya acuminata* DC.

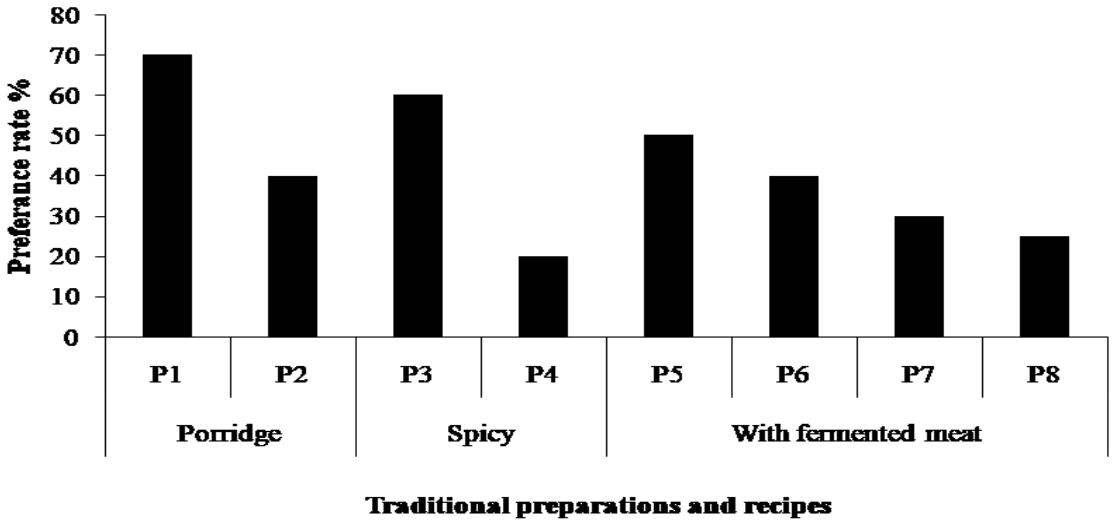

Figure 2: Preference rate of different traditional preparations and recipes during the study periods

Note: Sizo= *Eurya acuminata* DC.; P1= Sizo sa changal hmepawk; P2= Sizo si hmepak; P3= Chartang; P4= Sizo ra; P5= Sizo bal bekanthu; P6= Sizo thlaihna sithu changal; P7= Sizo changal hme; P8= Sizo zikdawng hmarcha deng

Sizo bal bekanthu changal is another prominent traditional dish of the Hmar tribe. It has been observed that in all the occasions where feasting is involved, atleast one or two preparation(s) of *Eurya acuminata* DC. is involved. This shows the attachment of the plant with the Hmar tribe. It could be also because it has no adverse affect on consumption. This was reported by the villagers during the study period. The wide consumption may be related to the taste as it is neither bitter nor sweet and odourless. From all the study villages, it was reported that these different preparations were transferred from generation to generation by word of mouth and has been followed till present days. However, this century old traditional knowledge is declining rapidly. Therefore, documentation of *Eurya acuminata* DC. is important for enhancing the understanding of traditional knowledge systems including their mode of preparations and consumption. The present study revealed that local inhabitants were dependent in this plant for different occassions. However, over utilization may affect the habitat due to anthropogenic and socio economic factors. Introduction of suitable technique for home garden cultivation is required in order to save the plant from deterioration. The study can also boost rural economy and provide high potential for conserving the valuable resources.

Acknowledgements:

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IMPACT OF COMPLEX MEDIA ON PRODUCTION OF CELLOBIASE AND SUCRASE FROM FILAMENTOUS FUNGUS *TERMITOMYCES CLYPEATUS*

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Abstract:

Fungi are long been known as potential producer of enzymes like invertase, beta-glucosidase, alpha-amylase, glucoamylase, endocellulase, exocellulase, carboxymethylcellulase, laccase, manganese peroxidase, ligninperoxidase, pectinase, inulinase, glycosyltransferase, dextransucrase, dextrandextrinase, transglutaminase, xylanase, protease, endoglucanase, polygalactouronase, asparaginase, penicillin acylase etc in presence of various carbon and nitrogen sources. As these enzymes are used heavily in Food and Feed, Pharmaceuticals, Textile and Agriculture, production optimisation was attempted by supplementing the growth media with micronutrients like amino acids, trace elements, vitamins and nucleic acids.

Termitomyces clypeatus has been exploited earlier to produce many industrially important enzymes in significant titre using several synthetic media. Present study describes the production of two crucial enzymes, cellobiase (β-glucosidase) and sucrase using malt extract and peptone as complex media. Cellobiose and cellulose are used as alternate carbon source. Titre was compared with both secreting and non-secreting control sets. Carbohydrate utilization and protein production was also estimated during the enzyme production days.

Introduction:

Fungi, especially filamentous fungi, are well known as a producer of a wide variety of extracellular enzymes of industrial importance since long (Crueger and Crueger, 1990). Invertase, beta-glucosidase, alpha-amylase, glucoamylase, endocellulase, exocellulase, carboxymethylcellulase, laccase, manganese peroxidase, ligninperoxidase, pectinase, inulinase, glycosyltransferase, dextransucrase, dextrandextrinase, transglutaminase, xylanase, protease, endoglucanase, polygalactouronase, asparaginase, penicillin acylase etc enzymes are utilized during production and processing steps in food and feed, beverage, textile, pharmaceuticals, laundry, leather, pulp and paper industry and alternative energy source as well (Crueger and Crueger, 1990, Ghorai *et al.*, 2009). Naturally, enzyme yield or specific activity is crucial for any

industry from economic point of view. Keeping that in mind, enzyme production was optimized by supplementing the growth media with micronutrients like amino acids, trace elements, vitamins and nucleic acids etc and choosing the best carbon and nitrogen sources while optimizing their concentration as well (Lee *et al.*, 1998).

Termitomyces clypeatus, one filamentous fungus from basidiomycetes class, has been found to produce cellulase, cellobiase, sucrase, endo-xylanase, xylosidase, arabinofuranosidase, acetyl esterase, alpha-amylase, amyloglucosidase etc extracellularly (Ghorai *et al.*, 2009). All of them have various industrial applications. As production cost is one key factor in industry, complex media can often act as a substitute for synthetic media. It also encourages production of multiple enzymes simultaneously which can be beneficial for hydrolysis of cellulosic mass (agro-wastes), saccharification and production of cellulosic ethanol etc. Present study describes production of cellobiase and sucrase in presence of Malt extract and peptone (1%) as complex media containing cellobiose, cellulose, (each 1%) as alternative carbon source. Both secreting and non-secreting controls were studied.

Materials and Methods:

Materials:

Cellulose (Sigma Cell), pNPG, D (+) Cellobiose was purchased from Sigma. Malt extract, peptone was purchased from Himedia. Other chemicals were of AR quality.

Mycelial Growth:

Termitomycesclypeatus was grown in shake-flasks at 30^0C in different complex media containing cellobiose (1% w/v) as the carbon source. Only cellulose (1% w/v) containing medium was without any additive. Micronutrients used for each medium were (% w/v) $CaCl_2.2H_2O$ - 0.037, KH_2PO_4 - 0.087, $MgSO_4.7H_2O$ - 0.05, boric acid - 0.057, $CuSO_4.5H_2O$ - 0.004, $FeSO_4.7H_2O$ - 0.025, $MnCl_2.4H_2O$ - 0.0036, $NaMoO_4.4H_2O$ - 0.0032 and $ZnSO_4.7H_2O$ - 0.03. Malt extracts (ME) and peptone was added (1%) in experimental sets whereas control flasks were without these two components. Both secreting (Sc) and non- secreting (NSc) controls were prepared. Sodium succinate (0.5%) was added to keep the medium in secreting condition, while in non-secreting medium it was omitted.

Enzyme Isolation:

Cell free culture filtrate was collected as extra-cellular enzyme.

Enzyme assays:

Cellobiase activity was measured by the amount of *p*-nitro phenol liberated from *p*NPG at 400nm described as before (Khowala and Sengupta, 1992). The reaction mixture (1ml) contained 4mM *p*NPG in 0.1M acetate buffer, pH 5.0 and an appropriate amount of the enzyme. Incubation was carried out at 50^0C for 30 min. Reaction was terminated by the addition of 0.5 ml

Na_2CO_3 (1M). Intensity of the yellow colour developed by liberation of *p*NP was measured at 400 nm. Enzyme activity was expressed as units per ml, which is the amount of enzyme that could produce 1 μ mole of *p*NP per minute under the assay condition. The absorbance values of the standard *p*NP solution was calculated on the basis of the extinction coefficient for *p*NP according to Bergmeyer and Bernt and the OD values were taken at 400 nm in 1 ml cuvette in Shimadzu spectrophotometer.

Enzyme activity (U/ml) was calculated by the following formula:

$U/ml = \Delta A / \Delta T \times 0.0719 \times l2 / \phi$.

ΔA = Net OD

ΔT = Time of incubation

ϕ = Ratio of enzyme sample to volume of incubation mixture used in assay

0.0719 = μ molar extinction coefficient of *p*-nitrophenol.

L = Path length of light i.e. 1cm.

Sucrase was assayed by estimating the liberation of glucose from sucrose by GOD-POD reagent according to the method mentioned earlier (Mukherjee and Khowala, 2002). The assay mixture containing 4 mM sucrose in 0.1M acetate buffer, pH 5.0 and an appropriate amount of the enzyme in a total volume of 40μl was incubated at 45^0C for 5 min. Reaction was terminated keeping the tubes at 100^0C for 5 min. Glucose liberated during the reaction was estimated by GOD–POD method. The standard curve was prepared by taking known dilutions of a stock glucose solution and adding GOD–POD reagent under conditions similar to those for the experimental. The absorbance at 505 nm was plotted against the corresponding sugar concentration to obtain the standard curve. The enzyme activity was expressed as units per ml, which represented the amount of enzyme that could liberate 1 μ mole of glucose produced per minute.

Enzyme activity (U/ml) was calculated by the following formula:

$U/ml = \Delta A \times L / \Delta T \times \Delta G \times \Delta GA$

ΔA = Net OD

L = Path length of light i.e. 1cm.

ΔT = Time of incubation

ΔG = Molecular weight of glucose

ΔGA = Absorbance of 1μg of glucose

Protein estimation:

Protein concentration was determined using the method of Bradford (1976) with bovine serum albumin as a standard. Coomassie protein assay reagent according to manufacturer's

technical bulletin (Pierce, USA). Assay mixture (0.5 ml) consisted of protein sample and water taken in glass tubes. 0.5 ml Coomassie protein assay reagent was added to them and absorbance at 595 nm was measured in between 15 – 60 min.

Carbohydrate estimation:

The carbohydrate content of the enzyme preparations were determined by orcinol-sulfuric acid reagent (Brown and Anderson, 1971)**.** 3 ml orcinol reagent was added to 0.1ml reaction mixture containing different volumes of sample. The resulting reaction mixture was kept on boiling water bath for 20 min. to develop the color and the intensity of the color was measured at 540 nm. Carbohydrate content of the sample was expressed in terms of glucose equivalent.

Results:

Cellobiase production in different media:

All the different media showed gradual increase in cellobiase activity up to 6^{th} day of growth except Sc medium. Production increased drastically (0.591 U/ml) on the 4^{th} day of growth. Among all the media peptone containing medium had the maximum cellobiase activity (0.827 U/ml) (Figure 1) on 6^{th} day. Malt extract was not effective as peptone as the activity of cellobiase in this medium was almost similar to that of non-secreting control till 6th day (0.027U/ml), whereas cellobiase activity was around 9 fold in secreting control (0.24U/ml) but was almost half compared to peptone medium on 5^{th} day (0.49 U/ml). Very poor enzyme activity was observed in case of cellulose containing medium. Highest cellobiase activity from this medium was 0.025 U/ml on 2^{nd} day.

Sucrase production in different media:

Earlier it was found that in the culture filtrate of *Termitomyces clypeatus*, cellobiase was associated with sucrose (Mukherjee and Khowala, 2002). So, sucrase activity was also measured along with cellobiase. Sucrase activity was high in both the control media (Figure 2) in comparison to their supplemented counterpart except peptone containing medium. Peptone medium attained highest sucrase activity (0.87U/ml) on 3^{rd} day, then activity decreased in the next four days but increased in the days onwards.

Almost similar activity (0.82U/ml) was achieved by NSc on the 4^{th} day. In secreting control the enzyme activity increased rapidly and highest was 0.69U/ml on the 2^{nd} day of growth. Activity decreased on 3^{rd} day onwards and again increased on 5^{th} day. Maximum activity was attained by all the media either on the 3^{rd} or 4^{th} day except Sc.

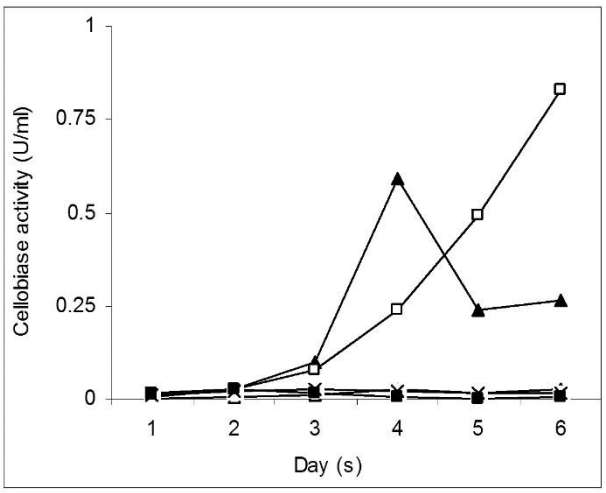

Figure 1 - Cellobiase production in different media

ME (X), peptone (-□-), and cellulose (-■-), secreting control (-▲-) and non-secreting (-Δ-) control

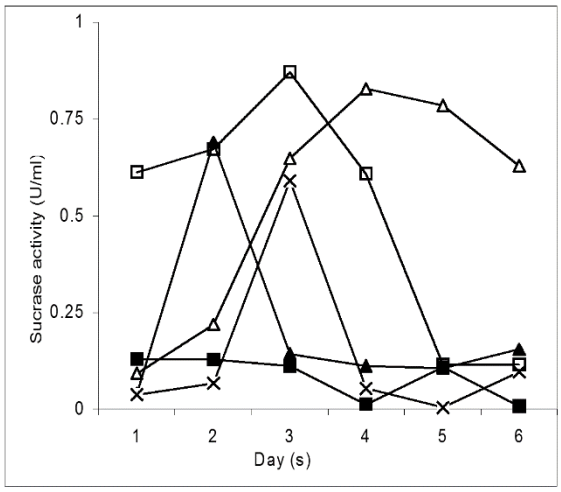

Figure 2 - Sucrase production in different media

ME (X), peptone (-□-), and cellulose (-■-), secreting control (-▲-) and non-secreting (-Δ-) control

Protein production in different media:

Protein production was almost similar in all the media except peptone containing medium (Figure 3). Peptone medium produced maximum protein (1.6292 mg/ml). Production was highest in all the media on the 4th day of growth except cellulose medium. It produced highest protein on 3rd day (0.6938 mg/ml). Total protein production was more than twofold higher in peptone medium (1.6292 mg/ml) compare to ME containing medium (0.5917 mg/ml) which was higher than Sc (0.4929 mg/ml) medium. All of the supplemented media produced higher protein than both the control media. Sc medium produced higher protein than NSc (0.186 mg/ml) medium. Maximum production was observed either on 3rd or 4th day of growth.

Specific activity in different media:

As Figure 4 shows specific activity was very low in all the media till the 3rd day of growth. Highest specific activity was observed in case of peptone containing medium on 6th day (1.4064 U/mg). All other media showed very insignificant results except Sc medium. On the 4th day there was steep rise in specific activity. in Sc medium (1.199 U/mg) which was decreased to 0.5228 U/mg in the next day. Much lower value (0.1334 U/mg) was calculated on the same day for NSc medium. ME and cellulose containing media showed highest specific activities of 0.1561 U/mg and 0.08896 U/mg respectively on the 2nd day of growth.

Carbohydrate utilization:

Soluble sugar was utilized from the very 1st day of growth in every medium (Figure 5). So the amount of residual sugar decreased gradually. Sugar utilization was highest in case of secreting control medium. ME, peptone and cellulose containing medium had almost similar

profile. In both the control medium, sugar utilization was higher compared to the other three media. Minimum sugar was utilized in cellulose medium.

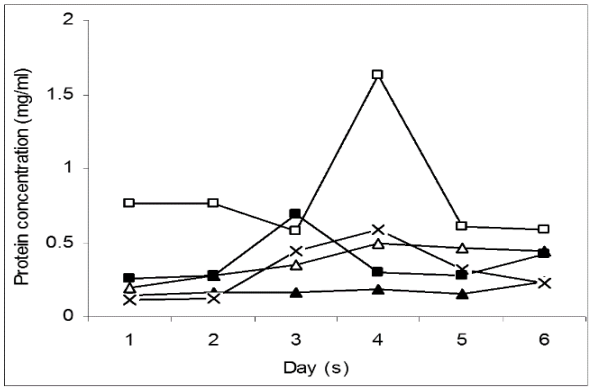

Figure 3 - Protein production

ME (X), peptone (-□-), and cellulose (-■-), secreting control (-▲-) and non-secreting (-Δ-) control

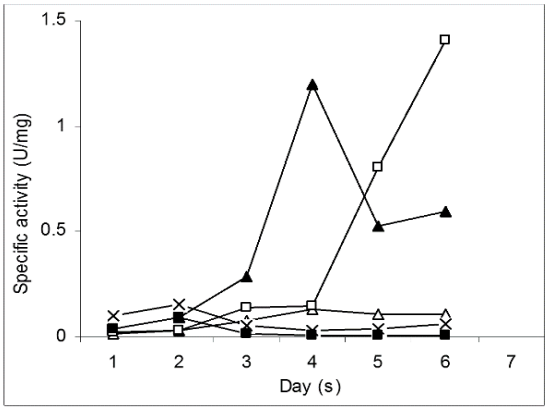

Figure 4 - Specific activity profile

ME (X), peptone (-□-), and cellulose (-■-), secreting control (-▲-) and non-secreting (-Δ-) control

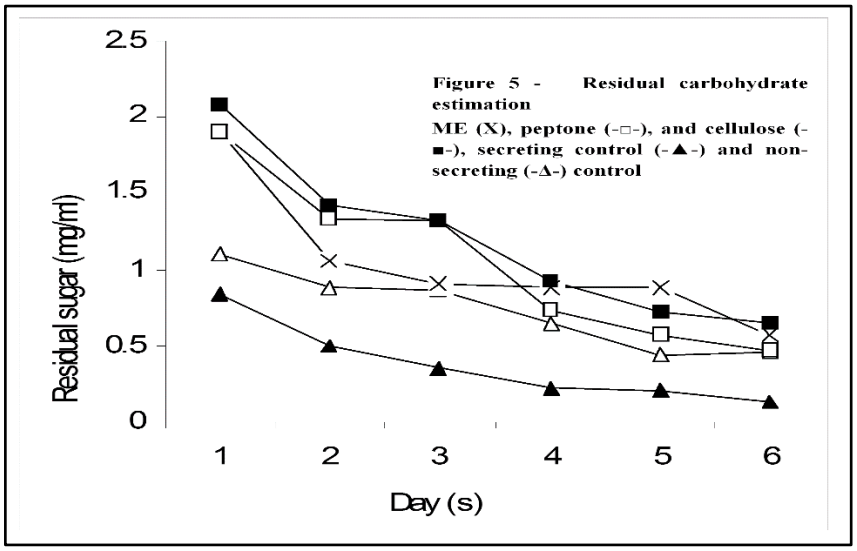

Figure 5 - Residual carbohydrate estimation

ME (X), peptone (-□-), and cellulose (-■-), secreting control (-▲-) and non-secreting (-Δ-) control

Discussion:

Complex media are exploited as an enriched media for production of various industrially important enzymes. Till date, cellobiase production was studied in presence of different synthetic media. So, we opted for various supplemented media for higher cellobiase production. Studies have proved that both the nature and concentration of carbon and nitrogen sources are powerful nutrition factors regulating the protein and enzyme production by different micro-organisms. Production varies greatly with the addition of various supplements like malt extract, peptone, yeast extract etc. When optimum culture conditions for the batch production of extracellular peroxidase by *Coprinus cinereus* UAMH 4103 and *Coprinus* sp. UAMH 10067 were explored

(Ikehata *et al.*, 2004) , the concentrations of carbon (glucose) and nitrogen (peptone or casitone) sources showed significant effects on the peroxidase production.

Some white rot fungi; *Daedalea avida, Phlebia brevispora, Phlebia radiata* and *Polyporus sanguineus* when grown under different nutritional conditions, mineral salts malt extract broth proved to be the best medium of the various basal media tested for laccase production (Arora and Gill, 2001). In our case ME was not so much effective for cellobiase production. Cellobiase activity was (0.025U/ml) comparable to the NSc (0.027 U/ml) and cellulose (0.025 U/ml) (Figure 1) containing media. With respect to protein, ME containing medium showed higher production (0.5917 mg/ml) compare to Sc (0.4929 mg/ml) (Figure 3). Moreover ME medium promoted higher sucrase production (Figure 2) (0.59 U/ml) than cellobiase (0.025 U/ml). It showed better production profile compare to the cellulose medium.

In *Schizophyllum commune* KUC9397, 7.2 fold higher betaglucosidase production was reported from 2.96% cellulose, 2.30% soy peptone and 0.11% thiamine HCl supplemented medium (Lee *et al.*, 2004). Feeding on glucose and cellobiose resulted in production of 0.198 g/L/h of protein and betaglucosidase 36.7U/L/h from *Trichoderma reesei* during 1st to 4th day of growth. 0.195g/L/h of protein and 51.9 U/L/h of betaglucosidase were produced during 4th to 8th days (Ike *et al.*, 2013). In *Termitomyces clypeatus* also, peptone induced higher enzyme production compare to all the other media. Cellobiase activity (0.827U/ml) was 1.4 times higher comparing to secreting control but 33 times higher than cellulose, ME and NSc medium. Similarly sucrase activity was higher in peptone containing medium. In that medium, on the 3rd day sucrase production was 1.3, 1.4, 6 and 7.7 times higher compare to NSc, ME, Sc and cellulose containing medium respectively. In case of total protein, peptone medium secreted 2.7, 3.3, 5.5, 8.7 times higher on the 4th day compare to ME, Sc, cellulose and NSc medium respectively.

In all experimental sets, *Termitomyces clypeatus* was grown in presence of 1% cellobiose as the carbon source except for cellulose containing medium. It induced cellobiase activity and it was almost 24 times higher (0.591 U/ml) compare to the cellulose (0.025 U/ml) (Figure 1) containing medium. Sucrase activity was more than 5 times higher in presence of cellobiose (Figure 2). Moreover specific activities for cellobiase (Figure 4) were higher in Sc, ME and peptone containing media. So, extracellular enzyme from cellobiose and peptone medium can be utilized for hydrolysis of different agro-wastes and bioethanol production after optimizing the chemical and physical components.

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CHARACTERIZATION OF A LOCAL RAW HONEY SAMPLE AND ASSESSMENT OF ITS ANTIMICROBIAL ACTIVITY

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Abstract:

Antimicrobial agents, specially antibiotics, are the first and the most important medicine to fight the infectious diseases worldwide. The emergence of antibiotic resistant pathogens is greatly reducing the efficacy of this wonder drug. With rampant and widespread misuse of antibiotics, antibiotic resistance nowadays is a global threat to human health. Hence, the search for newer strategies to combat antibiotic resistance and infectious diseases is gaining impetus with each passing day. The use of natural products is an attractive alternative due to its low toxicity, easy availability and potent activities. Honey, the concentrated sweetener of nature, has long been known for its medicinal values. In traditional Indian medicine honey had been used as an external wound healer as well as an internal medication. In recent years various reports have been published about the antimicrobial activity of different raw, unheated honey samples against both broad range of bacterial and fungal species. Here we report the antimicrobial activity of a raw, untreated honey sample procured from a local beehive. The honey showed significant inhibitory effect against *Pseudomonas* sp. and *Vibrio* sp. at 50% and 25% (v/v) concentrations. Antifungal activity against *Candida* sp. and *Aspergillus* sp. was also observed. The physical and chemical parameters of the honey were also characterized.

Keywords: Honey, traditional medicine, antimicrobial agents

Introduction:

Antibiotics, the wonder drug of modern medicine, are fast losing its efficacy due to emergence of antibiotic resistance. Antibiotic resistance is becoming a real threat to global health as well as food safety (Levy & Marshall, 2004; Ventola, 2015). Dissemination of antibiotic resistance genes through environmental bacterial sample by horizontal gene transfer is giving rise to new resistant population (Wintersdorff, *et al.*, 2016). Misuse and overuse of antibiotics is

further aggravating the situation with time. Thus the search for alternative antimicrobials is urgently needed and the repertoire of natural products can be used to find such as an alternative (Mahady, Huang, Doyle, & Locklea, 2008). The added advantage of the natural products is their low toxicity and easy availability. Various herbs, plant extracts, essential oils and honey are often used for their antimicrobial properties. (Slover, Danziger, Adeniyi, & Mahady, 2009)

Honey is probably the oldest known wound dressing used from ancient times (Molan P. , 2006; Molan P. , 2009). In Ayurveda honey has been used both as a topical medicine as well as an internal medicine (Ediriweera & Premarathna, 2012). Honey, the natural sweetener is produced by honey bees and can be classified into different categories according to their floral source. Manuka honey, the most -studied honey for its antimicrobial activity, has broad spectrum antibacterial activity against sixty different species including some multi-drug resistant bacteria (Molan P. , 1992). Tualang honey, a Malaysian multifloral jungle honey, has been reported to have variable activities against wound and enteric bacteria (Tan, *et al.*, 2009). Medihoney is a licensed medical wound dressing product in Europe and Australia (Simon, Traynor, Santos, Blaser, Bode, & Molan, 2009). Nowadays a lot of medical grade honey is sold with standardised level of antibacterial activity (Deb Mandal & Mandal, 2011). Raw, untreated, natural honey often shows good antimicrobial activity.

In this report we have characterized a locally available raw untreated honey sample procured from a local beehive. We have analysed the physical properties and sugar composition of the honey sample. Further studies were carried out to ascertain its antimicrobial activity,

Materials and Methods:

Sample Collection:

Raw untreated honey sample from blossoms of blackberry plant were procured from a local beehive. The sample was collected in a clean container.

Measurement of Physical Properties:

The pH of the honey sample was estimated by using pH paper strip of suitable range. The moisture content of honey was measured by incubating 1ml of honey sample in a dessicator and weighing the sample till no change in weight was observed in two successive days. The final weight was subtracted from the initial weight to determine the moisture content

Determination of Sugar Composition by Thin Layer Chromatography:

The sugar composition of the honey sample was determined by running different dilutions of honey samples, 5%, 10%, 25% and 50%, as well as raw honey sample on preformed silica TLC plates. n-butanol, acetic acid and water in the ratio (3:1:1) was used as a solvent and diphenylaniline, aniline and phosphoric acid as the detection reagent. Glucose, fructose, lactose and sucrose were used as standards.

Well Diffusion Assay:

Four dilutions of honey samples (5%, 10%, 25% and 50%) were prepared by using sterile LB-broth. 100 ul of overnight bacterial (*Pseudomonas aeruginosa., Vibrio cholerae., B. subtilis, E. coli, S aureus, Klebsiella sp. Salmonella sp.*) or fungal culture (*Aspergillus niger, Candida albicans, Penicillium notatum*) were plated on respective agar plates. Wells were prepared on the agar plates and 50 ul of each honey dilutions were added to the wells in duplicates. The plates were incubated at 37^{o}C for 24hrs and diameter of zone of inhibition (ZOI) was recorded in triplicate.

Spectrophotometric Assay of Growth Inhibition:

Four dilutions of honey samples (5%, 10%, 25% and 50%) were prepared by using sterile LB-broth. For each microbial culture a set of four different honey dilutions along with a suitable control where no honey sample was added were inoculated with 2% overnight culture. Samples were incubated at 37^{o}C overnight and O.D. was measured at 595 nm.

Results and Discussion:

Physical Properties of Honey:

The raw honey sample collected from blackberry blossoms was dark brown in colour. Other physical properties are listed in Table 1

Table 1: Physical Properties of Honey:

pH	Density (gm/cc)	Moisture Content
4.5	1.405	13.75 %

Moisture content is an important property of honey and determines its quality as higher moisture content (greater than 20%) facilitates fermentation of honey by yeasts (Binnie, 2018). This raw honey sample is quite good in quality as its moisture content is quite low. The dark colour of the honey indicates presence of pigments in it (Albaridi, 2019). The low pH of honey is often related to its antimicrobial property (Albaridi, 2019).

Sugar Composition of Honey:

The main component of honey is sugar. Different types of sugars in varied proportions are present in different types of honey samples. However the primary components are glucose and fructose (Ouchemoukh, Schweitzer, Bachir-Bey, Djoudad-Kadji, & Louaileche, 2010). The sugar composition of this honey sample was assessed by running a thin layer chromatography. Figure 1 shows the TLC plate profile. In case of the raw honey and 50% honey sample there was huge drag due to high viscosity.

Figure 1: TLC profile of honey sample

Lane1-Lane5 are dilutions 5%, 10%, 25%, 50% and undiluted honey respectively. The different sugars lactose (lane6), glucose (lane7) sucrose (lane8), fructose (lane9) were run as standards. Lower three dilutions migrated well and distinct spots were observed. From thin layer chromatography presence of glucose, fructose, sucrose and a slight amount of lactose can be confirmed in this honey sample.

Antimicrobial Activity of Honey:

Honey has long been reported to have wound healing activity (Molan P. , 2009). Many local honey samples have shown good antibacterial activity (Albaridi, 2019). Hence we have analyzed the antibacterial as well as the antifungal activity of this honey sample by two different methods. First well diffusion assay was performed with seven different bacterial strains such as *Pseudomonas aeruginosa., Vibrio cholerae., B. subtilis, E. coli, S aureus, Klebsiella sp. Salmonella sp.* and three different fungal strains *Aspergillus niger, Candida albicans, Penicillium notatum.* However zone of inhibition was observed for only two bacterial strains *Pseudomonas aeruginosa., Vibrio cholerae* and two fungal strains *Aspergillus niger, Candida albicans.* The diameters of zone of inhibition are given in Table 2.

Table 2: Zone of inhibition diameter

Honey Dilutions (v/v)	**Average diameter of zone of inhibition (cm)**			
	Pseudomonas aeruginosa	***Vibrio cholerae***	***Aspergillus niger***	***Candida albicans***
50%	**3.23**	**3.47**	**2.13**	**2.23**
25%	**2.9**	**2.8**	**1.83**	**1.73**
10%	**2.27**	**-**	**-**	

To further validate the observed results spectrophotometric assay for growth inhibition was performed for two bacterial and two fungal strains that have given zone of inhibition. Figure 2 shows the growth inhibition results for different strains at various dilutions of honey. For two bacterial species, *Pseudomonas aeruginosa.*, *Vibrio cholerae* good growth inhibition was observed at 50% and 25% dilutions of honey. For *Aspergillus niger* good inhibition was observed for all dilutions except 5%. Whereas for *Candida albicans* only 50% and 25% showed inhibition. Inhibition at 50% dilution may be due to high osmolarity. However growth inhibition at lower dilutions i.e. 25% and 10% proves that this honey has some additional component responsible for its antimicrobial activity.

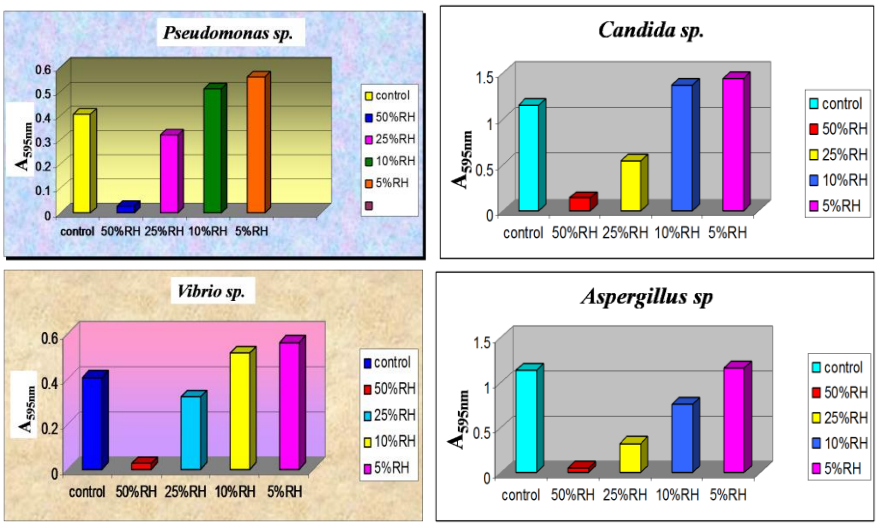

Figure 2: Graphical representation of zone of inhibition for two bacterial and two fungal cultures at different dilutions of honey

Conclusion:

The antimicrobial property of honey is often attributed to its high osmolarity, acidity, low water activity, hydrogen peroxide and non-peroxide phytochemical components like methylglyoxal (Deb Mandal & Mandal, 2011). Hydrogen peroxide in honey is produced by the enzyme glucose oxidase, which is naturally present in honey. However in undiluted honey it is inactive and gets activated only upon dilution. In diluted honey glucose oxidase acts on glucose of honey to produce hydrogen peroxide (Molan P. , 1992; White Jr., Subers, & Schepartz, 1963). Analyzed local raw honey sample showed significant antibacterial as well as antifungal activity at 50% and 25% (v/v) dilutions. To identify the origin of its antimicrobial activity well diffusion assay was performed with peroxidase treated honey that failed to show any zone of inhibition. Thus this particular honey sample when treated with peroxidase showed a drastic reduction in its

antimicrobial activity indicating hydrogen peroxide to be a major player in determining its antimicrobial activity.

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SULFOSALICYLIC ACID MEDIATED INDUCTION OF PR- PROTEINS IN GROUNDNUT

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Abstract:

Salicylic acid (SA) is an important signalling molecule, it plays an important defensive role in plants against various biotic and abiotic stresses. 5-sulfosalicylic acid (SSA) is one of the derivative of Salicylic acid. Although SA and its related compounds are well known for inducing various physiological and biochemical processes in plants, little is known about effects of SSA. A field experiment was, therefore carried out to evaluate the possible involvement of antioxidative enzymes and lipid peroxidation in SSA mediated induction of pathogenesis related proteins (PR-protein) in groundnut. The results showed that all the concentrations of SSA increased lipid peroxidation, activities of peroxidase and superoxide dismutase. In particular, the treatment of 50 ppm SSA found better influence. In contrast to it the activity of enzyme catalase was decreased with all the applied concentrations of SSA. The protein profile revealed appearance of relatively prominent PR-protein bands in 5 and 50 ppm SSA treated plant leaves. The detected protein bands are generally belonging to the PR-1 and PR-5 families having chitinase and glucanase activity. The results pertinent to antioxidative enzymes and protein profile indicated that SSA mediated induction in lipid peroxidation could act as translocated signal that may elevate PR- protein induction and establish systemic acquired resistance (SAR) in groundnut.

Keywords: Antioxidative enzymes, Groundnut, PR-proteins, Sulfosalicylic acid, Systemic Acquired Resistance

Introduction:

Salicylic acid (SA) is an important natural endogenous signal molecule (Raskin, 1992). It plays a major defensive role in plants against various biotic and abiotic stresses (Huang *et al*.,

2008; Sayeed *et al.*, 2011). SA has drawn the attention of researchers due to its ability to induce systemic acquired resistance in plants. Sulfosalicylic acid (SSA) is one of the derivative of salicylic acid. There are 33 analogs of salicylic acid and although they have been reported to be involved in various physiological processes of plants (Hayat *et al*., 2007; Cag *et al.*, 2009), not much work has been carried out on influence of 5-sulfosalicylic acid on physiological responses of plants. Groundnut is the major annual oilseed crop of India and it is cultivated mainly for oilseeds and food. Several studies revealed significant reduction in productivity potential of groundnut in India as compared to other countries due to reduction in acreage under peanut, insufficient rainfall, pests and diseases etc. (Ramkrishnan and Apparao, 1968; Kaushik, 1993). The groundnut production is affected by diseases throughout its life i.e. from planting to storage. Various strategies are now available to control these diseases to some extent including fungicides and genetic control. But chemical control is restricted due to its expensive and toxic as well environmental concerns. So as to control cost of chemical control and environmental safety, now there is need to develop alternative strategies to control diseases and enhance productivity of crop in limited land at highest potential. Keeping this view in mind the present investigation was conducted to assess the possible involvement of antioxidative enzymes and lipid peroxidation in SSA mediated induction of pathogenesis related (PR) proteins and thereby to reveal its role in disease resistance and adaptation of groundnut crop to environmental changes.

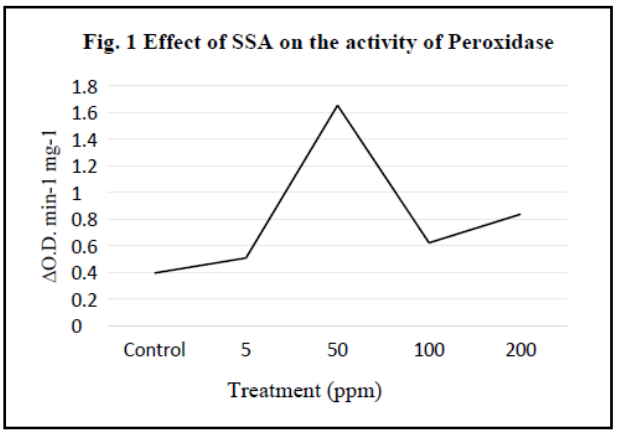
Material and Methods:

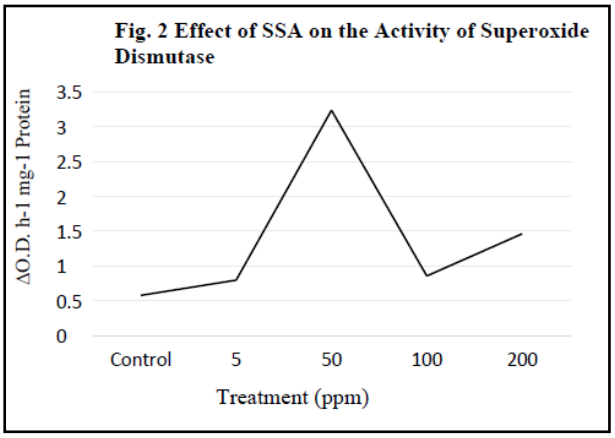
The seeds of groundnut cultivar SB-11 were collected from agricultural research station, Karad. The experiment was laid out in Randomized Complete Block Design (RCB) with three replications. Fifteen days old plants were sprayed with different concentrations (5, 50, 100 and 200 ppm) of SSA (40-50 ml/pl). The physiological parameters were studied at the end of exogenous foliar treatments.

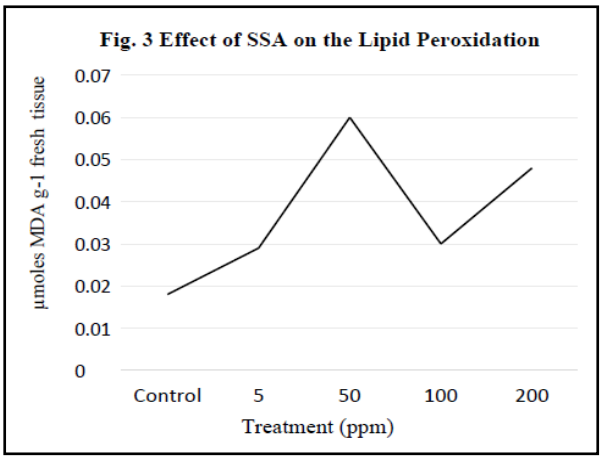
Lipid peroxidation was determined following the method described by (Cakmak and Horst, 1991). The activity of enzyme catalase was determined by the method described by (Sadasivam and Manikam, 1991). The enzyme peroxidase activity was studied by the method of Maehly and Chance (1954). To study the activity of enzyme Superoxide dismutase the method given by (Giannopolitis and Reis, 1977) was followed. The leaf protein profile of groundnut was prepared following the SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electophoresis) technique.

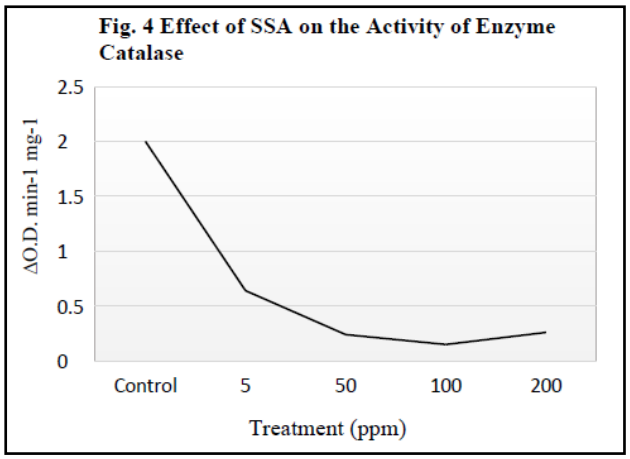
Result and Discussion:

Results showed that the foliar application of 50 ppm SSA noticeably enhanced MDA content, activity of enzyme peroxidase and superoxide dismutase (Fig. 1-3). Our earlier studies showed acetyl salicylic acid mediated increased activity of peroxidase, superoxide dismutase and lipid peroxidation in groundnut (Jadhav and Bhamburdekar, 2015).









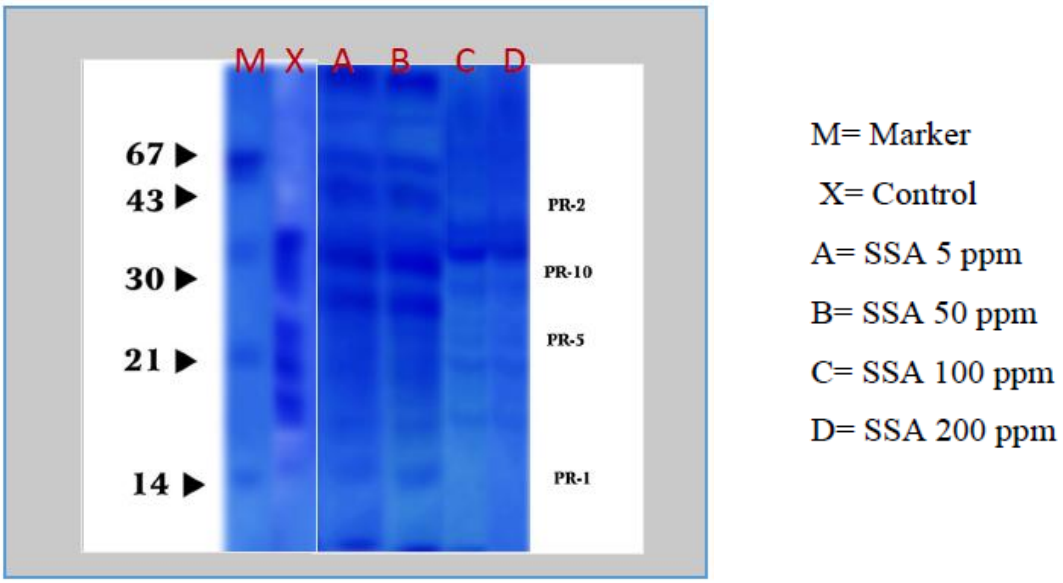


Figure 5: Leaf Protein Profile of Sulfosalicylic Acid Treated plants

Present studies show similarity with results of Radwan *et al.* (2007) and Rakhmankulova *et al.* (2012). It was found that all the applied foliar treatments of SSA decreased the activity of catalase enzyme (Fig. 4). The protein profile revealed appearance of relatively prominent Pathogenesis Related (PR) protein bands in 5 aand 50 ppm SSA treated plant leaves (Fig. 5). The detected protein bands belonging to the PR-1 and PR-2 families having chitinase and glucanase activity. It signifies the protective role of SSA against pathogenic infection. SSA-mediated catalase inhibition increases the accumulation of free radicals resulting in hypersensitive cell death. The administration of SSA may enhance H_2O_2 production by inactivation of H_2O_2 degrading enzyme-catalase which can alter the cellular redox state and may further enhance

generation of active free radicals of salicylic acid (SA*) as indicated by Rao *et al*. (1997) and Anderson *et al.* (1998).

Active free radicals of salicylic acid may further initiate lipid peroxidation and activation of secondary metabolites as well as PR-genes. Although SA is essential signal molecule for the establishment of SAR, it cannot be considered as translocated signal. Thus, its derivative, SSA-generated lipid peroxidation may act as translocated signal. In such a way SA and its derivatives such as sulfosalicylic acid (SSA) and acetyl salicylic acid (Jadhav and Bhamburdekar, 2015) in active forms induce accumulation of lipid peroxidation products via catalase inactivation and higher H_2O_2 production and efficiently enhance disease resistance in plants.

Conclusion:

The induction in lipid peroxidation in present study could act as translocated signal that may elevate PR- protein induction and establish systemic acquired resistance (SAR) in groundnut. The results pertinent to antioxidative enzymes and protein profile indicated that SSA mediated the induction in lipid peroxidation and PR-gene expression is closely related with catalase inhibiting action of SSA and confer resistance in groundnut against pathogen attack.

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UBIQUITOUS PHYTOHORMONE ABSCISIC ACID IN PHYTOREMEDIATION AND BIOMEDICAL APPLICATIONS: AN OVERVIEW

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Abstract:

Abscisic acid (ABA), a central regulatory hormone involved in seed dormancy, closure of stomata, plant growth and development is triggered as a plant defense mechanism against biotic and abiotic stresses, Heavy metals that include lead, mercury, cadmium, arsenic, zinc, are natural non-biodegradable constituents of the Earth's crust that accumulates and persists indefinitely in the ecosystem as a result of human activities, contaminates soil and water resources, leading to pollution and significant alteration in morphology, physiology, and biochemistry that yield losses in plants. Therefore, bioremediation being an effective and inexpensive technique has been utilized to detoxify the heavy metals. ABA may play a vital role in plant response to heavy metal toxicity by stimulating plant antioxidant defense system. The oxidative stress and inflammation are closely interconnected. There is gaining interest and pertinent search to use plants as multi-component agents to modulate the immune system and to prevent infections and inflammations.Hence, in this study, the plant hormoneABA which sustains the plant in stress condition has been explored for its potential therapeutic applications as an immunomodulator and anti-inflammatory agent in ischemic retinopathies, inflammatory bowel diseases, influenza- virus-associated diseases, fungal infections, malaria, diabetes, tuberculosis, atherosclerosis, cancer, Atherosclerosis, Alzheimer's and neurodegenerative diseases

Keywords: Abscisic acid, stress, heavy metal toxicity, phytoremediation, anti-inflammatory, immunomodulator

Introduction:

Plant hormones also are known as phytohormones/biological growth bioregulators are chemicals other than nutrients produced by plants at specific locations in very lower concentrations causing alterations at target cells at other locations that involvesthe regulation of germination, growth, metabolism, or other physiological activities (Sakthivel *et al.*, 2016)

Classification based on their actionas:

Promoters:

Auxins, Cytokinins, (CKs) Giberillins (Gas), Brassinosteroids

Inhibitors:

Abscisic acid (ABA), Salicylicacid (SA) Ethylene (ET), Jasmonates(JA)

- The positive or negative crosstalk between ABA, SA, JA, and ET with the major growth -promoting hormones, i.e. Auxins, Gas, and CKs determinesthe stressresponses inplants (Lin *et al.*, 2015)

Abscisic acid/Dormidin/Abscisin II:

Chemistry:

Chemically, ABA has a formula $C_{15}H_{20}O_4$, and its appearance is colourless crystals. ABA belongs to the terpenoid (also known as isoprenoid) class of plant metabolites.

Physiology/Functions of ABA:

Abscisic acid (ABA), a central regulatory hormone is involved in seed dormancy, closure of stomata, plant growth, and development istriggered as a plant defensive mechanism against biotic and abiotic stresses(Endo *et al.*, 2014)

Biosynthesis:

ABA is synthesized from zeaxanthin through a five-step biosynthetic process catalyzed by the proteins ABA1, ABA4, NCED, ABA2, and ABA3.

Zeaxanthin and initial biosynthetic reactions arelocalized in plastids. xanthoxin (ABA2, ABA3) and other reactions occur in the cytoplasm. Metabolism occurs through CYP707A mediated oxidative degradation or conjugation with glucose (Fig 1).Fungi has an alternative biosynthetic pathway (Liotenberg *et al.*, 1999; Endo *et al.*, 2014).

ABA in plants:

The responses include short term -closure of stomata to maintain water balance and long term -growth and development of plants. These responsesare mediated through

Upregulating the expression of ABA biosynthetic genes (*zeaxanthin epoxidase (zep)* geneand **Low Expression** Of Osmotic Stress-responsive Gene (6los6 */ABA1*,) *Aldehyde Oxidase* gene (*AAO3*), *9-Cis-Epoxycarotenoid Dioxygenase* gene (*NCED3*), *Molybdenum Cofactor*

Sulfurase gene (*MCSU*; LOS5*/ABA3*)(Bellaire *et al.,* 2000; Verma et al 2016; Hauser *et al.*,2017)

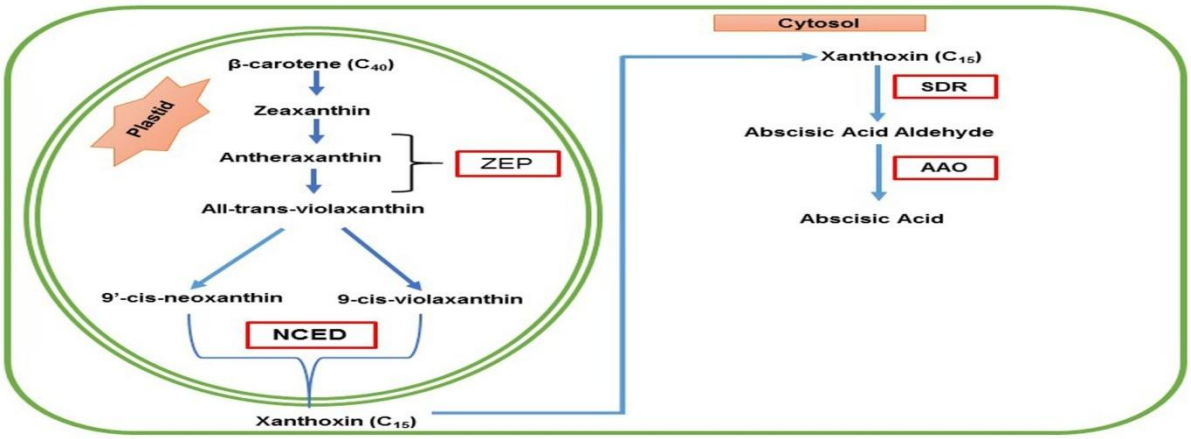

Figure 1: Biosynthetic pathway of Abscisic acid (ABA) (Courtesy, Sah *et al.,* 2016**)**

ABA – other than plant sources:

ABA is present in wide range of animals, sponges (Axinella polypoides),hydroids (Eudendrium racemosum), human parasites (Toxoplasma gondii), andvarious mammalian tissues and cells (leukocytes, pancreatic cells, and mesenchymal stem cells). It is also present indifferent dietary sources and endogenously produced by the carotenoid biosynthesis pathway ABA canbe administered either through different nutritional sources or as a drug (Magnone *et al.*, 2009; Li,et al 2011;Chaqour *et al.*, 2018)

Mechanism of action of ABA:

Through G-coupled membrane protein –Lanthionine synthetase C-like protein 2 (LANCL-2)

↓

ABA binds to LANCL-2

↓

Triggers a PKA-dependent cascade

↓

Activates the ADP-ribosyl cyclase, mobilization of cyclic ADP-ribose

↓

Increases cyclic AMP-dependent cellular calcium

(Sturla *et al.*, 2011; Fresia *et al.*, 2016; Hauser *et al.*, 2017)

Application of ABA in Phytoremediation of Heavymetals:

Heavy metals:

The contamination of natural ecosystems by heavy metals is a worldwide environmental concern endangering agricultural systems,Bioactive-metals are divided into two groups:
redox metals -Cr, Cu, Mn, and Fe, andnon-redox metals - Cd, Ni, Hg, Zn, and Al.

The redox metals directly generate oxidative injury in plants through Haber-Weiss and Fenton reactions stimulatingthe production of ROS and causes oxidative stress (Vanhoudt *et al.,* 2010; Zhao,et al 2012)

The non-redox metals are indirect oxidative stressors. The mechanisms involve glutathione depletion, binding to sulfhydryl groups of proteins, inhibiting antioxidative enzymes or inducing ROS-producing enzymes like NADPH oxidases (Yu *et al.,* 2019)

High concentrations of contaminants affect plants from molecular to physiological levels (DalCorso *et al.,* 2013; Li *et al.,* 2014).

Phytoremediation:

Phytoremediation is defined as plant-influenced biological, chemical, and physical processes that aid in the uptake, degradation, and metabolism of contaminants by either plants or free-living organisms in the plant's rhizosphere.

ABA Elevation in Heavy Metal toxicity:

ABA concentration has been observed to increase during Cadmium (Cd)- *Typha latifolia* and *Phragmites australis, rice plants*,Mercury (Hg), Cd and Copper (Cu) -wheat seeds during germination, Copperand Zinc -cucumbers, seed germination decreased,Lead (Pb) -chickpea (*Cicer arietinum*) seeds,Copper and nickel (Ni)-crowberries (*Empetrum nigrum*) toxicities which is a protective mechanism against heavy metal toxicity(Sah *et al.,* 2016;Singh *et al.,* 2016; Soti *et al.,* 2018).

ABA acts as chemical messengers with highly complex regulation thereby allow plants to retain growth plasticity during development and respond to abiotic and biotic stresses (Krämer *et al.,* 2007;Zhao *et al.,* 2012; Lin *et al.,* 2013; Ivanov et al 2016)

Stress-Ros- Inflammatory Responses:

ROS - Reactive Oxygen Species released during stress causes

- Lipid- peroxidation- causesan alteration in permeabilityof plasma membrane.
- Protein – causes adduct formation affecting signaling and enzymatic pathways
- DNA- causes mutation.
- Mitochondria stimulates cytochromes involved in apoptosis process and cell death.
- Myelin sheath – neurodegenerative diseases

Oxidative stress and inflammation are tightly interconnected (Allegra, 2019). Inflammation is a cellular response to external stimuli that compromises cell and tissue homeostasis, and acts as a defense mechanism to preserve the stability of cellular functions Identifying the natural phytochemicals, which generally act through multiple cell-signaling mechanisms, but minimally affects the overall health of tissues, seems a better alternative approach, ROS and inflammatory response (Jiang and Zhang, 2001,2003;Wang *et al.*, 2013; Ng *et al.*, 2014; Sakthivel *et al.*, 2016) is illustrated in following figures (2a,b, c)

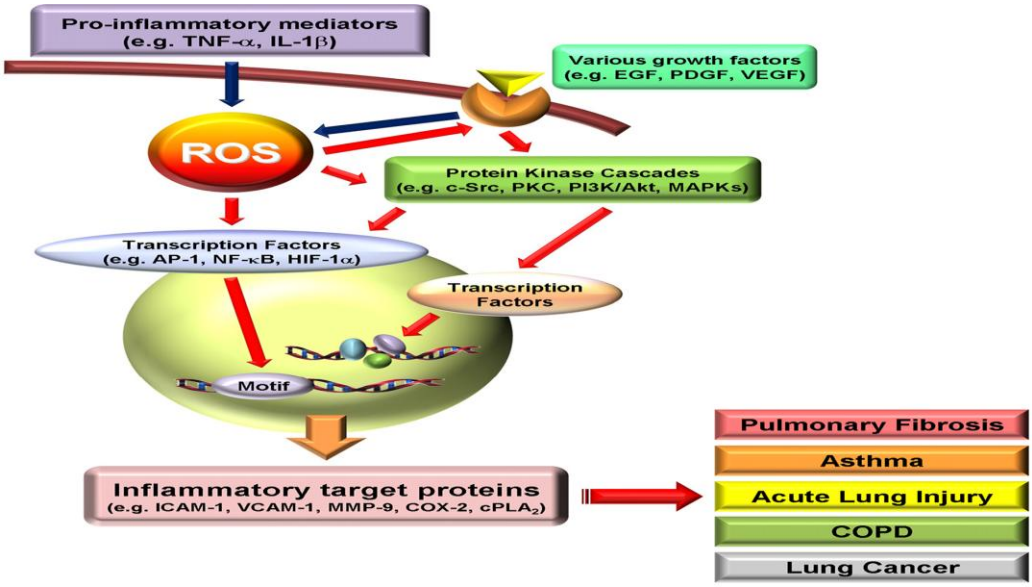

Figure 2a: Reactive Oxygen species (ROS) and inflammatory responses in lung (Lee and Yang, 2012)

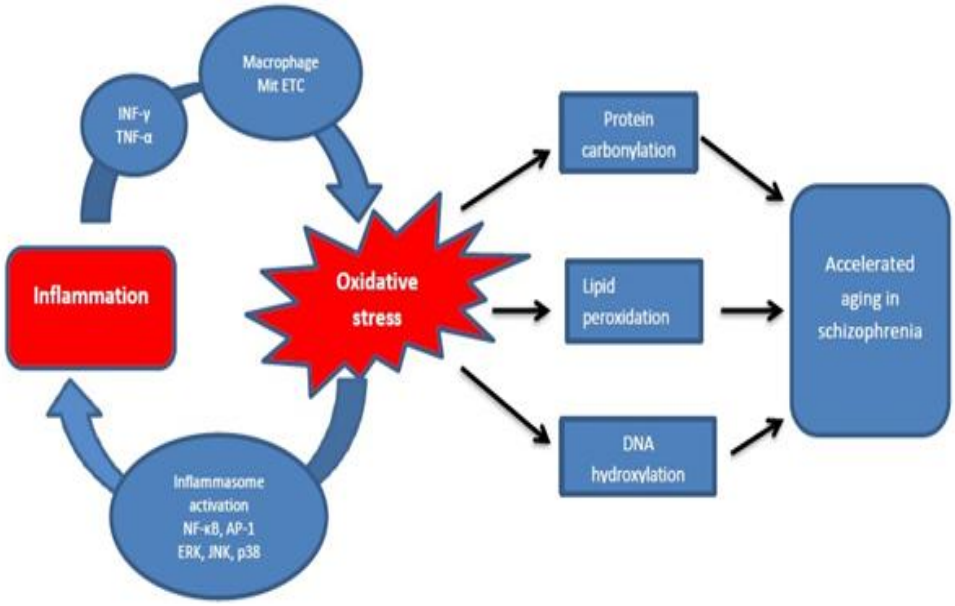

Figure 2b: Reactive Oxygen species (ROS) and neurodegenerative diseases (Olaoluwa O Okusaga, 2014)

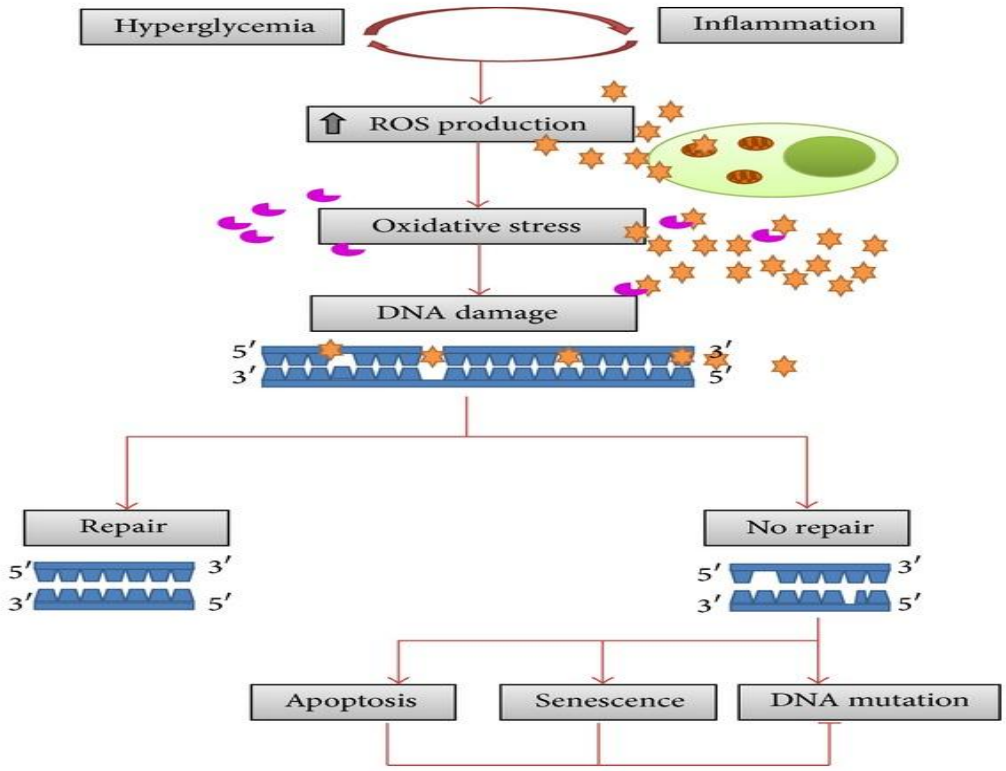

Figure 2c: Reactive Oxygen species (ROS), inflammationand Diabetes (Moreli *et al.*, 2014)

ABA, Anti-inflammatory activity and biomedical applications:

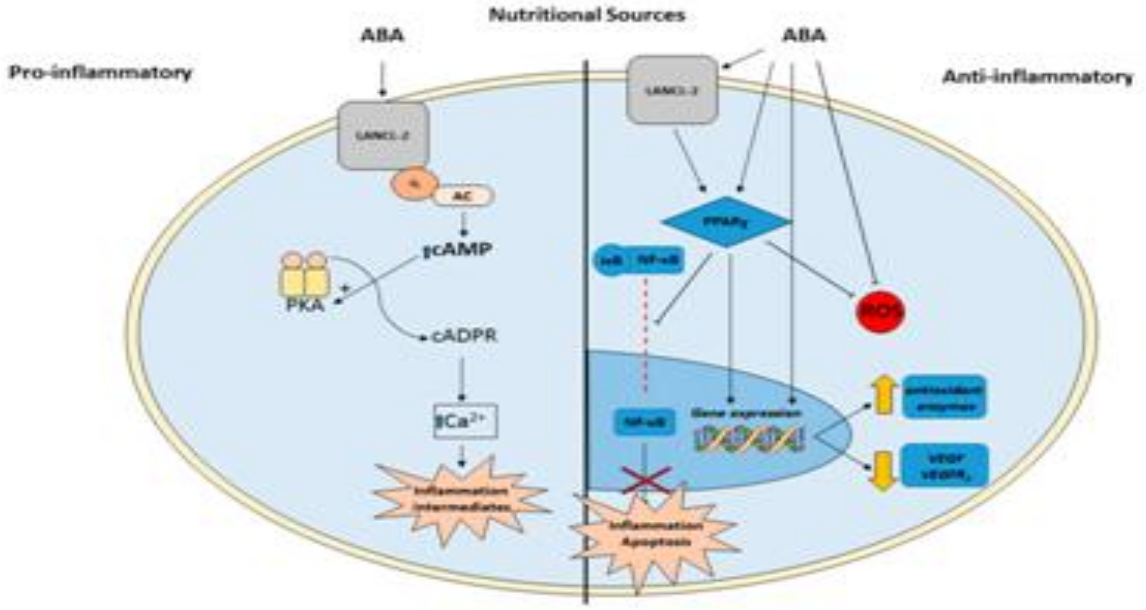

Figure 3: Abscisic acid and anti- inflammatory responses (Baliño et al 2019)

ABA plays a dual function and possesspro- and anti-inflammatory action, ABA causes knocking down endogenous pro-migratory genes in endothelial cells, thus promoting endothelial

quiescence. ABA reduces oxidative stress in rat brain by altering the MDA and H_2O_2 levels in rat diencephalon and elevates the activities of antioxidant enzymes catalase and peroxidase and overall prevents the release of ROS (Guan *et al.,* 2000)

ABA exhibits antiapoptotic activity throughupregulation of PPARs (peroxisome proliferator activated receptors) signaling cascade (Bassaganya-Riera *et al.,* 2010) (Fig. 3)

ABA- Biomedical Applications:

ABA due to its Antioxidant and Immunomodulating activity can be utilized in the treatment of Type-2 Diabetes (Guri *et al.,* 2007; Atkinson *et al.,* 2019). Atherogenesis (Magnone *et al.,* 2009), neurodegenerative diseases like Alzheimer and parkinsonism (Sanchez-Perez, 2020) and Ischemic retinopathy (Moran *et al.,* 2016; Baliño *et al.,* 2019). Additionally, ABA have antimicrobial (Hontecillas *et al.,* 2013; Khedr *et al.,* 2018), anticancer (Rafiepour *et al.,* 2019) and antimalarialactivity (Glennon *et al.,* 2016).

Conclusion:

To identify the drug with multifaceted functions will be a boon to the researchers to explore itfor therapeutic utilization. The recent findings of ABA, itssignaling pathway, mechanism of action in plants and animals reveals that ABA is the crucial hormone that plays a pivotal role in plants during unfavourable conditions and beneficial in phytoremediation of heavy metal toxicity. In humans with its extensive efficacy, ABA can be used in inflammatory, infectious and diseased conditions.Future prospective of the phytohormone is promising to be utilized asdrug or novel nutritional intervention for the management of several pathological conditions

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FLUORIDE INDUCED CHANGES IN ANTIOXIDATIVE ENZYMES OF MEDICINALLY IMPORTANT OIL YIELDING PLANT *SIMAROUBA GLAUCA*

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Abstract:

Fluoride occurs in the earth surface in very low amount, but it acts as pollutant. Fluoride is found in soil, water, air and also in plant with varying concentrations. This leads to the toxicity to animal and plants. The fluoride was potent metabolic inhibitor. Antioxidative enzyme activities of medicinally important oil yielding plant *Simarouba glauca* were evaluated under fluoride stress. One year seedlings of *S. glauca* were subjected to fluoride stress. In present study shows the effect of fluoride concentration (100mM, 200mM and 300mM) as the decrease in peroxidase activity of root tissue indicates generation of ROS. In catalase activity increases with increased concentration of fluoride this will helps to detoxify the H_2O_2. This develops the fluoride as oxidative stress tolerance mechanism, therefore this plant is used for phytoremediation.

Keywords: Antioxidative enzyme, *Simarouba glauca,* sodium fluoride, catalase, peroxidise.

Inroduction:

Peroxidase are heme containing glycoprotein which involves in the biosynthesis of lignin and ethylene, defense against pathogens and wounding, auxin metabolism and stress response (Halbrock and Gricebach, 1979; Lagrimini and Rothstein, 1987; O'Nell and Scoot, 1987 and Kim *et al.,* 1999). This enzyme has also a role in secondary cell wall formation (Thaker *et al.,* 1986), and suberization (Kollatukudy *et al.,* 1989). The peroxidase blockers ascorbic acid

(Castillo and Greppin, 1988) and isoflavones located in cell wall are involved in phenolic cross-linking reactions (Ferrer *et al.*, 1990). Catalase is found predominantly in leaf peroxisomes, where it functions chiefly to remove H_2O_2 formed during photorespiration in C3 species (Dat *et al.*, 2000). According to Guo *et al.*, (2006), catalases are localised in mitochondria of maize and in the apoplast. They have further added that preliminary scavenging of H_2O_2 done by catalase and ascorbate peroxidase. Sairam *et al.* (1998) speculated that plants have evolved a complex antioxidative defense system composed of antioxidant enzymes and metabolites like superoxide dismutase, catalase, peroxidase, glutathione reductase, ascorbate peroxidase, ascorbic acid, reduced glutathione and vitamin E to counter the harmful effects of ROS.

Materials and Methods:

The seed coats are removed and seeds were surface sterilized with 0.1% mercuric chloride (HgCl2) for 10 min and thoroughly washed with distilled water. The seeds were soaked in distilled water for 12 hrs and sown in poly-bag containing FYM and soil with the ratio of 1:3. The one year old fully grown seedlings of *Simarouba glauca* DC. were transplanted in earthen pots. Seedlings were settled by regular watering in polyhouse of Botany Department, Shivaji University Kolhapur. After one month plants were transplanted to the pots were irrigated alternating with tap water and 100mM, 200mM and 300mM sodium fluoride solution twice in a week for a period of 40 days. The leaves from each treatment were randomly selected and washed thoroughly with water and blotted to surface dry. The activity of enzyme peroxidase was determined with the help of the method of Horiguchi (1988). Catalase activity was assayed by following the method of Luck (1974) as described by Sadasivam and Manickam (1992).

Results and Discussion:

The activity of enzyme Peroxidase was increased by 2-3 folds in the leaf and stem tissues up to 200mM Sodium fluoride treatments and further it was considerably decreased in response to 300mM Sodium fluoride concentration. A group of peroxidases is responsible for development of oxidative stress tolerance in plants as well as the fluoride toxicity leads to the production of ROS that give the more production of peroxidises in the tissues of mulberry leaves as indicated by Kumar *et al.* (2009). Similar mechanism might be prevailing in case of *S. glauca* at lower levels of fluoride stress. The activity of enzyme peroxidase was elevated in the leaf and stem tissues at 100 and 200mM sodium fluoride treatments which might be responsible for the development of oxidative stress tolerance, while the reduction in activity of this enzyme at 300mM concentration might be producing ROS in excess in the leaf and stem tissues.

Continuous decrease in the peroxidase activity in the root tissue also indicates generation of ROS in fluoride exposed prime root tissue.

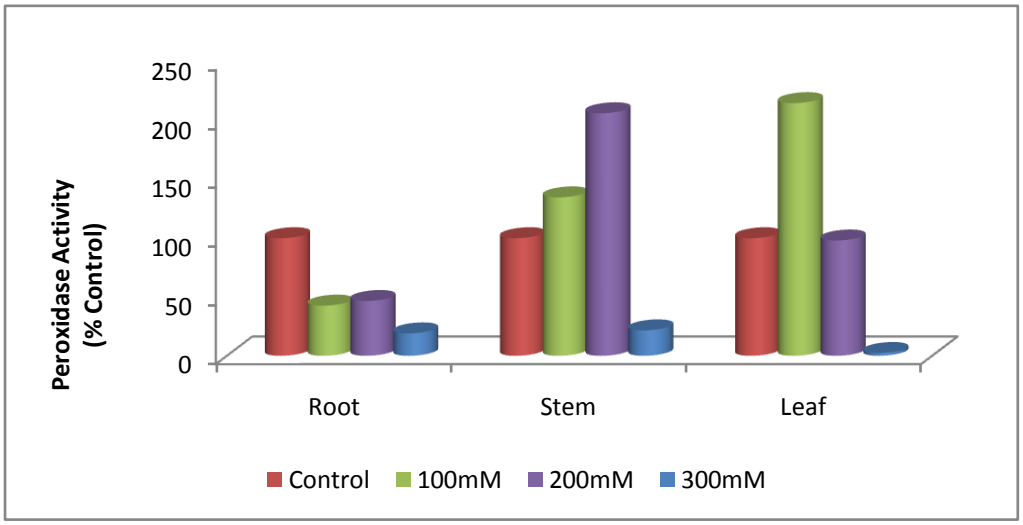

Figure 1: Effect of sodium fluoride on the activity of enzyme peroxidase in root, stem and leaves of *S. glauca*

Control value: Root- 15.16 ΔOD h^{-1} mg^{-1} protein, Stem-5.41 ΔOD h^{-1} mg^{-1} protein, Leaves-2.08 ΔOD h^{-1} mg^{-1} protein

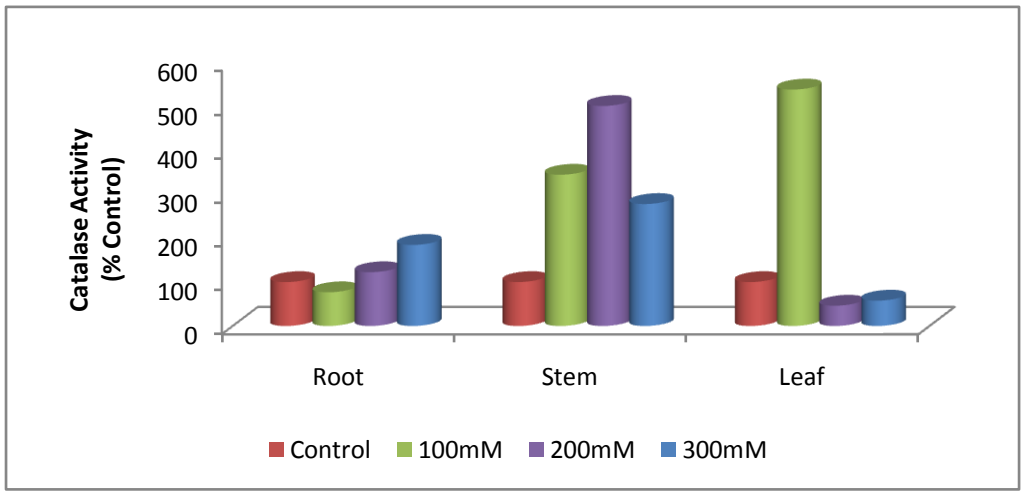

Figure 2: Effect of sodium fluoride on the activity of enzyme catalase in root, stem and leaves of *S. glauca*

Control value: Root- 2.80 ΔOD h^{-1} mg^{-1} protein, Stem-0.09 ΔOD h^{-1} mg^{-1} protein, Leaves-0.26 ΔOD h^{-1} mg^{-1} protein

The activity of enzyme catalase was increased in the root tissue with increasing concentration of sodium fluoride. Leaf and stem tissues showed an increase in the level of catalase upto 100mM sodium fluoride treatment, while at 200mM and 300mM concentrations it was drastically decreased. H_2O_2 acts as a precursor for cytotoxic ROS. Thus the elevation of activity of enzyme catalase in the root tissue with increasing concentration of sodium fluoride, might have helped to detoxify the H_2O_2 leading to the development of oxidative stress tolerance.

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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFICIENCY OF TWO MACROLICHEN SPECIES FORM KARNATAKA

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Abstract:

Lichens are nonflowering plants which consist of two partners living in symbiotic association. In the folklore of many countries, lichens were used as a remedy for pulmonary tuberculosis, Diahorria, epilepsy and in the treatment of wounds and skin disorders. Lichens produce diverse range of secondary metabolites, depsides, depadones, furans and pulvic acid which are unique to lichen symbioses. These compounds have attracted much attention in investigations because of their antiviral, antibiotic, antioxidant, antitumor, allergenic and plant growth inhibitory activities. The study was conducted for evaluating phytochemical and antimicrobial potential of *Parmotrema cristiferum* (Taylor) Hale (Parmeliaceae) and *Ramalina pacifica* Asahina (Ramalinaceae). It is established that Lichens have been used by various ethnic groups from the time of early civilization; however very less scientific reports on its medicinal properties. With this background, in the present investigation chloroform and methanol extracts of lichen thallus were screened for their antimicrobial activity. The antibacterial activity is tested by using four bacterial strains *S. typhimurium*, *K. pneumonia*, E.coli. and *P. auregionsa*. The methanolic extract of *P. cristiferum* show good activity against *E.coli* (14% of inhabitation at 25% concentration) and *S. typhimurium* but the chloroform extract *R. pacifica* had shown to very little activity against bacteria. The extracts were found to possess more activity against Gram positive bacteria than Gram negative bacteria. *A.niger, C. albicans, M. gypsium and T. rubrum* were selected for antifungal activity. Methanolic extract showed inhibition against selected fungi. The extracts could be used to treat infections caused by these bacteria. The presence of various secondary metabolites in the extracts highlights the antibacterial efficacy of the lichens.

Keywords: Lichens, *Parmotrema cristiferum*, antibacterial activity, *E.coli*

Introduction:

Lichens produce diverse range of secondary metabolites, depsides, depadones, furans and pulvic acid which are unique to lichen symbioses. The lichen derived compounds were gained more attention in investigations because of their antibiotic, antioxidant, antiviral, antitumor and antiallergenic activities (Boustie and Grube, 2005; Muller, 2001). But, lichen potentiality have been neglected by the current pharmaceutical industry, despite of novel lichen secondary metabolites with diverse structures and that studies have provided evidence of bioactivity in extracts from lichens (Behera *et al.* 2005). Lichens produce large number of chemical products many of which have been found to be antimicrobial activity (Elix, 1996; Lawrey, 1986) most lichen having phenol derivatives they having high antimicrobial activity (Elix, 1996). The present short communication deals with the evaluation of antimicrobial activities of *Parmotrema cristiferum* (Taylor) Hale (Parmeliaceae) and *Ramalina pacifica* Asahina (Ramalinaceae). The secondary metabolites are produced only by the mycobiont and they will be secreted onto the surface of lichen hyphae either they will be in amorphous or as crystalline forms (Srivastava, 2013). There are about 350 unique metabolites have been found in the lichen (Elix, 1996; Gaulan, 1988). These unique chemical compounds are also known to show valuable biological activities against microorganisms (Shukla, 2018). Usnic acid is the highly investigated lichenic compound with their bioactivity, because of their antiviral, antiproliferative, analgesic, anti-inflammatory, antipyretic and antimicrobial activities as well as their UV protective nature (Ingólfsdóttir, 2002; Cocchietto *et. al.* 2002).

Materials and Methods:

Collection of lichen:

Lichen material was collected from the Chikmaglur region of Western Ghats, India at an altitude of 750-900meters from the twigs and bark of *Ziziphus* and *Terminalina* tree. Collected materials representative samples were prepared herbarium and they were deposited in the Department of Botany, KU herbaria and Voucher specimen no. KU0023 and KU0075. The lichens species were identified by morphological, anatomical and chemical test and final confirmation by using standard manual (Awasthi, 2007).

Parmotrema cristiferum

Ramalina pacifica

Preparation of extract:

The lichen was air dried at room temperature under shade. After air-drying, the lichen material was ground to fine powder and extracted by soxhlet apparatus using chloroform and methanol as solvent. The lichen solvent extracts were filtered by using Whatmans filter paper no. 1 and they were concentrated at an temperature of 40^{o}C under reduced pressure (Manjunatha *et al*., 2006). The condensed chloroform and methanol extract were stored at 4^{o}C until use (Manjunatha *et al*., 2006).

Phytochemical analysis of solvent extract:

The lichen extract were obtained after solvent evaporation, later the extract was subjected to standard phytochemical tests for detection of saponins by frothing test, glycosides by Salkawski test, sterols by Burchard test, alkaloids by Mayer's reagent test, terpenoids by Salkowski test, tannins by ferric chloride test and flavonoids were detected by Shinoda test (Mallikarjuna *et al*., 2007; George *et al*., 2010).

Detection of lichen metabolites by (TLC) Thin layer chromatography:

TLC was performered in solvent A (180 ml toluene: 60 ml 1-4, dioxine: 8 ml acetic acid) this will help in detection of secondary metabolites and we have calculate the RF value and finalize the compound identification by using standard protocols (Culberson, 1972; Walker and James, 1980).

Antimicrobial assay:

The lichen was washed with distilled water then dried at room temperature. The powdered lichen was wrapped in a in a cylindrical pouch (Whatman filter paper grade 1) and kept inside the extractor arm of the soxhlet apparatus. Based on their polarity we were extracted in different solvents like Chloroform and Methanol. The final filtrate of each of the extraction obtained was concentrated using a Buchi Rotary Evaporator. A total of four bacterial cultures *Pseudomonas auregionsa* (MTCC1934), *Salmonella typhimurium* (MTCC1251), *Klebsiella pneumonia* (MTCC432), *Escherichia coli* (MTCC405), and the fungal cultures *Aspergillus niger* (MTCC478), *Candida albicans* (MTCC563), *Microsporium gypsium* (MTCC2819), *Trichophytum rubrum* (MTCC3272), were used in this investigation. All these cultures were obtained from MTCC collection IMTECH (Institute of Microbial Technology), Chandigad, India. The cultures were maintained at 4ºC and subculture frequently in respective media.

Antibacterial activity was tested using well assay method. The nutrient agar medium for bacteria and PDA medium for fungi was transferred in to one fourth volume of the petri plate. Inoculation of cultures (60µl) to this medium was carried out uniformly using a glass spreader. Different concentrations of crude extract of Methanol and chloroform (i.e. 5%, 10%, 15%, and 25%) were prepared as individual stocks in sterile vials and these were transferred (30µl) to the wells. These plates were incubated at 37ºc for 24hrs. The inhabitation of bacterial and fungal growth was determined by measuring the diameter of the clear zone around each well. In the centre well soaked with respective solvents that serve as a control. Chloramphenicol-50µg/well taken as standard antibiotic. An average of the triple reading for each microorganism was recorded. All data were expressed as mean ± SD of the number of experiments (n = 3).

Result and Discussion:

Phytoconstituents detected by chemical tests:

Preliminary phytochemical analysis of methanol extract of of *P. cristiferum* and *R. pacifica* was determined by chemical tests. Phytoconstituents like terpenoids, saponins, alkaloids and tannins were detected in the solvent extract **(Table-1)**.

Secondary metabolites detected by TLC:

TLC in solvent A showed the presence of *R. pacifica* showed presence of Usnic acid, Sekikaic acid and *P. cristiferum* showed presence of Atranorin, Salazinic acid in the lichen material.

Table 1: Phytochemicals test

Phytoconstituent	**Test**	***P. cristiferum***	***R. pacifica***
Alkaloids	Mayer's test	-	-
Saponins	Frothing test	+	+
Flavonoids	Shinoda test	-	-
Glycosides	Salkowski test	-	-
Tannins	Ferric chloride test	+	+
Sterols	Burchard test	-	-
Terpenoids	Salkowski test	+	+

'+' Present; '-' Absent

Antimicrobial activity:

The methanolic extract of *P. cristiferum* show good activity against *E.coli* (14% of inhabitation at 25% concentration) and *S. typhimurium* but the chloroform extract *R. pacifica* had shown to very little activity against bacteria (Table -2). The lichenic extracts were found to having possessed good activity against gram positive bacteria than the gram negative bacteria and result of the experiment found that the lichenic extract and standard (Chloramphenicol) have shown inhibition against the all tested bacteria (Table -2). Among bacteria, *P. aeruginosa* and *E. coli* were found to be more sensitive to lichen extract as revealed by larger inhibition zones. *A.niger, C. albicans, M. gypsium and T. rubrum* were selected for antifungal activity. Methanolic extract showed inhibition against selected fungi (Table-3). The lichen solvent extracts can be used to treat infections caused by above mention bacterial strains. The presence of various secondary metabolites in the extracts highlights the antibacterial efficacy of the lichens.

Humans have exploited lichens for various purposes among which most important use has been for dyeing purposes. Besides that, lichens were used extensively in traditional medicines and for cosmetic purposes (Huneck, 1999; Dayan, 2001; Hawksworth, 2003; Rojas, 2003). The antibacterial activity of the extracts tested could be due to the presence of inhibitory principles in the extracts. There are plenty of literatures available on antimicrobial activity of lichens and their purified compounds. The antimicrobial activity of the different solvent extracts of the lichen *Cladonia foliacea* and their isolated compounds like usnic acid, atranorin tested against nine bacteria and fungi (Yilmaz, 2009; Shukla 2018).

Table 2: Showing Antibacterial activity

Sr. No.	Organisms	standard	Diameter of zone inhibition (mm)															
			Parmotrema cristiferum **Methanol extract (%)**				***Parmotrema cristiferum*** **Chloroform extract (%)**				***Ramalina pacifica*** **Methanol extract (%)**				***Ramalina pacifica*** **Chloroform extract (%)**			
			5	10	15	25	5	10	15	25	5	10	15	25	5	10	15	25
1	*E. coli*	23±0.3	10 ± 0.1	12± 0.2	12± 0.4	14± 0.2	-	5± 0.3	6± 0.3	8± 0.2	6± 0.3	8± 0.2	10± 0.3	12± 0.2	5± 0.3	5± 0.2	6± 0.4	7± 0.3
2	St	21±0.4	5± 0.3	7± 0.3	8± 0.2	10± 0.4	-	5± 0.2	5± 0.5	7± 0.3	5± 0.2	7± 0.2	8± 0.2	11± 0.2	-	-	5± 0.2	8± 0.2
3	Kp	20±0.2	7± 0.4	5± 0.3	11± 0.3	11± 0.2	-	-	-	5± 0.1	5± 0.4	6± 0.4	6± 0.3	10± 0.4	-	-	-	5± 0.4
4	Pa	19±0.2	6± 0.3	7± 0.2	7± 0.1	8± 0.3	-	-	-	-	6± 0.6	5± 0.1	6± 0.4	10± 0.3	-	-	-	-

St- *Salmonella typhimurium*,

Kp- *Klebsiella pneumonia*,

E.Coli-*Escherichia coli*,

Pa-*Pseudomonas auregionsa*

Table 3: Showing Antifungal activity

Sr. No	Organisms	standard	Diameter of zone inhibition (mm)															
			Parmotrema cristiferum **Methanol extract (%)**				***Parmotrema cristiferum*** **Chloroform extract (%)**				***Ramalina pacifica*** **Methanol extract (%)**				***Ramalina pacifica*** **Chloroform extract (%)**			
			5	**10**	**15**	**25**	**5**	**10**	**15**	**25**	**5**	**10**	**15**	**25**	**5**	**10**	**15**	**25**
1	An	20±0.3	-	5± 0.2	7± 0.3	8± 0.4	-	-	-	6± 0.3	-	-	6± 0.2	10± 0.2	-	-	6± 0.4	6± 0.2
2	Ca	12±0.4	-	-	5± 0.4	6± 0.2	-	-	-	-	5± 0.3	-	-	8± 0.3	-	-	-	-
3	Mg	08±0.2	-	-	-	-	-	-	-	-	-	-	-	5± 0.3	-	-	-	-
4	Tr	09±0.2	-	-	-	5± 0.3	-	-	-	-	-	-	-	-	-	-	-	5± 0.3

An-Aspergillus niger,

Ca-*Candida albicans,*

Mg-*Microsporium gypsium,*

Tr- *Trichophytum rubrum*

Lichenic acid like usnic acid showed effective antimicrobial activity against *Bacillus subtilis*, *Candida albicans* and *Trichophyton mentagrophytes* (Perry, 1999). The antibacterial activities of usnic acid, vulpinic acid against to various aerobic and anaerobic microorganisms were reported (Ghione 1988; Lauterwein, 1995; Yilmaz, 2004). The most common five lichen compounds were screened for *in vitro* activity against *Mycobacterium aurum*. The usnic acid isolated from *Cladonia arbuscula* exhibited the effective activity against *M. aurum* with a MIC value of 32 μg/ml and this type of similar studies have been conducted by various researchers (Krishna, 1992; Ingolfsdottir, 1998; Kumar 2010; Demet and Mehmet, 2012; Srivastava, 2013). It has been shown that species of *Parmotrema* are reported to possess inhibitory activity against clinical isolates (Chauhan and Abraham, 2013). The present study revealed marked inhibitory activity of lichen extracts against tested microorganisms.

Conclusion:

The various extracts of the lichen selected for this study have shown a good activity against the tested bacteria. The present investigation showed that lichen extracts can be used to treat various infections caused by the different bacterial strains. The presence of various constituents in the extracts could be responsible for the antibacterial efficacy. The results of the study have justified the folkloric significance of lichens in curing diseases. Further studies on animal models will be carried out for effective discovery of novel activity of selected lichen samples.

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STUDY OF DIVERSITY OF COPROPHILOUS FUNGI FROM SELECTED DUNG SAMPLE

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Abstract:

The scholarly significance of coprophilous growths is the manure cherishing organisms. It speaks to an assorted network of morphologically and physiologically specific mycota, which gives a nuclear power to the deterioration and reusing of creature dung. Coprophilous parasites assume a significant job in the deterioration and reusing of creature dung, especially defecation of herbivores and in biodegradation of natural materials, particularly in intensely compost soils and mushroom beds. The current investigations are about the assorted variety, ID, and arrangement up to the class level of the coprophilous parasites found on the waste examples gathered from various territories. At long last, the fertilizer parasites are delightful life forms regardless of their ugly specialty. From this examination, it is presumed that creature excrement is a suitable substrate for the creation of coprophilous organisms. An aggregate of 12 spp. of coprophilous parasites were gotten, out of which 8 were microfungi, and 4 were macrofungi. The vast majority of the confined species have a place with Ascomycetes, followed by Basidiomycetes and Zygomycetes.

Keywords: Coprophilous fungi, *Pilobolus*, *Panaeolus*, domestic animals, stray animals

Introduction:

Dung-loving is the literary definition of coprophilous fungi. Coprophils speaks to a different network of morphologically and physiologically specific mycota, which gives natural

power to the deterioration and re-use of the defecation of life forms. Some physiochemical factors, such as temperature and humidity content, pH, rot phase, and type of creature, affect mycobiota compost. Numerous small-scale coprophilous creatures produce significant synthetic compounds that may hinder the contending and attacking of living beings or stimulate parasitic development and are considered to have a promising organic effect. (Abdullah, 1982) Forty species of coprophilous fungi (34 Ascomycetes, 3 Phycomycetes, 2 Basidiomycetes, and 1 Deuteromycetes) were established in the donkey, sheep, and camel dung laboratory collected from the semiarid desert areas of southern Iraq. Compositions of fungal species and their percentage concentrations in different dung media, along with the type of vegetation on which the above animals were grazed. (Island *et al.*, 2016) In diversity studies, sampling of several fresh and dried dung samples was done to achieve a comprehensive picture of the fungal diversity of the region.

In earlier studies, the author proposed that fungal diversity can be monitored by documenting coprophils from a selection of dung samples from a particular area or habitat (Richardson, 2001). Creating species abundance curves enables estimating the number of species. Upon hatching, with fresh or dried fertilizer, the entire infectious spectrum may be considered over some unknown timeframe, from lower to higher growths. Contagious progression architecture was tested using new compost experiments. Herbivore excrement is a partly processed, profoundly complex, natural problem. This consists of the remaining pieces of ingested plants as waste products, along with microbial population residing in herbivore rumen. Coprophilous species live in vast living spaces, fulfilling big employment in a number of environments. They are a subgroup of saprophytic parasites that can repress dung, often herbivorous defecation. As the waste results of the stomach related procedures, herbivore defecation is dominatingly made out of the most refractory and toxic pieces of the plants; the call divider polymers cellulose, hemicelluloses, and lignin. In this way, the potential for the secretomes of coprophilous organisms to contain novel compounds for productive plant cell divider corruption is high. So, the dung fungi are very nice organisms despite their unattractive niche.

The present studies are about the diversity, identification, and classification up to the genus level of the coprophilous found on the dung samples from different locations. Dung types of different herbivorous animals are partially digested highly complex, organic matters. They composed of the remains of ingested vegetation in the form of waste products, along with microbial population residing in the herbivore rumen. These dung types contain nitrogen, which is as high as 4 percent, a three to four-fold increase over the ingested material. So, the dung fungi

are very nice organisms despite their unattractive niche. The present studies are about the diversity, identification, and classification up to the genus level of the coprophilous found on the dung samples from different locations. Dung types of different herbivorous animals are partially digested highly complex, organic matters. They composed of the remains of ingested vegetation in the form of waste products, along with microbial population residing in the herbivore rumen. These dung types contain nitrogen, which is as high as 4 percent, a three to four-fold increase over the ingested material. Herbivore creatures brushing on vegetation ingest numerous contagious spores alongside their food. A portion of these spores will be of the coprophilous fertilizer parasites; others will be spores of non-coprophilous species (Ahmed and Khiralla, 2007). Microorganisms involved in the composting process have variable capabilities of decomposing organic matter (Noreen *et al.*, 2019).

Dung samples incubated in a warm, covered container exposed to indirect light will promote the growth of coprophilous fungi for at least a few weeks. Numerous dung sources are possible, with common herbivorous mammals such as horses, cattle, sheep, rabbits, and deer being the most accessible and productive species. (Chamuris and Counterman, n.d.).

Coprophilous fungi from wild herbivores moist chamber dung cultures from African elephant, Cape buffalo, dik-dik, giraffe, impala, Jackson hard beast, waterbuck and zebra found in Kenyan National Parks and Reserves were examined for sporulating coprophilous Sordariales. (Pg *et al.*, n.d.).

Materials and Methods:

Selection of study area:

Since the dung is a universal herbivore waste, it is readily available so that the dung samples were collected from Shrirampur, Rahuri, and Sangamner Tahsil. Dung samples from Cow, goat, and horse were collected as the animals were readily available in the study field. Dung samples from domestic and stray animals were obtained during the study period. The livestock was Cow (Bos taurus), Horse (Equus caballus) and Goat (Capra aegagrus hirctus).

Sampling materials for the study of fungal diversity:

Dung samples of three domestic and stray animals were collected. Their details are given in table no.1: Details of and their dung samples collected. During these studies, both domestic, as well as stray samples of dung, were collected. Each dung sample was collected in a clean, airtight polythene bag and taken to the laboratory at Loknete Ramdas Patil Dhumal Arts, Science

and Commerce College, Rahuri Department of Botany, Tal- Rahuri, Dist-Ahmednagar, Maharashtra.

Isolation of fungi from the dung samples:

Dung samples were subjected to insulation and enumeration of saprophytic and coprophilous fungi using a moist chamber system (Hawksworth, 1974). Growing dung samples of domesticated and stray animals were deposited in moist chamber plates equidistantly with full space for the growth of the fungi. Samples were incubated for 5 to 10 days at a temperature of 25±3 C for fungal growth and sporulation. Moist chamber plates do not require any special form of medium for the growth of both saprophytic and coprophilous fungi in the dung samples. In this process, the fungi grow on their own on the host, i.e. the dung. All plates were incubated at a temperature of 25±3 a.c. in the incubation chamber under dark conditions. After 5-10 days, fungal growth was developed on dung. The fungal mycelia and spores were lifted carefully using a sterilized fine-tipped needle from the dung surface and observed under a compound microscope and binocular light microscope. The photographs of the isolates were taken with the help of a mobile phone (Xiaomi Redmi note-1).

Identification of isolated fungi:

Preparation of the fungal slides was achieved by having the fungal mycelia and the spores coated with blue cotton and then placed in lactophenol on the slides. Such slides are then carefully examined under the uniocular compound microscope at various magnifications, i.e. X10, X45, X100. The identification of isolated fungi was made based on morphological peculiarities, such as color, texture and appearance, and was subsequently validated by consulting with experts and related literature.

Table 1: Showing details of animals and their dung samples collected

Sr. No.	Common name	Scientific name	Domestic/ Stray	Sample collection location
1.	Cow	*Bos taurus*	Domestic and Stray	Chanegaon, Tal-Sangamner and Satral, Tal – Rahuri, Dist- Ahmednagar.
2.	Horse	*Equus caballus*	Domestic	Shrirampur, Ahmednagar.
3.	Goat	*Capra aegagrus hirctus*	Domestic	Chanegaon, Tal-Sangamner, Dist- Ahmednagar.

Results:

Table 2: Coprophilous fungi obtained from different dung samples

Sr. No.	Name of Fungi	Dung Samples of			
		Cow		Horse	Goat
		Domestic	Stray	(Domestic)	(Domestic)
1.	*Pilobolus*	+	-	-	+
2.	*Parasola*	-	-	+	-
3.	*Mucor*	+	+	+	+
4.	*Rhizopus*	+	-	-	-
5.	*Panaeolus*	-	-	+	-
6.	*Podospora*	+	-	-	-
7.	*Chaetomium*	-	+	-	-
8.	*Ascobolus*	-	+	-	-
9.	*Cheilymenia*	+	-	-	-
10.	*Coprinellus*	-	-	+	-
11.	*Unidentified*	+	-	-	-
12.	*Unidentified*	+	-	-	-

Colonies appeared on dung samples after incubation:

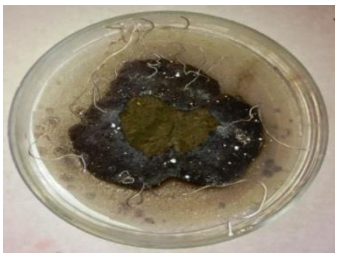


Plate 1: Showing Colonies of *Pilobolus* sp.

Plate 2: Showing magnified Colonies of *Pilobolus* sp

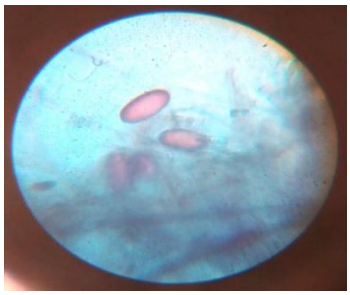
Plate 3: Showing Colonies of *Pilobolus* sp. and *Chaetomium* sp.

Plate 4: Showing Colonies of *Pilobolus* sp. and *Mucor* sp.

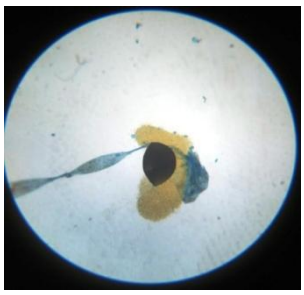
Slide mounts:

Slide 1: *Pilobolus* sp.
Sporangium with black cap

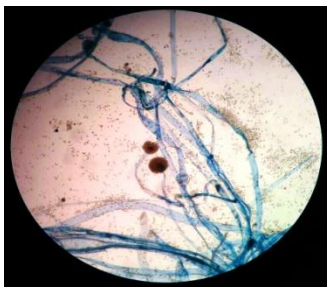
Slide 2: *Pilobolus* sp.
Sporangiophore



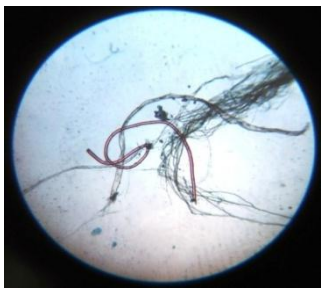
Slide 3: *Podospora* sp.
Ascospores 100X



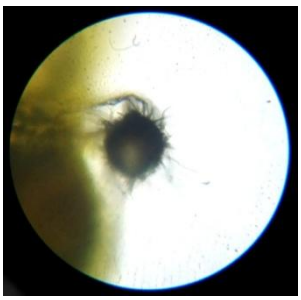
Slide 4: *Rhizopus stolonifer* and Sporangiophore



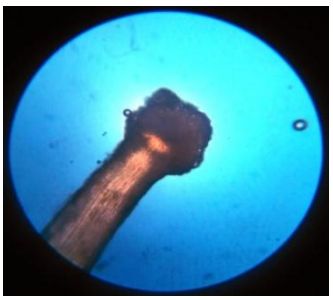
Slide 5: *Rhizopus* sp.
Sporangium and Rhizoids 45X



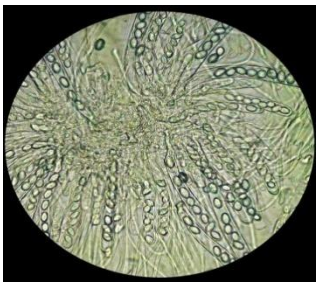
Slide 6: *Unidentified* sp.
Sporangium



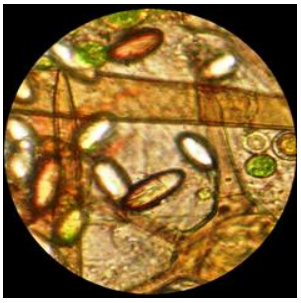
Slide 7: *Chaetomium* sp.
Perithecium 100 X



Slide 8: Unidentified sp.



Slide 9: *Ascobolus* spMature apothecium X100



Slide 10: *Cheilymenia* sp.
Marginal hairs

Specimens found on different dung samples during the study:

Specimen 1: *Ascobolus* sp. Fruiting Bodies

Specimen 2: *Parasola* sp. Fruiting Body

Specimen 3: *Panaeolus* sp. Fruiting Bodies

Specimen 4: *Coprinellus* sp. Fruiting Bodies

Discussion:

Pilobolus was found only on domestic cow and goat dung samples, while *Parasola* was found only on domestic horse dung. *Mucor* is the only species found to be widespread in all the dung samples obtained in the studies. Dung provides a significant amount of readily accessible nutrients such as carbohydrates, high nitrogen content, vitamins, and growth factors. (Webster, 1970).

Panaeolus, Parasola, and *Coprinellus* were found only on the domestic horse dung sample. The *Podospora, Rhizopus,* and *Cheilymenia* were found only on the domestic cow dung sample. The *Chaetomium* and *Ascobolus* were found only on the stray cow dung sample. Five fungal species were isolated, identified as *Aspergillus niger*, *A. flavus, A. fumigatus*, *Saccharomyces cerevisiae,* and *Mucor* sp. (Olahan and Idowu, 2018). Six species of plant pathogenic fungi were found on animal droppings, including *Alternaria alternata, Curvularia lunata, Exserohilum rostratum, Fusarium oxysporum, F. solani, Nodulisporium gregarium* and *Thielaviopsis* sp. (Jeamjitt *et al.,* 2006). (Piasai and Manoch, 2009) studied diversity and distribution in samples of dung fungi collected from four species of wildlife and domestic animals (barking deer, buffalo, Cow, and elephant). This study showed that the isolated species are distributed in three classes, i.e., Ascomycetes, Basidiomycetes, and Zygomycetes.

Most of the isolated species belong to Ascomycetes, followed by Basidiomycetes and Zygomycetes.

Conclusions:

This study concludes that animal dung is an excellent substrate for the growth of coprophilous fungi. Total of 12 spp. Of the coprophilous fungi, 8 were micro fungi and 4 were macro fungi. Considering the value of these fungi, some are edible; some are poisonous, and some are enzyme producers. They play an essential role in the decomposition of organic matter and thus measure soil fertility. Simply coprophilous fungi are pleasant species that are known to be essential factors in ecosystems.

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IMPACT OF MARINE POLLUTION ON SEA WEEDS- A THREAT TO MARINE BIODIVERSITY

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Abstract:

Marine pollution is a growing problem in today's world. Our ocean is flooded with different types of pollutants ranging from chemicals to waste like plastics. Global climate change caused due to pollution also causes acidification of the water bodies. Seaweeds which are one of the major architects of the marine environment get heavily impacted by the disturbances caused due to pollution. Here an attempt has been made to discuss how anthropogenic activities affect seaweeds which indirectly or directly disturb the marine ecosystem.

Keywords: Marine, pollution, seaweed, ecosystem

Introduction:

Most of our earth surface is occupied by oceans. Less than 3% is present in ice, groundwater, freshwater lakes and rivers (Tom *et al.*, 2008). Oceans play a very integral part in all living organisms. Their physical and chemical characteristics directly and indirectly affect living organisms that inhabit them. Ocean productivity is a prominent research area where the amount of food produced and the number of organisms it can support is studied. Oceans accounts for a significant amount tothe world's food supply.

Algae are a diverse group of photosynthetic organisms which range from unicellular (microalgae) to multicellular (macroalgae) forms and inhabit freshwater and marine water ecosystem. Marine macroalgae are better known as seaweeds (Kaladharan and Kandan, 1997). Seaweeds are classified according to the coloured compounds present in them. These coloured compounds are associated with chlorophyll molecules and help in enhancing photosynthesis. The coloured compounds may be brown, green, or red as shown in Figure 1. Brown seaweeds (Phaeophyceae) are usually large and range from 20m long to 30–60 cm in size. Red seaweeds

(Rhodophyceae) are smaller in size, and range from a few centimetres to a meter in length. Green seaweeds (Chlorophyceae) are small in size similar to red seaweeds.

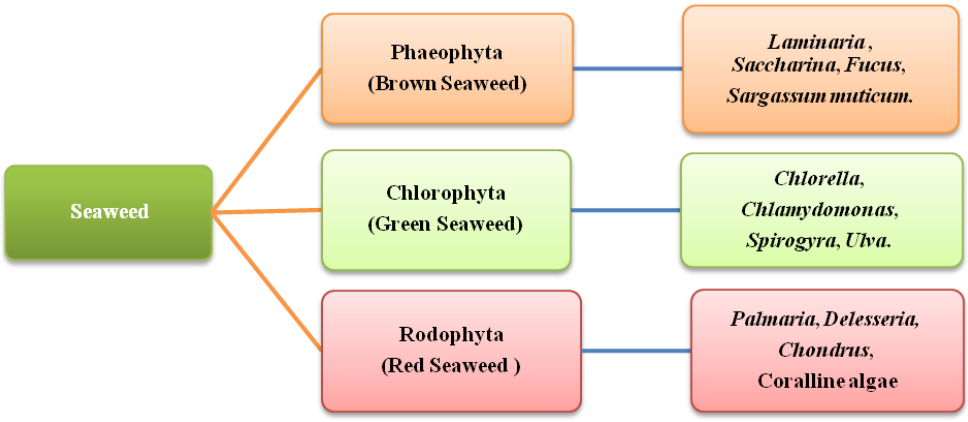

Figure 1: Classification of seaweeds on the basis of colour

Seaweeds are important as they are the major primary producers in the oceans andarchitects which play a major rolein these ecosystems (Sano *et al.*, 2001; Graham, 2004). Changes in the marine environment, both physical and chemical can influence the nature and characteristics of seaweeds. The impact of anthropogenic stress to seaweed ecosystems is widely understood (Xia *et al.*, 2004). This paper describes the ways in which changes in the environment due to pollution can directly affect the seaweeds and can be a threat to marine biodiversity.

Role of seaweeds in marine ecosystem:

The seaweed have a great influence on the marine ecosystem, as it plays important role by providing food and habitat for marine organisms (Estes and Peterson, 2000; Christie, 2009)

Food and Food Web:

Seaweed is present both floating and in the deep ocean and are the producers in the marine ecosystem. Seaweeds form the base of productive food webs (Norderhaug *et al.*, 2003). They are the major primary food source as they have the ability to photosynthesise due to the pigments in them. These seaweeds produce a lot of organic matter which is consumed by the marine animals to give nutrition and energy. They feed on them either directly when they are eaten or indirectly when they are decomposed and brokendown into fine particles and are eaten by filter feeders (Bustamante and Branch, 1996; Duggins *et al.*, 1989). The unconsumed organic matter also plays a very important role as it is exported to adjacent food webs in the form of detrital algae which is transported to various other ecosystems like subtidal, intertidal, pelagic and terrestrial (Dethier *et al.*, 2014; Krumhans and Scheibling, 2012). Apart from this the

decomposed seaweeds which is very nutritive settle downs on the ocean floor and help the organisms present on the floor of the oceans. They also mix with the sediments and increase the fertility.

Habitat and Sheltar:

Seaweeds help in protecting the marine animals and facilitate in the support and maintenance of biodiversity of the ecosystem. Marine animals are always in search for shelter which they use for resting, breeding, protection from predators or lurking for prey.

Many authors have reported the use of seaweeds by juvenile fishes as a habitat (Sofie *et al.,* 2007). Masuda and Tsukamoto (2000) have reported that association of fishes with seaweeds starts at an early stage of development as it gives protection from predators and gets shade from detection of prey. Kokita and Omori (1998) have reported that seaweed beds dominated by *Sargassum* spp. also act as habitat for the juvenile reef fishes. They are complex and have a large surface for association of various other invertebrates and filamentous algae. Tano *et al.,* (2017) have shown the presence of fishes which feed on invertebrates in the seaweed beds. Seaweeds also act as important nurseries for many other species such as the rock lobster, abalone, mollusks and mussel.The major reason for the above is the fact that seaweed are abundant food source for the developing forms. Davenport and Rees (1993) have reported that seaweeds are also used as a potential for passive transport by the animals and in some migrating animals it act as a temporary shelter. Apart from these seaweeds also act as meeting places for formation and maintaining of schools or for spawning (Masuda and Tsukamoto, 2000)

Ecological importance of seaweeds:

Seaweed play a very important part in maintaining the ecological balance and have a positive environmental impact on the marine ecosystem were they are present. They are autotrophic, produce large quantity of organic matter through photosynthesis and one of the major sources associated with oxygen production and uptake of CO_2 (Duarte and Cebrian, 1996). Giant seaweeds absorb large amount of CO_2 and also trap waste and make water clean and healthy. Seaweeds also help to stabilise marine rocks which are used for protection by many marine animals and prevent erosion as they hold the sediments together.

Ecological importance of seaweeds (Source: deepoceansfact.com**):**

- Keep Ocean Healthy
- Make Marine Animals Healthy
- Maintain Stability
- Prevent Erosion
- Traps Parasites
- Traps Waste

- Take In Excess Nutrients
- Determine Ocean Condition
- Support Biodiversity
- Produce Oxygen

Effect of marine pollution on seaweed:

Seaweeds have extensively been used in the biomonitoring of contaminants due to their potential to accumulate pollutants on their surface or in their tissue (Roberts *et al.*, 2008). Environmental changes like heavy metals, climate change, plastics and other types of pollutions pose serious challenges to all organisms including marine species which have to tolerate and adapt to new challenges produced (Ritter *et al.*, 2008). Seaweeds are good indicators of pollution as they are easily accessible and are sensitive to the changes in environment.

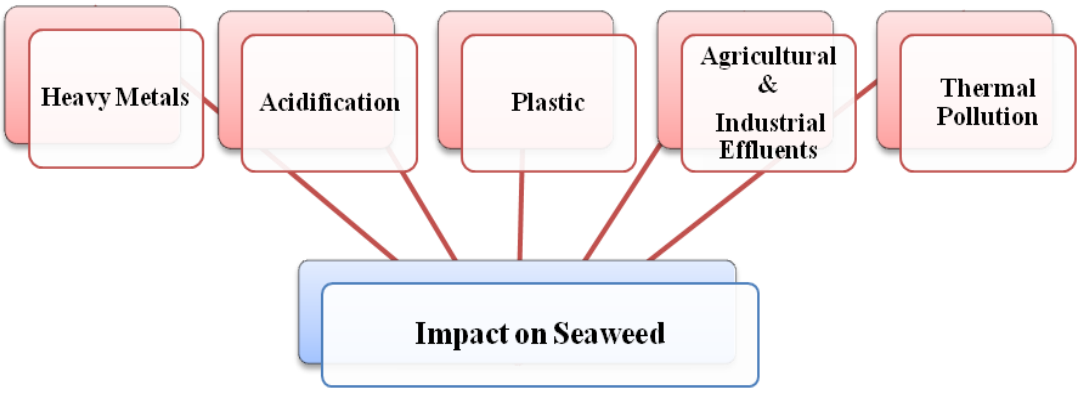

Figure 2: Pollutants which impact the seaweeds

Heavy metal:

They are highly toxic and are one of the major pollutants which is continuously released into the biosphere (Walker *et al.*, 2012). Metals cause formation of Reactive oxygen species (ROS) which at certain levels causes oxidative stress in seaweeds.

Deposition of heavy metals on seaweeds can adversely affect macroalgae and can in turn affect other organisms at different trophic levels. Medina *et al.* (2005) have reported that deposition of copper in the Chañaral Bay due to mining activity negatively influenced algal biodiversity and eliminated several benthic herbivores and all benthic carnivores.

These heavy metals inhibit photosynthesis (Nielsen *et al.*, 2003), can reduce the pigment concentration and the growth in seaweeds. Brown and Newman (2003) have shown that heavy metals like copper and cadmium can affect seaweeds at cellular levels.

Acidification:

The concentration of atmospheric CO_2 is continuously increasing due to various human activities. The excess CO_2 causes ocean acidification (Feely *et al.*, 2004). In marine environment

CO_2 is one of the factors which can affect the physiology of seaweeds (Kroeker *et al.*, 2010). CO_2 is used by the seaweeds for photosynthesis, but excess of CO_2 released due to various anthropogenic emissions reduces the pH of water and can dis-balance the availability of carbonate ions (Orr *et al.*, 2005). This results in change in calcification in seaweeds. Kroeker *et al.* (2010) have reported that ocean acidification is related to reduce growth rates in certain calcified macroalgae. A similar condition of reduced growth rates was reported by Hofmann *et al.* (2012) in some species of red algae *Corallina offcinalis*. Gao *et al.* (1993) have shown the reduction in calcification rate at higher CO_2 in some red and green algae. All these changes can affect the seaweed ecosystem directly or indirectly.

Thermal pollution:

Thermal pollution is one of the major environmental pollutants. Thermal pollution occurs through letting off warm water produced during the cooling of industrial machinery and power plants which flow back into the water bodies (Eggert *et al.*, 2012). Thermal effluents also include waste containing petroleum products like oil and grease. This warming of water can result in less dissolved oxygen and cause algal blooms which becomes a threat to marine life. This new condition can result in migration of aquatic forms from one place to another which in turn leads to disruption of the ecosystem that is left behind. Some authors have also shown that there is a decrease or absences of some seaweed in water bodies were there is discharge from thermal power plants.

Agricultural runoffs and industrial effluents:

Ananthropogenic activities generate a lot of waste in the form of effluents from industries, sewage treatments, agricultural and urban runoffs. These have affected the different ecosystems at varied levels. They have varying pH, density, total dissolved solids, heavy metals, pesticides, fungicide etc. (Pinto *et al.*, 2003). Jadeja and Tiwari (2007) have shown *in vitro*the effects of effluents on seaweeds like *Sargassum, cystoseira*. They showed that long term exposure could result in change in biomass of seaweeds. These effluents hamper the growth of seaweeds. All these above can harm the other diverse communities which are interdependent on each other for their survival.

Plastics:

Plastic waste is a major concern for all types of ecosystem. In marine ecosystem plastics are a major threat to all life forms present in it. The plastic debris which is added to the environment has led to progressive accumulation of microplastics in the marine waters. Plastics of all sizes are found at all levels in the marine habitats from the surface up till the sediments of ocean floor. Seaweeds present near rocky shores provide habitat, food, and shelter for a large number of associated organisms (Taylor and Cole, 1994). Rocky shores enhance formation of

microplastcis which can get deposited on the benthic seaweeds present there and could pose a threat to the organisms present. Additionally the suspended particles also get accumulated in the macrophytes of the seaweeds due to their complex morphology and cause its retention (Gutow, 2015). Many studies have been done in laboratory conditions to show that seaweed like *Fucusvesiculosus* can retain suspended microplastics on its surface and the amount adhered could be correlated with the suspended particles in water (Gutow, 2015). Invertebrates like mesoherbivores feed on an algal diet, and any contamination in the algal tissue can deter them from feeding on it or it may affect their physiology upon consumption. These mesoherbivores can lead to transfer of the microplastcis into marine food webs and transfer to the different trophic levels (Farrell and Nelson, 2013).

Conclusion:

Seaweeds are important components of marine ecosystem as they are primary producers and form a structure which helps the ecosystem to function. They provide number of basic requirement like habitat, shelter, feeding and breeding ground for vast number of species. They form important link in the marine food web. Although they are known to remove toxins from water, increased marine pollution can affect seaweeds in different ways which indirectly will have adverse effects on other various communities associated with it. The marine ecosystem is becoming vulnerable due to all these factors which could result in change in community composition of the ecosystem. Hence there is a need to check and monitor the extent of marine pollution

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PHYTOCHEMICAL SCREENING OF *CUCUMIS MELO* (L). FRUIT EXTRACT

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Abstract:

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance. In olden civilization, plants were used in the treatment of various diseases. Since, plants are a source of natural remedies, due to the presence of potential of bioactive compounds or extract which provide new and novel products for disease treatment and prevention is still enormous. Phytochemicals are naturally occurring substances with wide range of biological potential. Fruits are rich source of diverse antioxidants, and phytochemical compounds which are useful for public health. Fruits and vegetables are mostly used for diet by most of people in throughout the world. They contain important chemical compounds which are called as phytochemicals with antioxidant activity. The present investigation was designed to evaluate the phytochemical analysis of the primary and secondary phytochemicals in different solvent extracts such as ethanol, methanol, chloroform, ethyl acetate, and aqueous extract of *Cucumis melo* fruit. These results clearly indicate that ethanolic extract of *C.melo* fruit has different types phytochemicals and it can be used as a bioactive source of natural antioxidants for pharmacology.

Keywords: Phytochemicals, Alkaloids, Phenols, Flavonoids, Antioxidants, *Cucumis melo* (L)

Introduction:

India is bestowed with the enormous biodiversity of medicinal plants. According to the World Health Organization estimates that up to 80 percent of people still rely mainly on

traditional remedies such as herbs for their health needs due to better cultural acceptability, fewer side effects and better compatibility with the human body (Thevasundari and Rajendran, 2011). Medicinal plants have been widely used for the treatment of diseases in a traditional way for several years. An interaction between ancient medicine and biotechnological tools is to be established towards newer drug development (Arunkumar and Muthuselvam, 2009).

Plants produce an array of active ingredients that are known as secondary metabolites and a major part of traditional therapy involves the use of plant extracts or their active principles are being beneficial to combat cancer (Altundad and Ozturk, 2011). Phytochemicals can be defined as substances found in edible fruits and vegetables that may be ingested daily by humans in a small measure, and that exhibit a prospective for modulating the human metabolism in a favorable manner for the prevention of diseases. Plants, particularly fruits and vegetables, have many phytochemicals that possess various bioactivities, including antioxidant and anticancer properties (Rabeta *et al.*, 2013).

Our human body has an elaborate antioxidant defence system. Antioxidants are manufactured within the body and may be even be extracted from the food humans eat like fruits, vegetables, seeds, nuts, meats and oil. Certain foods like high fiber, low-fat diets and fresh fruits and vegetables provide protection against cancer. Fresh fruits and vegetables are a rich source of antioxidant vitamins like A, C and E, that prevent cellular damage associated with cancer incidence. The scavenging capacity of the antioxidant vitamins prevents oxidative damage by neutralizing the free radicals (Taraphder *et al.*, 2001).

Cucumis melo (L.) is commonly known as musk melon, cantaloupe. They belong to the family of Cucurbitaceae and are cultivated in all tropical regions of the world. They are rich sources of vitamin C, vitamin E, polyphenols and carotenoids, which have been suggested as natural sources of antioxidants (Lester *et al.*, 2009). Many phytochemicals having potential benefits are present in *Cucumis melo* fruit. The fruits are often used as a cooling, light cleanser or moisturizer for the skin and have stomachic properties. Traditionally, it is used for treatment of kidney stones, cancer, cardiovascular disorders and stroke. The fruits can be used as a light cleanser or moisturizer for the skin and has stomachic properties. Traditionally, it is used for treatment of kidney stones, cancer, cardiovascular disorders and stroke (Ritschel *et al.*, 2004). The present study was conducted to evaluate the phytochemicals present in the solvent extracts of *Cucumis melo* fruit.

Materials and Methods:

Collection and authentication of plant material:

Cucumis melo (L.) (Family - Cucurbitaceae) fruits were collected from the local markets of Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr. M. Palanisamy, Scientist-C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/482.

Preparation of extracts:

The pulp of fresh fruits of *C. melo* was chopped into pieces and dried at room temperature for 24 hours. The air-dried pulps were kept at 40 °C in hot air oven for 24 hours to remove moisture content. The completely dried fruits were ground into powder by using a mixer grinder and stored. 10 g of the dried fruit powder was successively extracted with 100 ml of selected solvents (water, methanol, ethanol, chloroform and ethyl acetate) using soxhlet apparatus and filtered through Whatmann No 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of fruit were stored in small vials at 20º C and used for further analysis.

Qualitative Phytochemical Screening of *Cucumis melo* Fruit extract (Peach and Tracey, 1955; Raaman, 2006**):**

The phytochemical screening of aqueous, ethanol, methanol, chloroform and ethyl acetate extracts of *Cucumis melo* fruit was carried out for the detection of different phytoconstituents such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, carbohydrates, amino acids and proteins using standard procedures given in Table 1.

Chemicals and Reagents

All the reagents and solvents used were of analytical grade and highly pure.

Table 1: Methods of phytochemicals screening

Sr. No.	Phytochemicals	Methods
1	Alkaloids	1.3 g of mercuric chloride was dissolved in 60 ml water and 5.0 g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water. To 1.0 ml of fruit extract, few drops of reagent were added. Formation of white or pale-yellow precipitate showed the presence of alkaloids.

2	Phenols	**Ferric chloride test:** To 1.0 ml of fruit extract 2.0 ml of distilled water, followed by a few drops of 10 % aqueous $FeCl_3$ solution was added. Formation of blue or green colour indicates the presence of phenols.
3	Flavonoids	In the test tubes containing 0.5 ml of fruit extract, 5-10 drops of dilute HCl and a piece of zinc or magnesium were added and therefore the solution was boiled for a few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.
4	Tannins	**Ferric chloride test:** To 1- 2 ml of fruit extract, few drops of 5 % aqueous $FeCl_3$ solution was added. A bluish black colour, which disappears on the addition of a few ml of dilute H_2SO_4 was followed by the formation of a yellowish-brown precipitate.
5	Saponins	In a test tube containing about 5.0 ml of fruit extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.
6	Terpenoids	**Salkwski reaction:** 5.0 ml of fruit extract was mixed in 2.0 ml of chloroform and concentrated H_2SO_4 (3.0 ml) was carefully added to form a layer. A reddish-brown coloration in the inter phase formed to show positive results for the presence of terpenoids.
7	Steroids	**Libermann-Burchard's test:** To 1.0 ml of fruit extract, 1.0 ml of conc. H_2SO_4 was added, followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and turned blue indicates the presence of steroids.
8	Carbohydrates	**Benedict's test:** 173 g of sodium citrate and 100 mg of sodium carbonate was dissolved in 500 ml of water. To this solution 17.3 g of copper sulphate dissolved in 100 ml of water was added. To 0.5 ml of the fruit extract, 5.0 ml of Benedict's reagent was added and boiled for 5 minutes. Formation of a bluish green colour showed the presence of carbohydrates.
9	Glycosides	A small amount of fruit extract was dissolved in 1.0 ml of water and then an aqueous sodium hydroxide solution was added. Formation of a yellow colour indicates the presence of glycosides.
10	Amino acids and Proteins	**Biuret's test:** To 1.0 ml of fruit extract, 5-8 drops of 5 % sodium hydroxide solution was added, followed by one or two drops of 1 % copper sulphate. Formation of pink or purple colour confirmed the presence of proteins and amino acids.

Results:

Preliminary phytochemical analysis of the *Cucumis melo* fruit extract using various solvent systems revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids etc. Table 2 depicts the qualitative analysis of the primary and secondary phytochemicals which confirms the presence of alkaloids, phenolic compounds, flavonoids, terpenoids, steroids, tannins, glycosides, saponins, carbohydrates, amino acids and proteins in different solvent extracts such as ethanol, methanol, chloroform and ethyl acetate, and in aqueous extract.

Table 2: Qualitative analysis of phytoconstituents present in different solvent extracts of *cucumis melo* fruit

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform	Ethyl acetate
Alkaloids	+	++	-	-	+
Phenols	+	++	+	-	-
Flavonoids	+	++	+	-	-
Tannins	-	+	+	+	++
Saponins	-	-	++	-	-
Terpenoids	-	+	+	+	-
Steroids	+	+	++	++	++
Carbohydrates	++	++	+	-	-
Glycosides	-	+	+	-	+
Amino acids	+	+	+	-	-
Proteins	+	+	+	-	-

(+) present in small concentration; (++) present in high concentration; (-) absent

In the present phytochemical study, aqueous extract revealed the presence of carbohydrates in higher concentration whereas alkaloids, flavonoids, phenols, steroids, amino acids, and protein in smaller concentration. Ethanol extract showed the presence of alkaloids, flavonoids, carbohydrates, phenolic constituents in high concentration while the tannins, steroids, terpenoids, glycosides, amino acids, proteins and carbohydrates were present in smaller concentration. Methanol extract showed the presence of saponins and steroids in moderately high concentration whereas flavonoids, phenols, tannins, terpenoids, glycoside, carbohydrates, amino acids and proteins were in smaller concentration. Chloroform extract showed the presence of

steroids in moderately high concentration whereas tannins and terpenoids were present in smaller concentration. Ethyl acetate extract showed the presence of tannins, steroids and glycosides.

Discussion:

Phytochemical screenings are carried out with the purpose of detecting a diverse group of naturally occurring active phytochemicals. There, discovering the bioactive profile of plants with therapeutic value is prominent. The alkaloids which are nitrogen-containing compound, commonly found to possess antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Kasolo *et al.*, 2010). Alkaloids are abundant secondary metabolites found in plants and represent one of the most widespread classes of compound endowed with multiple, varied pharmacological properties including cancer (Stevigny *et al.*, 2005).

Polyphenols are important components in fruit tissues. These compounds are thought to be instrumental in combating oxidative stress. They can prevent some oxidation-related diseases such as atherosclerosis, cardiovascular and neurodegenerative diseases and cancer (Sun *et al.*, 2009). Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). Flavonoids are the most common group of polyphenolic compounds in the human diet, and they are found ubiquitously in plants (Davis *et al.*, 2009). Flavonoids are naturally occurring phenolic phytochemicals which are contained in many fruits, vegetables, and beverages reported to possess various biologically important properties Flavonoids are the molecules responsible for the color of fruit and flowers. Flavonoids are of great interest for their bioactivities, which are basically related to their antioxidant properties (Cote *et al.*, 2010). Flavonoids in the plants have strong free radical scavenging properties. The multiple pharmacological properties of flavonoids, like anti-inflammatory, antibiotic and cardiovascular activities are to a large extent, linked to their polyphenolic and hence radical scavenging nature and act as primary antioxidants or free radical scavengers (Jose and Radhamany, 2012).

Tannins are complex moieties produced by majority of plants as protective substances, they need wide pharmacological activities. They have been used since past as tanning agents and they possess astringent, anti-inflammatory, anti-diarrhoeal, antioxidant and antimicrobial activities. Saponins and tannins are known for a wide range of pharmacological effects like anti-inflammatory, antimicrobial, antispasmodic, antidiabetic, anticancer, anticonvulsant and cytotoxic activities (Supradip *et al.*, 2010).

Terpenoids play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions (Bassole *et al.*, 2003). Also, terpenoids are attributed for analgesic and anti-inflammatory activities. From clinical studies, it is shown that terpenoids can increase the concentration of antioxidants (Krishnaiah *et al.*, 2009).

From this Table 2, the solvent ethanol has more phytochemical solubilizing capacity than other solvents. The high efficiency of ethanol can be attributed to its intermediate polarity leading to the extraction of both polar and non-polar compounds. The presence of phytochemicals was found to be maximum in the ethanolic extract when compared to other extracts. This present study clearly indicates that phytochemical screening of primary and secondary metabolites of *Cucumis melo* fruit in different solvents (aqueous, ethanol, methanol, chloroform and ethyl acetate) revealed the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, glycosides, saponins, carbohydrates, proteins and amino acids. The results of the analysis indicated that the fruit is a copious source of phytochemicals in various extracts.

Conclusion:

Based upon the results obtained in the present study, it is concluded that ethanolic extract of *Cucumis melo* fruit contains considerable number of flavonoids, alkaloids and phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These indicate that the plant is a significant source of natural antioxidant, which may be helpful in preventing the progress of various oxidative stresses and treating many diseases. *Cucumis melo* (Musk melon) a short duration vegetable crop belonging to family Cucurbitaceae. The fruits are extracted in various solvents and evaluated for phytoconstituents present in them. The present study provides evidence that solvent extract of *Cucumis melo* fruit contains medicinally important bioactive compounds and this justifies the utilization of plant species as traditional medicine for treatment of various diseases. However, further isolation of bioactive compounds would assist to determine its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

Conflicts of Interest:

The authors declared that there is no conflict of interest.

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MYCOALLERGENS STUDY IN LIBRARY ENVIRONMENT

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Abstract:

Present study deals with the release of Allergenic fungal spores in library environment and their effect on human health. The studies were carried out during 1st Jan 2016 to 31st Dec 2017 with the help of "Tilak air Sampler", and Exposing "Potato- dextrose agar "containing Petri plates at fortnightly intervals. DNA Barcoding method is used for identification of taxon up to species level. Altogether twenty six types were recorded. Aeroallergens contributed (81.84%) to the total airospora. Allergenic fungal spores reported are *Chetomium* (21.88), *Alternaria* (20.71%), *Aspergillus* (12.50%), *Curvularia* (9.82%), *Fusarium* (7.45%), *and Cladosporium* (2.07%).The higher incidence of these types correlated with Metrological conditions and their effect on human health is discussed in paper.

Keywords: Airspora, Aeroallergens, Fungal spores, Sampler

Introduction:

Books and documents in libraries are valuable cultural heritage, as books and papers carry all kinds of knowledge through the barriers of time and have a capacity to pass them to future generation. It is precious legacies that remind people of their culture, religion and ethnic tradition. They deserve to be maintained and conserved in their original condition in libraries. (Kalbende *et al.*, 2012). Air is cheap component of microorganisms and many particles. Fungal spores have special important because of their application in diagnosis and treatment of allergic disorders. Fungal spores are not only responsible for paper deterioration and ageing of books but also significantly affect health of the library staff. Fungi present in the library causes bio-deterioration of books and damping materials.

Indoor environment provides favorable conditions for allergens. Whenever the weather conditions are changed percentage of airspora inside the library also changed. The library is public place so people and workers frequently exposed to allergens. Library employees have adverse health effects including infectious diseases like skin irritation, reddening of eyes or respiratory allergies like asthma and bronchitis etc. (According to oral survey and questionnaires). Fungal spores count directly influences the allergenic symptoms in sensitive individuals hence present study was undertaken to monitor the conditions and causes, responsible for deterioration of library material and fungal spores release in relation to their allergic effect.

Materials and Methods:

Present study was carried out in a Government library at Aurangabad during the 1st Jan.2016 to 31st Dec. 2017 by using Tilak air sampler and Anderson's sampler. Anderson's sampler was used for exposing "Potato dextrose agar" containing Petri plates at fortnightly interval (Plate I). The identifications and descriptions of spore types are essentially based on spore morphology (Barnett, 1962) and DNA Barcoding method. Specimens are sequenced with amplified ITS gene [Intratranscribe spacer gene] ≥450 bp for all and used to delimit barcode taxa (Ratnasingham and Hebert, 2007).

Plate 1: Petri plate exposure in library with Anderson's sampler and isolation of sp

During present study altogether twenty Six types of fungal spores were identified in the sampling site. Out of 26 fungal spore types, 15 species of 07 generaare known to be potentially allergenic. They have contributed (81.84%) to the total airspora. Taking the number into

consideration, *Chetomium* (21.88), *Alternaria* (20.71%) spores have contributed highest percentage to the total airspora, followed by *Aspergillus* (12.50%), *Curvularia* (9.82%), *Nigrospora* (7.77%), and *Fusarium* (7.45%) (Table 1).

Table 1: Percent Contribution of different spore types to the total airspora inside the Govt. Library

Sr. No.	Name of spores /genus	Percent /m^3 to the total airspora (%)
1	*Chetomium*	21.88
2	*Alternaria*	20.71
3	*Aspergillus*	12.50
4	*Curvularia*	9.82
5	*Fusarium*	7.45
6	*Cladosporium*	2.07
7	*Nigrospora*	7.77
8	*Rhizopus*	6.30
9	*Tetraploa*	2.19
10	*Colletotrichum*	0.37
11	*Brachysporium*	0.00
12	*Dracheslera*	0.25
13	*Pithomyces*	0.22
14	*Torula*	0.25
15	*Blastomyces*	0.13
16	*Memnoniella*	0.00
17	*Melamospora*	0.28
18	*Monillia*	0.51
19	*Epicoccum*	0.99
20	*Basidiospora*	0.87
21	*Speguzinia*	0.96
22	*Heterosporium*	1.52
23	*Monochetia*	0.38
24	*Myrothecium*	0.76
25	*Exosporium*	0.90
26	*Cercospora*	0.92

The role of fungal spores in deterioration is due to their hydrolytic enzyme activity. The cellulolytic activities cause maximum damage to paper. Petriplate expose method helped in determining the composition and percent contribution of the various airspora inside library. Isolated fungal types were studied up to the species level with the help of DNA bar coding method. Indoor environment provides congenial environment for allergens it shows higher concentration inside the library environment due to low temperature and humid climate during the study. Deuteromycotina spores dominated in the airspora by exhibiting the highest percentage contribution (69.94%), it was followed by Ascomycotina (28.92%) and Basidiomycotina (1.12%) (Table 2).

Table 2: Percentage Contribution of each spore group inside the Govt. Library

Sr. No.	Fungal group	Total no. of Spores/m^3	Percentage
1	Deuteromycotina	58496	69.94
2	Ascomycotina	24191	28.92
3	Basidiomycotina	942	1.12

Dominance of spore types in the airspora was determined by its percentage contribution to the total airspora. Lignocelluloses degrading *Chetomium* fungus has been found to be dominant in library environment *Chetomium*, *Aspergillus* and *Alternaria* was present throughout the period of investigations. It was major component of library and reported on many old books, on damping paper and on old rakes. Survey of library showed that highest catches were obtained in the month of June and July 2017. It was due to moist climate (Fig. 1) and damping material present along with the paper. It was also depends on the quality of the paper.

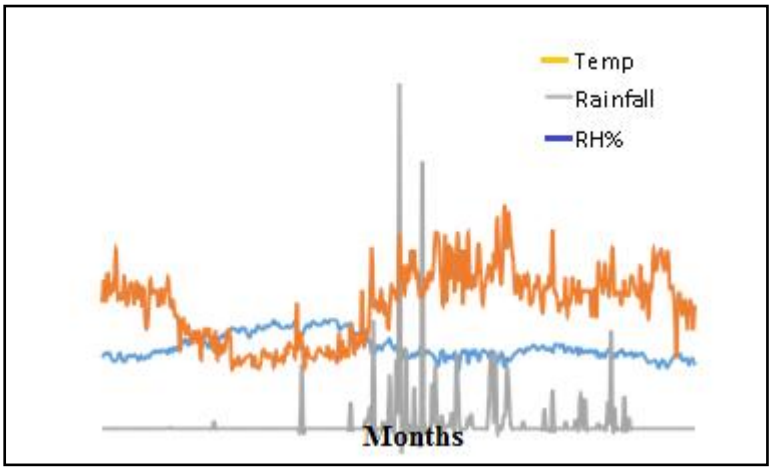

Figure 1: Graph showing the climate for the year 2016 - 17

Fungal colonies found on Petri plate were studied up to species level. Specimens are sequenced with amplified ITS gene and used to delimit barcode texa. In the petriplate exposed method altogether 176 colonies of dominant fungus were recorded (Table 3). Out of these total 52 colonies of *Chaetomium globosum and Chaetomium cellulolyticum were obtained on potato dextrose agar plate. Chaetomium globosum* is the most common species of the *Chaetomiaceae* in the indoor environment (Vesper *et al.,* 2007, Ayanbimp *et al.,* 2010, Straus 2011, McMullin *et al.,* 2013, Miller and McMullin, 2014) highest percentage of *Chetomium* in Aeromycoflora was mainly due to paper material dumped in side library. *Chaetomium* species have been reported to be important inhalant allergens. They develop the symptoms of rhinitis and asthma due to the production of Mycotoxins and microbial volatile organic compounds. *Chaetomium* liberates ascospores and hyphal fragments in the indoor environment. (Gonianakis *et al.,* 2005, Mason *et al.,* 2010, Andersen 2011). Similarly 46 colonies of *Alternaria alternate, Alternaria tenuissima, Alternaria macrospora* were develop on exposed plate.

Table 3: Total number of fungal colonies

Sr. No.	Name of species	No. of colonies	Total no. of colonies
1	*Chaetomium globosum*	24	**52**
2	*Chaetomium cellulolyticum*	28	
3	*Aspergillus fumigatus*	09	**43**
4	*Aspergillus flavus*	06	
5	*Aspergillus nidulans*	08	
6	*Aspergillus wentt,*	05	
7	*Aspergillus niger*	15	
8	*Cladosporium cladosporioides*	12	**23**
9	*Cladosporium tenuissimum*	11	
10	*Alternaria alternate,*	21	**46**
11	*Alternaria tenuissimum,*	13	
12	*Alternaria macrospora*	12	
13	*Curvularia sps.*	04	**04**
14	*Nigrospora sps.*	03	**03**
15	*Fusarium proliferatum*.	05	**05**

Total 43 colonies of five species of *Aspergillus* were found during an entire period of study on culture medium. These species are *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus wentti*, *and Aspergillus niger*, *Alternaria* and *Aspergillus* spores are the causative factor for respiratory diseases in human (Wioletta and Zukiewicz-Sobczak, 2013). *Aspergillus* was found to be predominant in library, because waste material or old books were not kept properly. Rapidly spread inoculums cause's diseases to human. In allergic subjects *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus niger* spores, act as allergens and give rise to allergic Rhinitis, Asthma (Citron, 1962), Broncho pulmonary aspergillosis and Allergic Aspergillosis (Mishra and Kamal, 1971). The highest concentrations of Aspergillus were recorded in the month of August. In this month average temperature was 28^0C and average humidity was (80 %) (Fig. A) meteorological conditions are favourable for sporulation and development of fungus. Air conditioners play important role in keeping the temperature low and moist. Such an ideal environment is suitable for spreading the spores in library. *Cladosporium* is also allergenic to the human beings (Jenna Fletcer, 2017). Spores are recorded throughout the period of investigations. Maximum concentration of *Cladosporium* was recorded, in the months of June and July. Spores of *Cladosporium* are allergenic to the workers in the library, which poses a serious problem of lung diseases (Talde *et al*,. 1987). 23 colonies of *Cladosporium cladosporioides and Cladosporium tenuissimum* were found on culture plate. Deuteromycotina spores were trapped maximum during the rainy season followed by winter season. Meteorological conditions also favour the release of spores.

The library airspora is thus continuously fluctuating as season change. It is bound to contain an abundance of fungal spores due to a constant built up of spore population from fungi, growing on stored and decayed paper material. The airspora of the library also have some implication on health of people working in the library. It is observed that they suffer from many respiratory problems and skin irritation. The percentage of workers was reported to be about 40% [Personal Communication and Questioners provided to them in local language] which indicates that, airspora within the library is very important in causing allergy. The people between the age 40-55 years shows high percentage of sensitivity to the allergic agents and the percentage of sufferers vary between 15 to 80% under different environmental conditions.

Present study reveals that, the airspora of library were responsible to enhance the allergensity in the people between the age group 40-55 years. It is also observed that the normal person sneezes more in the library atmosphere indicating their sensitivity to such allergic agents present inside. Necessary arrangements and modifications are to be provided in such libraries.

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MICRO AND NANOSENSORS AS A BETTER MONITORING SYSTEM

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Abstract:

Micro and nano electronics plays a vital role almost in every field. The centre of attraction is its uses in healthcare sector as a biosensor. Recent circumstances of COVID-19 gave a lesson to every individual how to be more cautious about our own health so it will be helpful for others to be healthy and finally the entire world will be safe if every individual is following some kind of care which is very common in nature. In this study primary focus has been made to highlight the unique features as well as challenges and opportunities of micro and nano sensors. Through deep literature survey and analysis one conclusion is very clear that using different kind of micro and nano sensors better kind of monitoring is possible. In a very simple language because of the uniqueness of micro and nano devices and MEMS (Micro-Electro-Mechanical Systems) structures, it may utilize as a best healthcare or environmental monitoring system. There are different kinds of micro and nano devices as example micro cantilever, nanowires, carbonnanotube, electronic nose and many more unique devices may use to do so. Through this paper different scopes and research opportunity has been highlighted in this field. The role of micro and nano science is very promising especially in the field of bioengineering. The beauty of micro and nano devices are it can be used as biosensor, chemical sensor or gas sensor and it can be used in agriculture, food industry, health or environment monitoring. Development of nano biosensor is one of the most recent advancement in the field of Nanotechnology as well as

bioengineering. By using attractive advantage, features and unique properties of the nanomaterials, faster and sensitive micro or nano biosensors can be developed. The study provides a brief view of utilization of microtechnology, nanotechnology and Nanosensors to sense monitor and detect with better selectivity and sensitivity.

Keywords: MEMS, Microtechnology, Nanotechnology, Sensors.

Introduction:

The impact of micro- and nanosensors on the elementary understanding of major biomedical challenges, clinical diagnosis, and care are highlighted here (Wei Chen *et al.,* 2020, Yoon *et al.,* 2019, Wang *et al.,* 2019). Routine clinical care of patients currently benefits from the use of macro- and micro scale sensors based on electrical, electrochemical, acoustic, piezoelectric and optical principles (Magna *et al.,* 2017, Paolesse *et al.,* 2016). The major research focus is to develop a chemical biosensor which may useful for multiple purposes like clinical diagnosis, water and environment monitoring, pharamaceutical, agriculture and food industry (Pang *et al.,* 2018, Kim and Lee, 2014, idtechex.com). To understand it in an easier way it has been shown in figure 1 below.

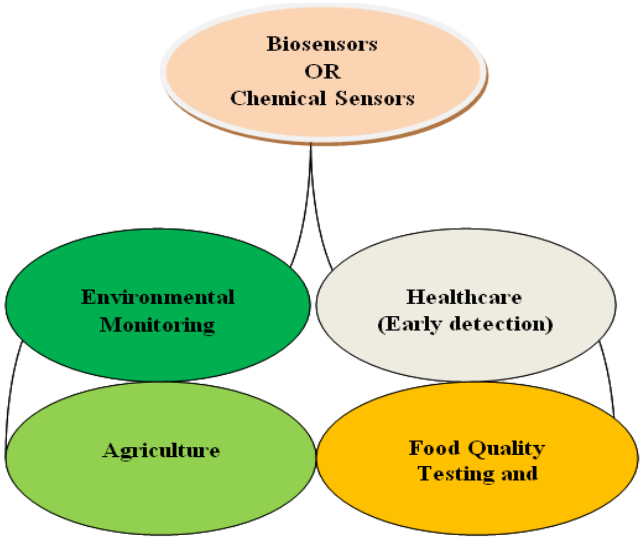

Figure 1: Versatile Chemical and biosensors

Micro sensors are miniature in size but these kinds of sensors are very much suitable to detect even small amount of changes in a physical variable like light, temperature and many more (Borisov *et al.,* 2011, Cha *et al.,* 2010, Tolentino and Park, 2010). To understand all these analysis need to understand the difference between sensor and transducer. The concept behind a

sensor is it is a device which is enough capable to receive a signal and respond to the received signal. Transducer is a sensing device which converts a physical input into an output. Transducer can convert one form of energy into another form. Mostly the physical dimensions are in the range of sub micrometer to millimetre. Through figure-2 which is given below it has been shown that the working functionality of a sensor device how it is converting nonelectrical quantities (it may be physical or chemical) into electrical signal (Assuncao *et al.*, 2009, Oberg *et al.*, 2004, Comini *et al.*, 2013).

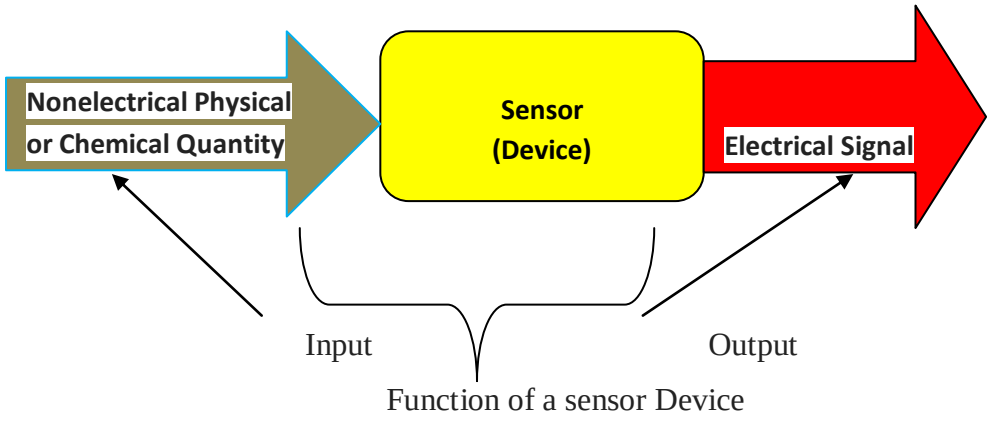

Figure 2: Function of a Sensor Device

MEMS (Micro-Electro-Mechanical-Systems) play a vital role in the field of environmental monitoring, agriculture and healthcare (Toko *et al.*, 2015, Raza *et al.*, 2014). This concept is shown in figure-3 and there are lot of MEMS structures to do so.

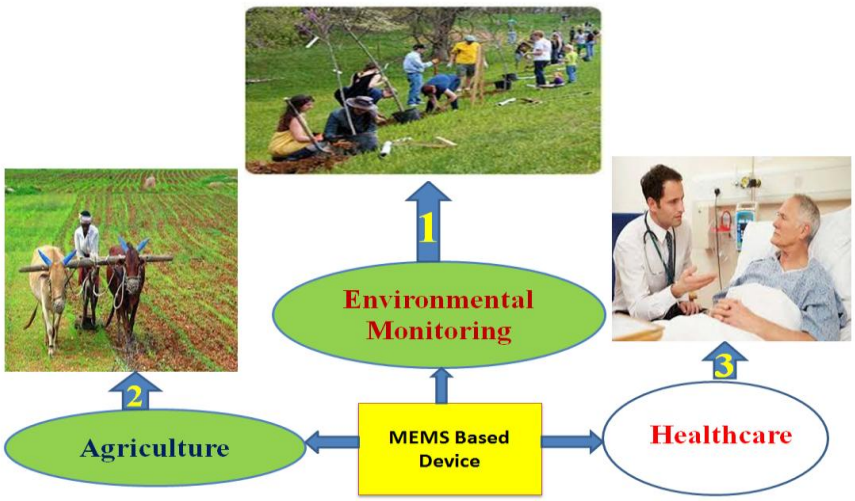

Figure 3: MEMS Technology for multipurpose

Research challenges and opportunities:

In clinical monitoring system there are lot many factors involved but one of the very important parameter is to measure the temperature apart from that as a physical variable measurement concentrate on measuring displacement, pressure, force, acceleration and flow (Magna *et al.*, 2017, Paolesse *et al.*, 2016, Pang *et al.*, 2018). Now days using MEMS technology there are so many structures are available as example micro cantilevers to measure these things but the major challenges are better selectivity and sensitivity (Kim and Lee, 2014, idtechex.com). Apart from that choosing a suitable sensing material is also one kind of challenge while doing this kind of research. Using nanowires, carbonnanotubs and electronic nose different kind of sensing and monitoring is possible, but the major challenges in front of researchers are fabrication of nano and micro devices (Borisov *et al.*, 2011, Cha *et al.*, 2010, Tolentino and Park, 2010, Assuncao *et al.*, 2009). Thus majority of good researchers are limited up to simulation and analysis report only (Oberg *et al.*, 2004, Comini *et al.*, 2013). Somehow because of multidisciplinary research work people can combine multiple ideas and may get help from multiple resources (Toko *et al.*, 2015, Raza *et al.*, 2014). Finally the latest trends in this field is to design an unique type of micro or nano sensors using MEMS technology or nanotechnology to monitor the environmental conditions,soil,air,water monitoring, plants and agriculture monitoring. The reason behind it is the entire life's are depending on these parameters so need to maintain balance between each and everything through detection, monitoring` and control. As example using gas sensor different gases like CO_2,O_2 can be monitored which is very helpful in clinical care.

Design of micro and nano structures:

To understand the different level of research work few of the structures are simulated using COMSOL multiphysics software.

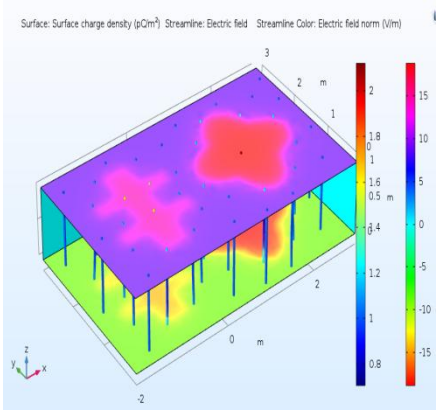

Figure 4(a): Electric Sensor

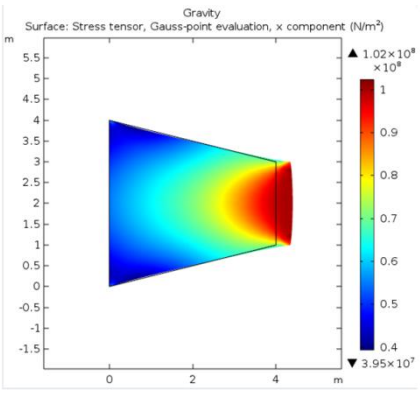

Figure 4(b): Tapered Cantilever

Above figures 4(a) and 4(b) showing the design of different structures in multiphysics environments. Using this tool different micro or nano scale structures can be design and it can be tested as its mechanical effects like stress, strain, displacement, pressure, force and many more. Beyond this temperature effects, materials sensitivity may also analyze in this multiphysics environment tool.

Conclusion:

Principal aim of this article is to make the people awareness about the unique and promising nature of micro and nano level devices. As the technology is growing faster, need to concentrate more on multidisciplinary kind of research. Nanotechnology and MEMS covering almost all applications and it is essential in everyday's life. Finally researchers and scientists have to to focus more on deigning some kind of unique devices by using nanotechnology and MEMS to sence,detect,monitor and control purpose to make the entire world to lead in a suitable way. To get success in this mission people may concentrate any one of the devices like micro cantilever, carbon naotube, nano wires, accelerometers and many more devices are available.

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TAXONOMIC ENUMERATION OF SOME BLUE GREEN ALGAE FROM KARAD AND IT'S ADJOINING AREA

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Abstract:

Blue green algae are the first photosynthetic prokaryotes cosmopolitan and occur in different types of habitats. Survey of localities like pools, puddles and marshy places was made to observe the BGA. Frequent visits were made and 14 taxa were recorded belonging to 09 Genera and 14 species. Among these recorded forms genus *Oscillatoria*found more frequently and showing abundance in all localities.

Keywords: Puddles, Marshy places, BGA, Karad.

Introduction:

Algae are ubiquitous cosmopolitanorganisms found in all habitats throughout the world.They have capacity to convert atmospheric free nitrogen in to fixed organic form like nitrate or ammonia (De and Mondal, 1956). Blue green algae also known as Cyanobacteria, Cyanophyta or oxygenic phototrophs. They are widely distributed in all types of habitats i.e. temperate, tropical and also polar region. Mostly they are free living found in aquatic, terrestrial or specialized habitats as well as find in symbiotic association with Fungi, Bryophytes, Gymnosperms and Angiosperms. They survive in soil, rocks, tree trunks, many water bodies, on moist walls and wherever they get moisture show their occurrence. They remain dormant in dry period and awaking at wet periods and grow.

Studies on blue green algal flora frompaddy fields of Maharashtra was undertaken by (Gonzalves *et al.,* 1949, Sardeshpande and Goyal, 1981, Kolte and Goyal, 1985, Patil and Satav 1986, Madane and Shinde 1993, Auti and Pingle, 2006, Patil and Chougule, 2009) as well as blue green algal diversity from Satara district was also studied by (Ghadage and Karande, 2008, 2012, Kamble *et al.,* 2014, Kamble and Karande, 2014). So, present work was taken up with a view systematic enumeration of blue green algae from different wet places from the study area.

Materials and Methods:

Karad is 52 km far to the south-east of Satara.Karad city situated at southern part of Satara district near Agashiva, at the confluence of Koyna and Krishna rivers; this confluence called as 'Preetisangam'. Screening of samples from different localities for blue green algal occurrence from Karad and its adjoining area was done viz. pools, puddles and marshy places. The blue green algal samples were collected in glass vials and brought in laboratory. These samples were washed thoroughly and were examined microscopically and identified with the help of standard literature (Dasikachary, 1959, Anagnostidis and Komarek, 1985, Santra, 1993, Anand, 1990). Photographs were taken by using photomicrography unit of Olympus CH20i. (Photoplate)

Results and Discussion:

Systematic account:

***Microcystis* Kutzing**

1. *Microcystis pulverea* (wood) Forti

Cyanophyta: T. V. Desikachary, 1959, p-96

Rounded to ellipsoidal Colonies, many together, Withdistinct colonial mucilage; spherical or ellipsoidalcells, arranged closely 2-3µm broad, blue green or olive green, gas vacuoles absent.

Locality: In Plastic Bucket, Malkapur Karad

2. *M. elabens* (Breb.) Kutz

Cyanophyta: T. V. Desikachary, 1959, p-97

Pl.- 18 fig-12 and Pl.-20 figs.- 6,7.

Spherical, or flat colonies, blue- green in color, Number of daughter colonies come together when they are old; oblongcells, 5.68 µm broad9.94 µm long, gas vacuoles present.

Locality: On moist ground of SGM College, Karad.

***Chroococcus* Nag**

1. *Chroococcus minutus* (Kutz) Nag.

Cyanophyta: T. V. Desikachary, 1959, p-103Pl.- 24, fig-4 and Pl.- 26, figs- 4,15. Photoplate: fig. 5

Spherical to oblongCells, in groups of 2-4or single, blue-green, with sheath 4-6 µm diam., without sheath 2-4 µm diam., colonies 10-15×15-20 µm; sheath unlamellated, colourless.

Locality: On moist ground of SGM College, Karad.

***Gloeocapsa* Kutzing**

1. *Gloeocapsa polydermatica* Kutz

Cyanophyta: T. V. Desikachary, 1959, p-114Pl.- 25, fig-1.

Mucilaginous thallus, spherical cells without sheath 5.68 µm diam., blue-green; colorless sheath, very thick, and lamellated many times.

Locality: on wet pull of Alpha Nursery, Malkapur area.

2.*Gloeocapsa aeruginosa* (Carm.) Kutz

Cyanophyta: T. V. Desikachary, 1959, p-115.

Crustaceous mucilaginous thallus, cells 2.84 µm broad, with sheath 4.8 µ broad, in colonies; spherical colonies, 16-50 µm diam., indistinctly lamellated sheath.

Locality: In wet earthen pot, Malkapur, Karad.

***Microchaete* Thuret**

1. *Microchaete violacea* Fremy

Cyanophyta: T. V. Desikachary, 1959, p-511

Mostly single filamentsor with other algae, curved slightly, up to 2mm long, broad uniformly, 11.36-12 µmbroad, thin sheath, colourless, unlamellated; basal heterocysts.

Locality: On moist ground, S.G.M. College, Karad.

***Oscillatoria* Vaucher**

1. *Oscillatoria subbrevis* Schmidle

Cyanophyta: T. V. Desikachary, 1959, p-207Pl.- 37, fig-2 and Pl.- 40 fig 1

Photoplate : fig-6

Single trichomes, 5-68 µm broad, straight,cells 1-2µm long, ungranulated, end cell rounded, absence ofcalyptra.

Locality: In wet earthen pot of Alpha Nursery, Malkapur area, Karad.

2. *O. obscura* Bruhl et Biswas

Cyanophyta: T. V. Desikachary, 1959, p-207

Trichomes 4.26 µm broad, at the apexrounded and attenuated,bent slightly,unconstricted at the cross-walls.

Locality: On temporary puddle, MalkapurKarad.

3. *O. fremyii* De Toni, J.

Cyanophyta: T. V. Desikachary, 1959, p-225

Bright blue greent hallus; trichome straight, at the cross walls constricted, 1.42 µm broad.

Locality: On temporary puddle, MalkapurKarad.

4. *O. amphibian* Ag. ex Gomont

Cyanophyta: T. V. Desikachary, 1959, p-229Pl.- 37, fig-6

Deep blue greenthallus, straight trichome, apices unattenuated, unconstricted at the cross walls, 2-3 µ long, 2-4 times as long as wide.

Locality: On wet ground, S.G.M. College, Karad

***Lyngbya* Ag.**

1. *Lyngbya spirulinoides* Gomont

Cyanophyta: T. V. Desikachary, 1959, p-289Pl.- 52 fig-4,5Photoplate : fig-2

Free floatingthallus, olive green in color; thin colourless, sheath, mucilaginous; trichome unconstricted at the cross walls,14.20 µm broad.

Locality: In wet earthen pot of Alpha Nursery, Malkapur area.

***Microcoleus* Desmazieres**

1. *Microcoleus paludosus* (Kutz) Gomont

Cyanophyta: T. V. Desikachary, 1959, p-344Pl.- 56, fig-2Photoplate : Fig-4

Single filaments not branched, 5.68 µm broad; cells as long as broad, 4-13 µ long, blue green; end cell conical.

Locality : On moist groundMalkapur, Karad.

***Anabaena* Bory**

1. *Anabaena khannae* Skuja

Cyanophyta: T. V. Desikachary, 1959, p-396Pl.- 75, fig-2Photoplate : fig-3

Creeping filaments, attached, elongated, straight trichomes 4.26-5.60 µm broad, barrel shaped cells terminal or intercalaryheterocyst, apex rounded 3-3.5 µm broad, 7-10 µm long.

Locality: On moist ground, S.G.M. College, karad

***Rivularia* (Roth) Ag**

1. *Rivularia aquatica* De wilde

Cyanophyta: T. V. Desikachary, 1959, p-552Photoplate: fig-1.

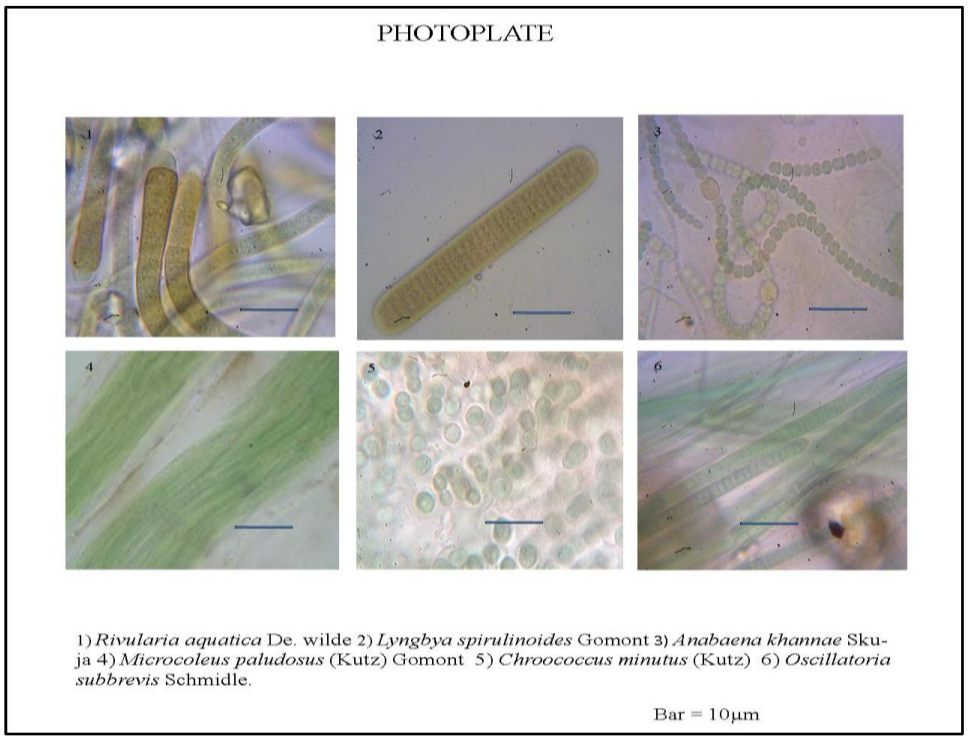
Spherical thallusup to 2mm diam., trichome 7-9 µm broad, ending in a long thin hair; at the base cells longer than broad.

Locality: On moist wall, Malkapur, Karad.

Total 14 species belonging to 9 genera viz *Gloeocapsa, Chroococcus, Oscillatoria, Microcystis, Microcoleus, Microchaete, Anabaena, Lyngbya* and *Rivularia* of blue green algae

were recorded from different localities. Among these species genus *Oscillatoria* found more frequently and showing abundance in all localities.

Blue green algal diversity from some cultivated soils of Marathwada was studied by Chaporkar and Gangawane (1984). Madne and Shinde (1993) studied blue green algae from salt affected soils of Kolhapur. Blue green algal occurrence recorded from industrial and domestic sewage from Satara district by Ghadage and Karande (2012), Cyanobacterial abundance from fresh water reservoirs of different localities of Nasik was recorded by Behere Patil and Deore (2014). Our selected study area also showed diversity of blue green algae from varied habitats. This proves that wherever possible these blue green algae showed their occurrence.



Conclusion:

Remarkable diversity is recorded from study area. Species richness is observed in *Oscillatoria*. Their occurrence from varied situations proves their cosmopolitan ability.

Acknowledgement:

Authors are thankful to Dr. Mohan Rajmane, Principal, Sadguru Gadage Maharaj College, Karad and Dr. K. G. Kanade, Principal, Y. C. Institute of Sciencs, Satara for the encouragement during the work. We are thankful to staff members and colleagues for the keen interest and suggestions during this work.

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EVALUATION OF SELECTED HEAVY METALS IN MEDICINAL PLANTS COLLECTED FROM DIFFERENT GEOGRAPHIC POINTS IN LUCKNOW, UTTAR PRADESH

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Abstract:

The present study was carried out to determine the heavy metal concentration in medicinal plants collected randomly along the highway. The concentration of four heavy metals such as Fe, Cu, Cd, and Pb were investigated in twenty medicinal plants (*Ocimum tenuiflorum, Azadirachta indica, Allium sativum, Curcuma longa, Catharanthus roseus, Cannabis sativa, Datura stramonium, Rosa rubiginosa, Calotropis gigantean, Aloe vera, Tagetes patula, Lantana camara, Rauvolfia serpentine, Withania somnifera, Bacopa monnieri, Asparagus racemosus, Tinospora cordifolia, Eclipta prostrate, Hibiscus rosa-sinensis, and Solanum nigrum*) using Atomic Absorption Spectrometry. The average concentration of metals among all the plants displayed the following order: Fe> Pb> Cu > Cd. Highest concentration of Fe (322.62 µg/g) and Cu (19.14 µg/g) was observed in *Asparagus racemosus,* Cd (2.1 µg/g) was observed *in Tagetes patula* and Pb (54.47 µg/g) was observed *in Catharanthus roseus.* Among the metals, Fe, Cd and Pb were found beyond the permissible limit recommended by World Health Organization (WHO).The main source of heavy metals in the studied area seems dust deposited by vehicular emissions due to high traffic on the highway. The mechanisms by which heavy metals introduce into the environment via vehicles consist of fuel incineration, engine oil consumption, crash barriers corrosion, tire wear, brake lining wear, and road abrasion. The finding of this study suggests that a high level of contamination of heavy metals in medicinal plants, which is not suitable for human consumption. So, regular monitoring and protective measures need to be taken to protect these important medicinal plants.

Keywords: Medicinal Plants, Heavy metal, Iron, Cadmium, Copper, Lead, Contamination

Introduction:

Medicinal plants have been traditionally using worldwide for manufacturing of large number of medicines. From the ancient period, medicinal plants have widespread applications in the treatment of several diseases around the world. Plant parts such as leaves roots, bark, fruits, and seeds are used in the herbal medicines. According to the World Health Organization (WHO) report, about 80% of the world's marginal communities use only medicinal plants for the treatment of various diseases due to limited medical facilities (Annan *et al.*, 2013). In developing countries, until now medicinal plants are used for the remedy of diseases like skin infections, malaria, fever, diarrhea, diabetes, respiratory disorders, and bacterial and fungal infections (Fernandez-Luqueno *et al.*, 2013).

From past decades, there is a growing concern due to increased environmental pollution by the rapid urbanization, population growth, and intense industrialization. In recent years, heavy metal contamination has gain global attention, mainly because of the toxicological risks posed by such metals to human health (Ayodeji and Olorunsola, 2011).The primary sources of metals are fossil fuel burning, mining, smelting of metals and ores, application of synthetic fertilizer and pesticide in agriculture, improper disposal of contaminated water, municipal and sewage waste, industrial residues, vehicular emissions (Liliane *et al.*, 2016).Increased concentrations of heavy metals in the air, soil, and water threatens human health both directly and indirectly via accumulation in the food chain. Although trace amounts of some metallic elements are essential for human health, they become toxic when their concentrations exceed their permissible limits (Elekes *et al.*, 2010). Plants are a good accumulator of heavy metals they can accumulate much higher concentrations than the metal present in the environment. Due to the great ability to bioaccumulation of metals in the plant, they can increase the potential harmful risk to health (Rao and Kumar Meena, 2011).

Heavy metals such as Pb, Cd, As, and Hg are non-essential and more toxic to the plants and no significant use in the biological system (Ji *et al.*, 2012). Whereas, heavy metals such as Cu, Zn, Fe, and Co are essential for the human body in trace amounts for the regulation of enzyme, vitamin synthesis, and hemoglobin formation, and also requisite for growth, development, and photosynthesis in plants. But their high concentrations may cause damage to the central nervous system, liver, heart, kidney, and brain, which further leads to hypertension, skin infections, intestinal ulcer, abdominal pain, and cancers (Fernandez-Luqueno *et al.*, 2013).

Medicinal plant contamination with heavy metals is an issue of serious concern to public safety worldwide. WHO has set the heavy metal limits to ensure the plant's suitability for medicinal purposes. WHO also recommends that heavy metal concentration should be examined

in the raw material before going for further processing.The present study aims to quantify the heavy metal concentration in some important medicinal plants. Four heavy metals (Fe, Pb, Cu, and Cd) concentration in 20 medicinal plants has determined.

Material and Methods:

Sample collection:

Leaf sample of different medicinal plants (*Ocimum tenuiflorum, Azadirachta indica, Allium sativum, Curcuma longa, Catharanthus roseus, Cannabis sativa, Datura stramonium, Rosa rubiginosa, Calotropis gigantean, Aloe vera, Tagetes patula, Lantana camara, Rauvolfia serpentine, Withania somnifera, Bacopa monnieri, Asparagus racemosus, Tinospora cordifolia, Eclipta prostrate, Hibiscus rosa-sinensis, Solanum nigrum*) were collected nearby the National Highway (NH-27) VidhyaVihar and South City, Lucknow, Uttar Pradesh. All the collected fresh leaf samples were transferred to the sterile polyethylene sampling bags and immediately transferred to the laboratory.

Preparation of samples:

The leaf samples were washed thoroughly with running tap water to remove the dust particle deposited on a leaf then further washed with deionized water to get refined samples. The washed samples were air-dried in shade and then placed into hot air oven at 80-90^{o} C for 48 hours to remove the moisture content. The oven-dried samples were immediately grind using pestle and mortar. Homogenized samples were sieved through 2-mm sieve. Then sieved samples were stored in the clean and dried plastic bottles and used for further heavy metal analysis.

Analysis of the samples:

One gram of each plant samples was precisely weighed on an electric balance and transferred into a 100 ml digestion flask. A digestion mixture of per-chloric acid ($HClO_4$) and concentrated nitric acid (HNO_3) in the ratio of 1:3 was used for the digestion of the samples (Annan *et al.,* 2013). 20 mL of digestion mixture was added to each of the flasks. The samples were placed on the hot plate in a fume chamber and the samples were heated for 1 hour at 90ºC, then the temperature was gradually increased to 100ºC-120ºC, the samples were heated until a clear and semi-dried solution was obtained. The digested samples were allowed to cool down at room temperature. The semi-dried residue was dissolved and made up to 20 mL with 0.1 N HNO_3 solution in a digestion flask. Then the solution was filtered through Whatman filter paper no. 42 and stored into the amber glass vials. A blank solution was prepared by the same

procedure as used for plant samples. The plant samples and standard of various heavy metals analyzed in the atomic absorption spectrometer.

Results and Discussion

Heavy metal (Fe, Pb, Cu and Cd) concentrations of 20 medicinal plants are shown in figure 1.Among all the plants, total metal concentration in *Asparagus racemosus* was found highest. Whereas, the lowest total metal concentration found in *Bacopa monnieri*.

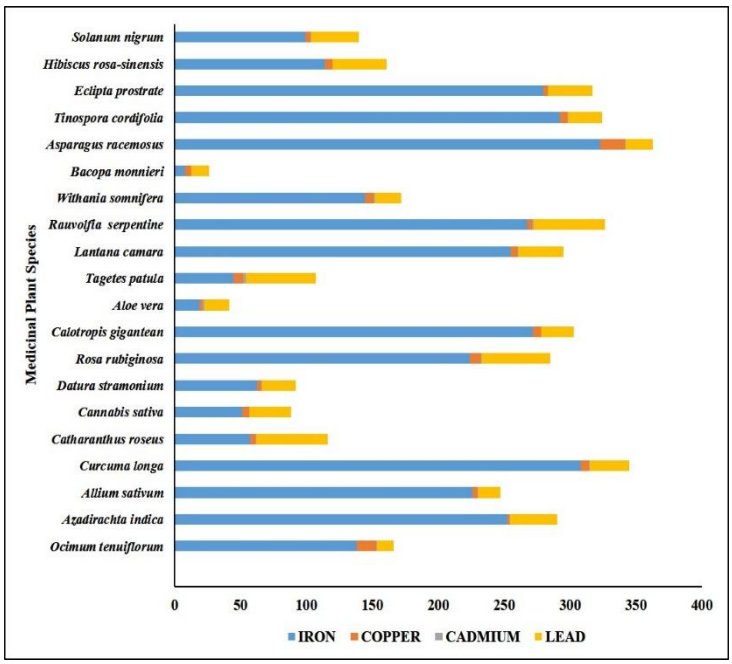

Figure 1: Heavy metal (Fe, Pb, Cu and Cd) concentration of the medicinal plants

The experimental results showed that Iron (Fe) concentration in medicinal plant samples found between the range of 322.62 µg/g and 7.61 µg/g. The highest concentration of Fe found in *Asparagus racemosus* (322.62 µg/g), whereas the minimum content of Fe found in *Bacopa monnieri* (7.61 µg/g). The permissible limit of Fe in the medicinal plants is 20 µg/g, which is set by WHO (WHO, 2007). In this study, most of the plants found above the permissible limit. Iron is a vital element and has several functions in the human body but its higher concentrations can cause health problems such as nausea, vomiting, diarrhea, joints pain, shock, myocardial infection, and liver damage (Nkansah *et al.*, 2016). The major source of Fe is earth crust as it is the fourth abundant metal of earth crust. Whereas anthropogenic sources are vehicle emissions and motorway road dust. The mean concentration of Fe found in different 20 medicinal plants and its permissible limit depicted in figure 2.

The experimental results showed that Copper (Cu) concentration in medicinal plant samples found between the range of 19.14 µg/g and 1.88 µg/g. The highest concentration of Cu found in *Asparagus racemosus* (19.14 µg/g), whereas the minimum content of Cu found in *Azadirachta indica* (1.88 µg/g). The permissible limit of Cu in the medicinal plants is 10 µg/g, which is set by WHO (WHO, 2007). In this study most of the plants found below the permissible limit. However Cu is an essential element, but its excessive concentration can cause skin and hair decoloration, respiratory problems, nausea, and dermatitis (Khan *et al.*, 2008). Vehicular emission and fungicides could be the source of Cu in the plants (Maffo Maffo *et al.*, 2016). The mean concentration of Cu found in different 20 medicinal plants and its permissible limit depicted in figure 3.

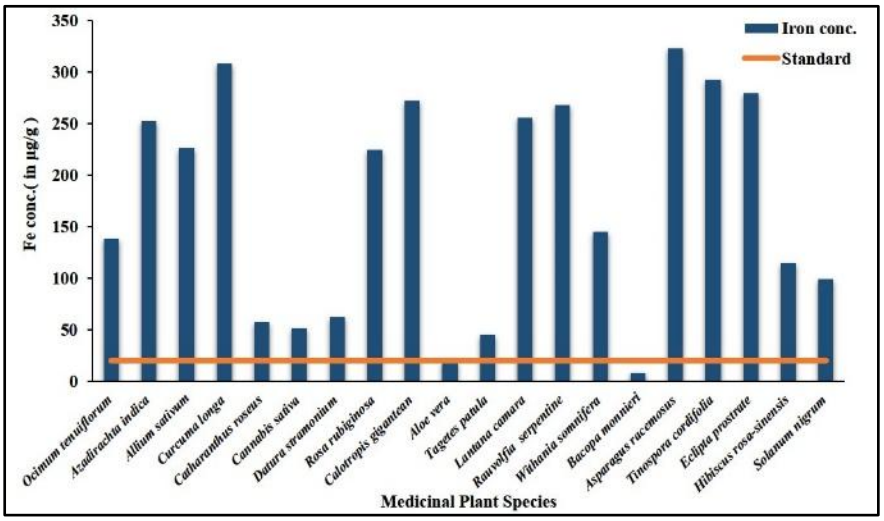

Figure 2: Graph showing Fe concentration of medicinal plants

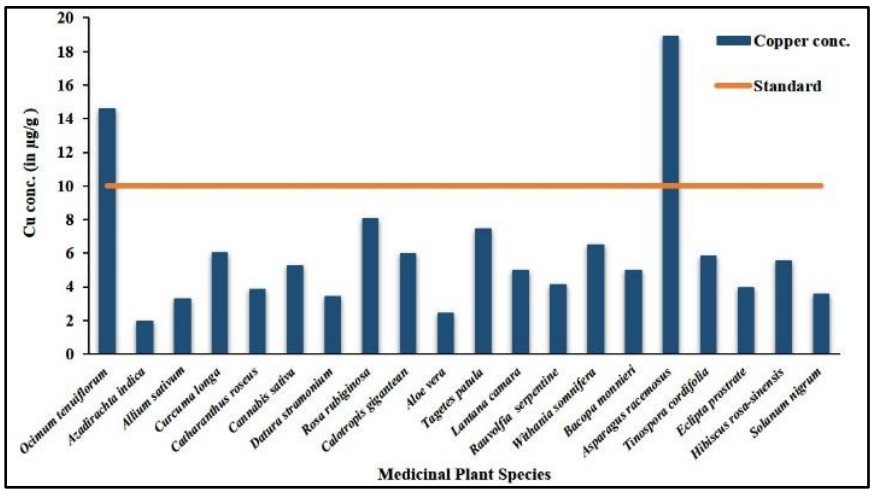

Figure 3: Graph showing Cuconcentration of medicinal plants

Cd is a non-essential element with no function in plants. It is very toxic even in low concentration, its accumulation in the human body can cause liver and kidney damage (Annan *et al.*, 2013). The experimental results showed that Cadmium (Cd) concentration in medicinal plant samples found between the range of 2.1 µg/g and 0.001 µg/g. The highest concentration of Cd found in *Tagetes patula* (2.1 µg/g), whereas the minimum content of Cd found in *Hibiscus rosa-sinensis* (0.001µg/g). The permissible limit of Cd in the medicinal plants is 0.3 µg/g, which is set by WHO (WHO, 2007). In this study most of the plants found above the permissible limit. The major source of Cd can be fossil fuel combustion and application of phosphate fertilizers in nearby agricultural filed (Annan *et al.*, 2013). The mean concentration of Cd found in different 20 medicinal plants and its permissible limit depicted in figure 4.

Pb is one of the highly toxic heavy metals in the environment. Its higher concentrations can cause brain damage, nervous disorders, anemia, kidney damage, reproductive defects carcinogenic, skeletal, muscular, and gastrointestinal symptoms(Nkansah *et al.*, 2016).The experimental results showed that Lead (Pb) concentration in medicinal plant samples found between the range of 54.47 µg/g and 13 µg/g. The highest concentration of Pb found in *Catharanthusroseus* (54.47 µg/g), whereas the minimum content of Pb found in *Ocimumtenuiflorum* (13 µg/g). The permissible limit of Pb in the medicinal plants is 10 µg/g, which is set by WHO (WHO, 2007).

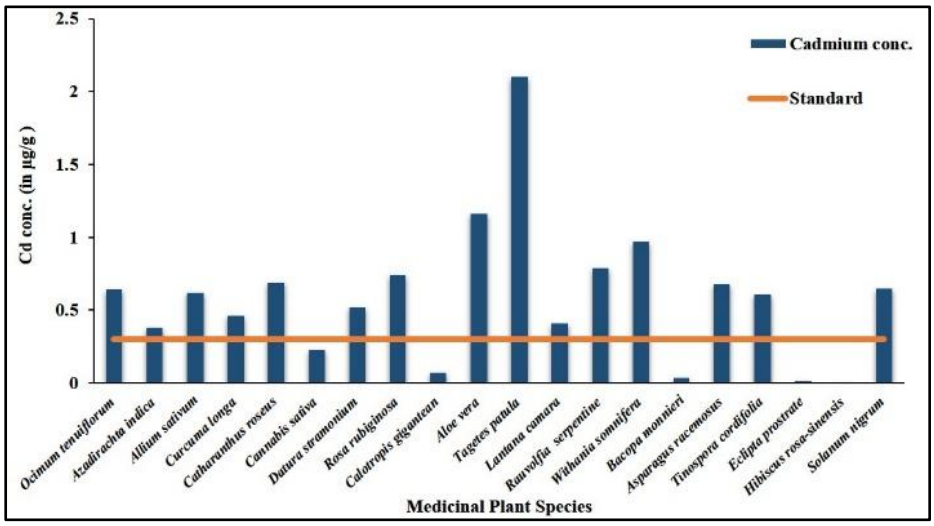

Figure 4: Graph showing Cd concentration of medicinal plants

In this study most of the plants found above the permissible limit. The main source of Pb in plants is contaminated soil, which can be polluted by different sources. In the study area, vehicular emission due to the nearby highway, and insecticides and fungicides used in the

agricultural field can be the source Pb in the plants (MaffoMaffo *et al.,* 2016).Fuel incineration, engine oil consumption, crash barriers corrosion, tire wear, brake lining wear, and road abrasion are also a source of heavy metal in the environment. The mean concentration of Pb found in different 20 medicinal plants and its permissible limit depicted in figure 5.

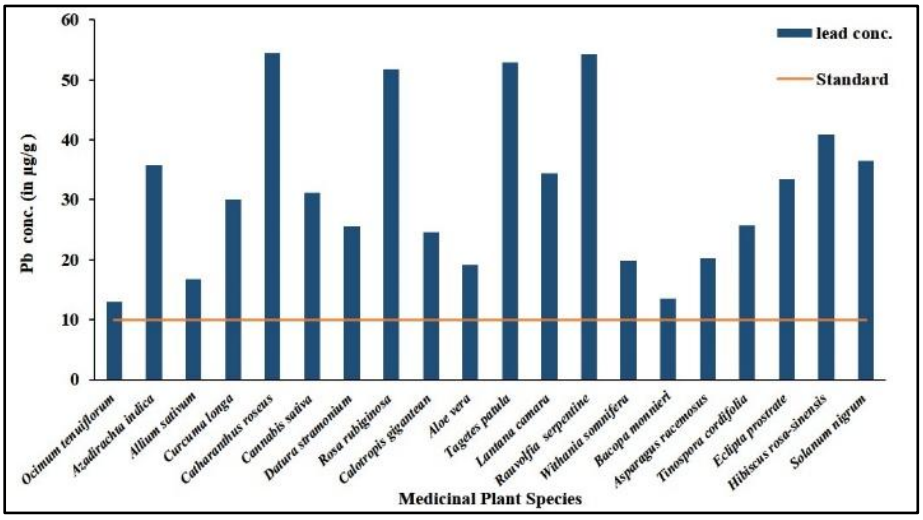

Figure 5: Graph showing Pbconcentration of medicinal plants

Conclusion:

In the present study, heavy metal concentrations in medicinal plants observed beyond the permissible limits. Among the metals, Fe content found highest followed by Pb, Cu, and Cd. The highest concentration of Fe and Cu found in *Asparagus racemosus*. Whereas the high Cd content has found in *Tagetes patula*, and high Pb content has found in *Catharanthus roseus*. The significant sources of heavy metals in the medicinal plants are dust deposited by vehicular emissions due to high traffic on the nearby highway. Besides, contaminated soil, fertilizer application in agriculture and groundwater could be a probable source of metals. The present study suggests that a high level of heavy metals contamination in medicinal plants is not suitable for human consumption. So it is recommended that medicinal plants for the formulation of herbal remedies should be harvested from pollution-free natural habitat.The studied plants are commonly used by the community, so it is essential to detect the chemical constituents in these plants for the well-being of the public health.

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SCREENING AND CHARACTERIZATION OF POLY-β-HYDROXYBUTYRATE (PHB) PRODUCING BACTERIA FROM FRESH WATER

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Abstract:

Poly-β-hydroxybutyrate (PHB)which is also known as bioplatic or biodegradable polyester is the need of time and a study was undertaken to identify the potential bacteria for production of PHB from freshwater. It is a substitute for conventional non-biodegradable plastics and has many applications in various areas of different industries. The present study reports the isolation, screening and identification of PHB producers from fresh water. Nine isolates were screened using sudan black staining method. PHB fermentation media was used to culture the selected isolates and the product obtained was purified and quantified using standard methods. Three out of nine strains were found to exhibit better productive capabilities as isolate 2,5 and 9 showed 7.79%,7.53% and 9% respectively. The strains were characterized morphologically and biochemically and were recorded as Bacillus species.

Keywords: Polyhydroxybutyrate, PHB production, biodegradable, Sudan black staining

Introduction:

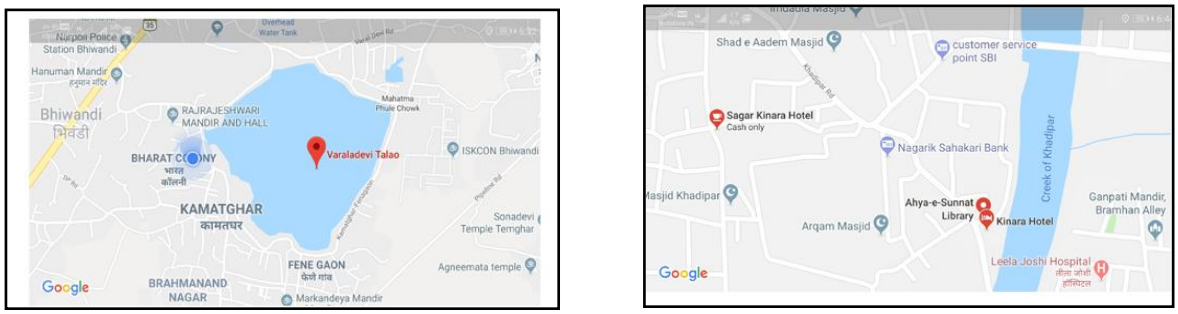
Human being is cleverer among the animal kingdom and creates many luxuries material for benefit of itself and from which millions of tones waste is generated so nature has solution within it. To overcome it we humans have to search solution, many more pollutants are been generated in due course of development, Plastic is the most talked. Properties of Bio-plastics are comparable in all respect synthetic plastics. Biodegradation can be explained as a chemical

process during which micro-organisms that present in the environment convert materials into natural substances such as water, carbon dioxide, and compost. Synthetic plastics remain in the environment for long time as they are resistant to degradation (Aminabhavi *et al.*, 1990). There are more than 300 of such species known to produce PHBs such as Azotobacter, Bacillus, Pseudomonas, Aeromonas sp., Rhodopseudomonas sp., Halomonas sp., Methylobacteria, transgenic Escherichia coli etc. (Berlanga *et al.*, 2006). Microbes have been reported to be the potent producers of PHB because of eventful changes in surrounding condition leads to susceptibility towards adverse conditions. PHB is biocompatible as it is soluble in chloroform and chlorinated hydrocarbon so can be used in different industries including medical industry. The study aims to screen fresh water bacteria for its bioplastic production ability as fresh water is not explored for this application.

Materials and Methods:

Sample collection:

The fresh water samples were collected from the Varaldevi Lake, Bhiwandi, Thane, Maharashtra. The water samples were collected from the collected from the areas like Varaladevi Lake and Khadi par. The samples were collected in bottels and carried to the laboratory of Department of Biotechnology, K.M.E.Society's G. M. Momin Women's College and Bhiwandi. These samples were serially diluted in Saline Solution.



A) Varaldevi lake **B) Khadi par**

Figure 1: Water sample collection sites

Screening of bacterial isolate for PHB production:

The isolates were screened using Sudan Black Staining Method. For this method, the plates containing nutrient agar were divided into 4 parts. The bacterial isolate were spotted in duplicates and incubated at 30◦C for 24 hours. To check the exhibition of PHB production ethanolic solution of (0.02%) Sudan Black were poured over the colonies and kept for 30

minutes. They were washed with ethanol (96%) to remove the excess stain from the colonies. PHB producing colonies were identified as dark blue stained colonies in the plate. These isolates were sub-cultured and maintained at 4^0C (Kalaivaniand and Sukumaran, 2013).

Fermentative PHB Production:

PHB production is a two-step process. The first step includes use of 100ml broth having rich carbon content with inoculums for growth The medium was prepared using $(NH_4)_2SO_4$, 20g/L; KH_2PO_4, 13.3 g/L; $MgSO_4.7H_2O$, 12 g/L; Citric Acid, 1.7 g/L; trace element solution, 1.0 ml/L containing $FeSO_4.7H_2O$ 10g/L; $ZnSO_4.7H_2O_2$, 25g/L; $CuSO_4.5H_2O$, 1.57 g/L; $MnSO_4.5H_2O$, 0.5 g/L; $CaCl_2.2H_2O$ g/L; $Na_2B4O_7.10H_2O$, 0.23 g/L; $(NH_4)6Mo70_{24}$,0.1 g/L; pH 7.2 at 29^0C at 120 rpm for 24 hours. After incubation culture broth was centrifuged at 10,000 rpm for 15 minutes further biomass was collected and weighed .Then in second step cell growth was done in Nitrogen Deficient Minimal Medium (NDMM) with some modification in standard medium (Montaser and Luesch, 2011).

Extraction and Quantification of PHB:

Centrifugation of carbon rich and nitrogen deficient broth culture of respective fermentation media were carried out at 10,000 rpm for 10 min and further washed with acetone and ethanol. The pellet was suspended in 4% Sodium hypochlorite and incubated at room temperature for 30 min. Centrifugation was repeated of suspension at 10,000 rpm for 10 min and supernatant was collected and discarded while pellet washed with acetone and ethanol respectively. 10ml of chloroform was added to the tube by placing it to separate PHB from liquid broth,10ml chloroform was added by placing tube in hot water bath (60^0C),then to obtain PHB crystals chloroform was evaporated.

$$\text{PHB accumulation (\%)} = \frac{\text{Dry weight of extracted PHB (g/L)} \times 100\%}{\text{CW (g/L)}}$$

10 ml of concentrated H2SO4 was used to extract PHB from the isolate under study and the tube was heated in a water bath kept at 60^0C for 1hr to complete the conversion of PHB crystals into crotonic acid. Ice bath was used to cooled the sample then sample was vortex and the amount of PHB was determined spectrophotometrically at wavelength 234nm against H_2SO_4 using 200 mg/ml standard crotonic acid (Sindhu *et al.,* 2013). The standard curve was used for the estimation of PHB yield (Nehra *et al.,* 2015).

Characterization and identificationof Bacteria:

The morphological characteristics of the isolate were examined. The potent isolate were observed for colony characterization by determining size, shape, Gram`s nature of the cells. The

biochemical characterization was in reference with the method of Bergey's Manual of Determinative Bacteriology.

Results and Discussion:

Isolation and Screening of PHB producers:

Efforts are made to replace plastic from petroleum origin so biodegradable plastic production to be developed for sustenance. Present study deals with isolation, screening, extraction and quantification of PHB producing microbes from freshwater environment as very few reports available on fresh water studies than marine water. Water samples were used as the source of microbes as they are known to produce variety of compounds in stressed condition in which they survive. Samples of 10^{-7} dilutions were cultured on nutrient agar plates and incubated at room temperature for 24 hours to isolate bacterial colonies.

The primary screening was done for PHB production following viable colonies using Sudan black staining method, use of dye was based on the principle that artificial organic compounds stains the plastic into blue or black colour. Out of 15 isolates nine were recorded to be positive for PHB accumulation.

Sudan Black, in particular, has been utilized as a presumptive test for the presence of PHB even though it stains all lipophilic storage materials (Serafim *et al.,* 2002).

Extraction of Polyhydroxy butyrate:

PHB produced by all the nine isolates was purified using sodium hypochlorite-chloroform method. The PHB accumulation was calculated for all the nine isolates and it was found to be two promising bacterial isolates which showed increased production of PHB of 12.08% of 15.14g/L CDW in carbon rich medium (Fig. 2) and 9.00% of 15.55g/L CDW in nitrogen deficient minimal medium (Fig. 2). The model of two-stage fermentation operation where cell growth phase is separated from production phase can improve the performance of PHB synthesis and may improve the yields further (Chen *et al.,* 2013).

Quantification of PHB:

The quantification method involves the breakdown of the PHB polymer into its monomers. PHB is polymer and to know the amount produced it need to be converted into its monomer and these monomers are then measured using crotonic acid as the standard on a calibration curve. PHB present in the sample was estimated by using the UV absorption by monomer which is directly proportional to the amunt of PHB (Fig. 3).

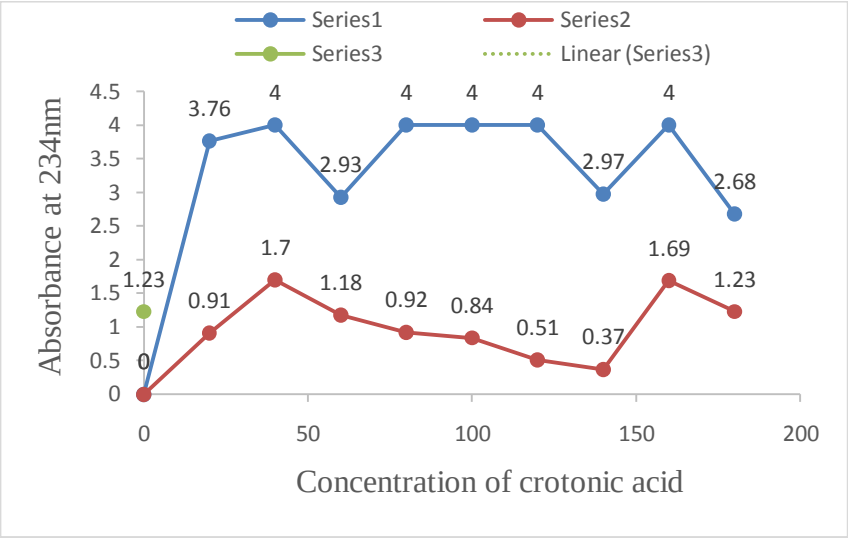


Figure 2: Amount of PHB produced in Carbon rich and NDMM growth medium

Series 1—Carbon rich, Series 2– NDMM

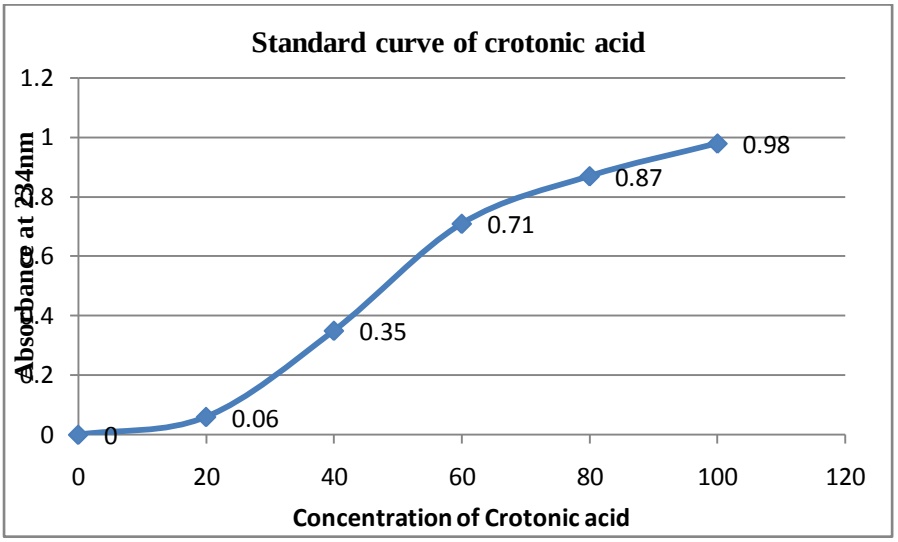


Figure 3: Standard Calibration curve of crotonic acid

Three out of nine strains were found to exhibit better productive capabilities as isolate 2, 5 and 9 showed 7.79%, 7.53% and 9% respectively (Table 1). The strains were characterized morphologically and biochemically and were recorded as Bacillus species. Bacillus strains have been reported possessing tremendous potential of PHB accumulation in their cytoplasm under nutrient limiting conditions at a level of 6–97% of dry cell weight (Hori *et al.*, 2002, Hikmet *et al.*, 2003, Rohini *et al.*, 2006). In present investigation also most potent isolates were identified as Bacillus.

Table 1: Residual biomass and PHB accumulation of the potent isolates

Sr. No.	Isolate No.	Weight of PHB	CDW (g/L)	PHB Accumulation %
1	1	0.21	11.69	1.79%
2	2	1.02	13.08	7.79%
3	3	0.36	14.05	2.56%
4	4	0.35	12.65	2.76%
5	5	1.12	14.87	7.53%
6	6	0.63	13.00	4.84%
7	7	0.23	11.72	1.96%
8	8	0.23	13.62	1.68%
9	9	1.4	15.55	9.00%

Characterisation of PHB producers:

According to Burgeys manual of Bacteriology the basic characterization of PHB producer were carried out which is depicted in Table 2. The genera Azotobacter, Alcaligenes, Pseudomonas, and Bacillus were reported to producer of PHB by many investigator (Singh and Parmar, 2011, Aarthi and Ramana, 2011, Babruwad *et al.*, 2015).

Table 2: Morphological and biochemical characteristics of isolates

Isolate No.	Grams Nature	Cit	TSI	catalase	Oxi	Nit	Gel	Ure
1	Positive	-ve	A/A	+ve	+ve	-ve	-ve	-ve
2	Positive	-ve	K/A	+ve	+ve	-ve	-ve	-ve
3	Positive	+ve	A/A	+ve	+ve	-ve	+ve	-ve
4	Positive	+ve	K/NC	+ve	+ve	-ve	+ve	-ve
5	Positive	-ve	A/A	-ve	-ve	-ve	+ve	-ve
6	Positive	+ve	K/NC	-ve	+ve	-ve	+ve	-ve
7	Positive	+ve	A/A	-ve	+ve	-ve	+ve	-ve
8	Positive	-ve	K/NC	+ve	+ve	-ve	-ve	-ve
9	Positive	+ve	K/NC	-ve	+ve	+ve	+ve	-ve

Keys: Cit: Citrate utilization Gel: Gelatin hydrolysis Oxi: Oxidase test,
Nit: Nitrite reductase test Ure: Urease test TSI: Triple sugar iron test,
A/A: Glucose, sucrose, and/or lactose fermenter K/A: Glucose fermentation only
K/NC: Glucose, lactose and sucrose non-utilizer (alkaline slant/alkaline butt)
+: Positive -: Negative

Conclusion:

The efficiency of PHB production using biomass collected from fresh water system may reduce the operational costs, making it an interesting prospect for many industrial, therapeutic, and diagnostic applications. The production of bioplastics is still under research and is far from commercialization. The significance of bacteria in bioplastics industry will make bio plastics a reality in the near future.

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SYNTHESIS AND CHARACTERIZATION OF 2, 2'- [1, 2-PHENYLENEBIS (AZANEDIYL METHYLENE)] DIPHENOL AND THEIR METAL COMPLEXES

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Introduction:

Schiff base ligands have the disadvantage of their propensity of hydrolysis. This instability will be conquered by reduction of the Schiff base to convert into an amine. This boons interesting possibilities since the reduced Schiff base are more flexible and not forced to stay planar when coordinated to a metal center. $NaBH_4$, as a typical reducing agent, has been used widely in organic synthesis. There are several reported within the literature for reduction of imines by sodium borohydride (Cho and Kang, 2004, Keskioglu *et al.*, 2008). Now it is reported that reduced Schiff base showed significant antimicrobial activity at low micromolar level (Cho and Kang, 2005, Musa *et al.*, 2010, Patil *et al.*, 2018). Amongst the ligand systems, derivatives ofsalophen are extremely important because of theirvaried chelating ability, structural flexibility and catalytic activities. Also, severalvarieties of reduced Schiff base ligands and their Fe complexes (III) Fig. 1 have attracted continued attentionwithin the medicinal field due to their effective DNA cleavage activity (Voronova *et al.*, 2014). It is evident from the literature that transition metal complexes of partially reduced Schiff base scaffolds (IV) are known to exhibit excellent catalyst for oxidation of varied phenols (Routier *et al.*, 1999).

Experimental work:

2, 2'-{1,2-phenylenebis [azanylylidene (E) methanylylidene]} diphenol Schiff base has been prepared from the reaction of two equivalents of salicylaldehyde with one equivalent diamine derivatives followed by reduction with $NaBH_4$. The Co (II), Ni (II) complexes of these ligands are prepared. All the synthesized ligands and metal complexes were characterized by FTIR, ^1H and ^{13}C-NMR, MS.

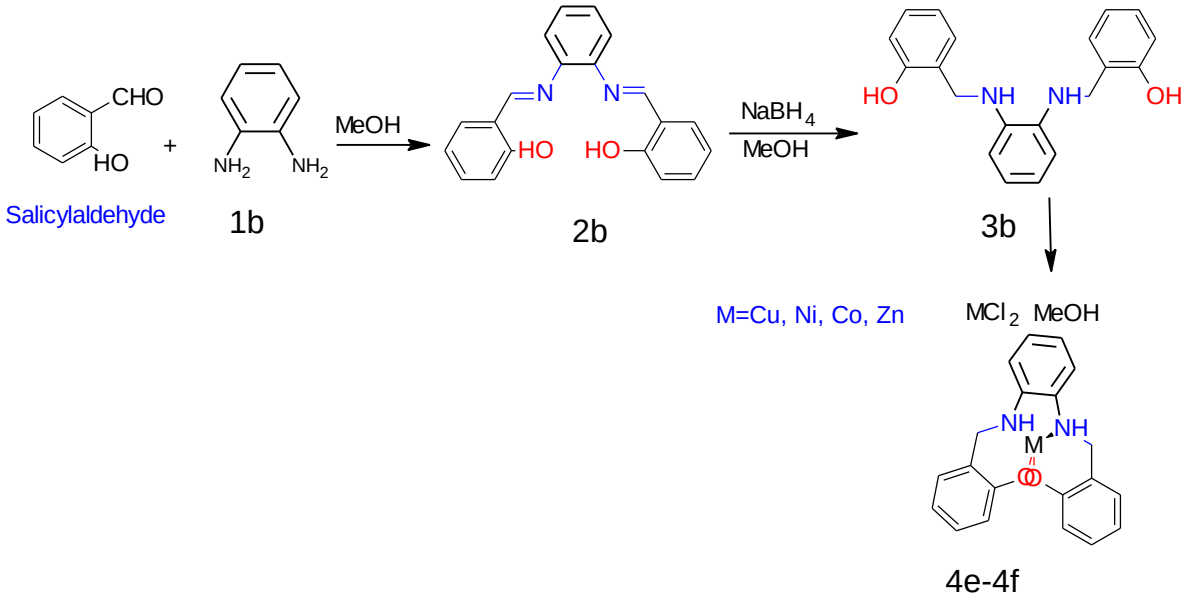
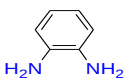
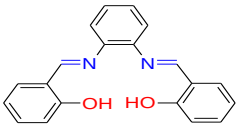
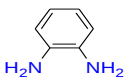
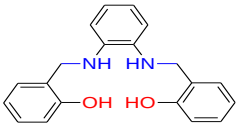


Figure1: Preparation of hydrazones and metal complexes

Procedure for preparation of Schiff bases:

A solution of salicylaldehyde and 1, 2-diamine derivative 1b (4.0mmol:2.0 mmol respectively) in EtOH (8 mL) was stirred for two hours.The solid obtained was filtered and dried.

Table1: Structure of Schiff base [2b] and its reduced form [3b]

Entry	Diamine	Product	Yield (%)
2b			94
3b			81

Procedure for preparation of reduced Schiff bases:

Methanolic solution of $NaBH_4$ (2.0 mmol) containing some drops of concentrated KOH solution was added to a solution of the Schiff base(2.27 mmol) in dichloromethane (10.0 mL) at 0°C. The pH was set to six and also the solution stirred for several hours until the yellow colour had disappeared. The solvent was evaporated to dryness and cold water (10.0 mL) was added to the residue. The pH was set to 4–5 by addition of 3.0 M HCl. The white solid was filtered, washed with cold water, ethanol and diethyl ether and dried under vacuum.

Preparation of 2, 2'- [1, 2-phenylenebis (azanediyl methylene)] diphenol [3b]:

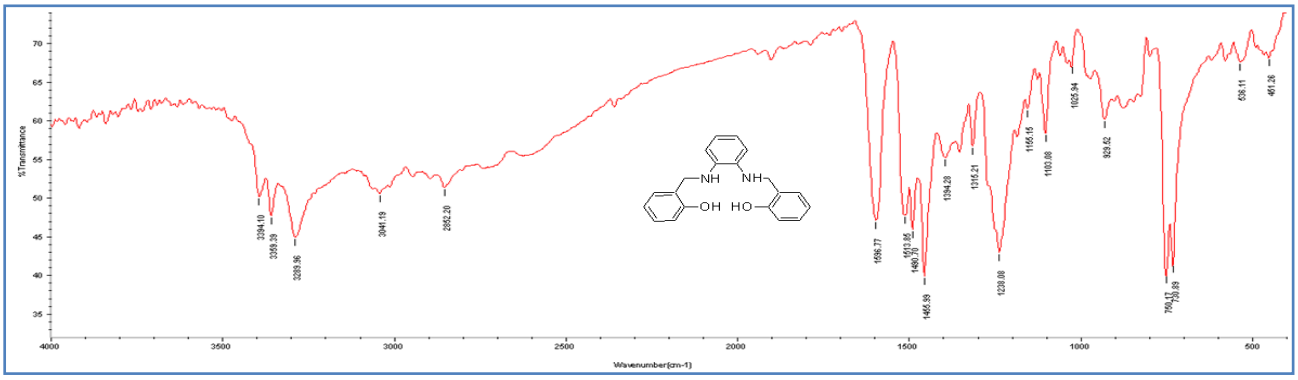


Figure 2: FTIR spectra of 3b

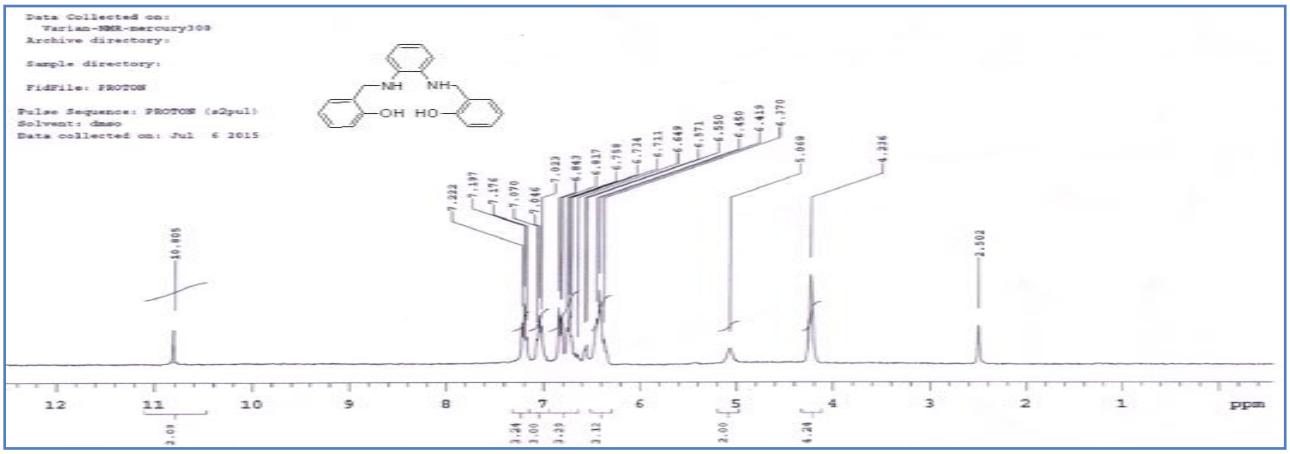


Figure3: ^{1}H-NMR spectra of 3b

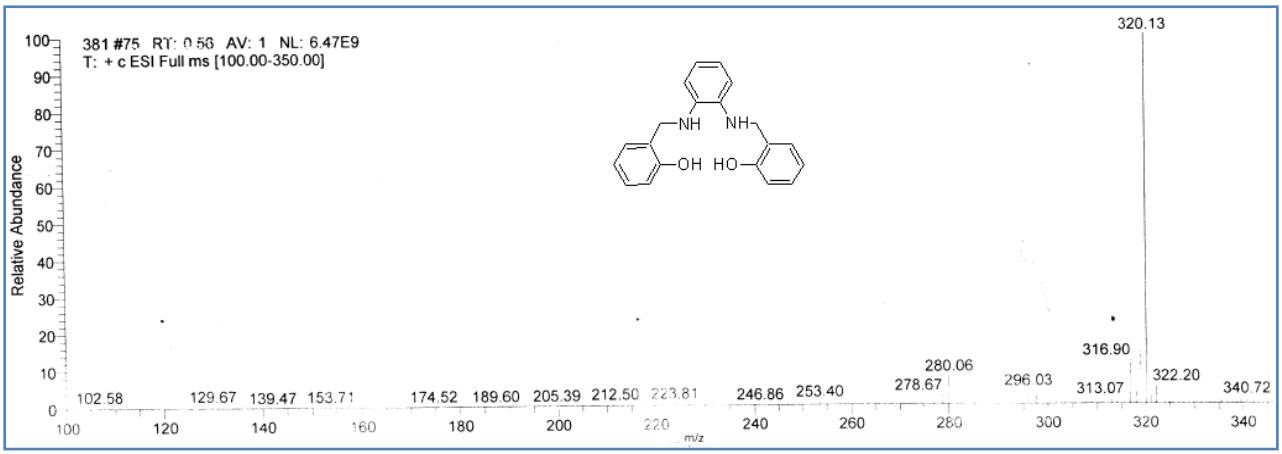


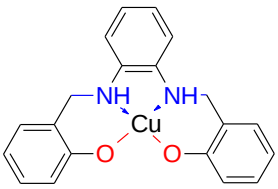
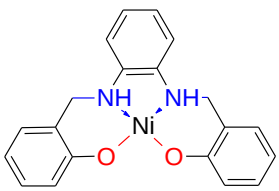
Figure 4: Mass spectra of 3b

Colour: White; MS [M+]: 320.13; FTIR(KBr cm^{-1}): 3394, 3359, 3289, 3041, 2852, 1696, 1456, 1315, 1238, 1103, 1025, 929,750 and 636; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.236(s, 4H), 5.068(s, 2H), 6.758-6.843(m, 3H), 6.37-6.450(m, 3H),7.023-7.070(m, 3H), 7.176-7.222(m, 3H), 10.805(s, 2H);

Procedure for preparation of metal complexes:

Schiff base (0.050 mmol) was dissolved in 10.0 ml of methanol in round bottom flask fitted with condenser and calcium chloride salt guard tube. Metal salt such as $CuCl_2$, $NiCl_2$; (0.050 mmol) was added and stirred the reaction mixture, finally Potassium hydroxide (0.050 mmol) in methanol was added and reaction mixture was refluxed for 3-5 hours in water bath. It had been cooled and filtered the solid separated and dried in oven.

Table 2: Structures of metal complexes of reduced Schiff bases [4e-4f]

Entry	Complex	Colour	Entry	Complex	Colour
4e		Grey	4f		Brown

Results and Discussion:

The primary step involves the synthesis of Schiff base derivatives by condensation of salicylaldehyde with diamines 1b in warming ethanol at 70^0 C afforded Schiff bases 2b. On

action with sodium borohydride imine bond undergo reduction to yield reduced Schiff base ligands 3b. Spectral data (FT-IR, ^{1}H and MS, UV-Visible and Fluorescence) confirmed the structure of the synthesized products. The FTIR spectrum region of Schiff bases 2b shows strong absorption bands at 1614-1616 cm^{-1} because of imine (-HC=N-), which disappearswithin the IR spectra of reduced Schiff bases 3bapproves the reduction of Schiff bases 2b. FTIR spectra of reduced Schiff base ligands 3bexhibitedcomprehensivepeak within the region 3390-3394 cm^{-1} and 3359 cm^{-1} due to amino (-NH-) group and hydroxyl group, respectively. Schiff bases 2b discovered the ^{1}H-NMR spectrumregion of expected aromatic signals, three singlets at δ 8.90 and 12.93 ppm of assignable proton to the azomethine (-CH=N-) and hydroxyl proton (-OH) respectively. The new singlets at δ 4.2 and 4.9 because of $–CH_2$- and –NH- group respectively, authorizes the formation of reduced Schiff bases 3b. Furthermore, the mass spectrum of Schiff bases exposed molecular ion peak confirming corresponding relative molecular mass of target compounds.

The Schiff bases 2b and reduced Schiff base ligands 3b were refluxed with suitable transition metal chlorides in methanol with molar ratio 1:1 offer metal complexes 4e-4f respectively. Due to water of crystallization, metal complexes show broad peak within the region of 3330-3517 cm^{-1}. The coordination mode of hydrazones with central metal ion iselucidated on the premise of FTIR spectral study. The low frequency area of the spectra indicated the presence of two new medium intensity bands at about 443-485 cm^{-1}because ofυM–O vibrations. Due to υM–N stretching,all the metal complexes show prominent band of IR spectra of at about 501-620 cm−1.

From this experimental learning, it's clear that practical observations and remarks are in good treaty with the theoretical values calculated for 1:1 ratio of metal: ligand stoichiometry. The overall discussion of the results of numerous spectroscopic details; it may be concluded that the projectedgeometry for the transition metal complexes with general formula $ML{\cdot}2H_2O$ is octahedral for metal complexes. The probable structures are shown in table 1 and table 2.

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RARE AND ENDEMIC SPHAEROPLEACEAE, ULVACEAE AND SCHIZOMERIDACEAE FROM DHULE AND NANDURBAR DISTRICT, MAHARASHTRA (INDIA)

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Abstract:

During the study of systematic account of algae of Sakri and Navapur taluka, district Dhule and Nandurbar respectively, Maharashtra the author collected three taxa belonging to three families i.e. Sphaeropleaceae, Ulvales and Schizomeridaceae. Out of three taxa Sphaeroplea soleiroti var. crassisepta (Rieth) Ramnathan have been recorded first time from India. Very small attempt have been made to explore these three families from region i.e. Jaiswal (2017). The Sphaeropleaceae, Ulvales and Schizomeridaceae of this region have not been studied earlier. This is the first ever attempt to explore, enumerate and taxonomically evaluate these families from this area. Distribution of taxa in India has been discussed. The quantitative availability of species is also noted.

Keywords: Sphaeropleaceae, Ulvales and Schizomeridaceae, systematic accout, Sakri, Navapur.

Introduction:

The good amount of literature is available on algal flora of this region viz. Barhate and Tarar (1985), Jaiswal (2005, 2009, 2013, 2013a, 2013b, 2013c, 2014, 2014a, 2017, 2017a, 2017b, 2017c, 2017d, 2017e, 2017f, 2017g), Jaiswal and Pathak (2005), Jaiswal *et al.* (2011), Jaiswal *et al.* (2012), no information exists on Sphaeropleaceae, Ulvales and Schizomeridaceae of Sakri and Navapur taluka. Three taxa belonging to three families collected from different places. Out of three taxa one is reported for the first time from India. During taxonomic evaluation of algae from this region it is observed that all these three taxa are on the verge of extinct. A serious step should be taken for the conservation of algal wealth of this region.

Otherwise in short period of time these species will be endemic for this region as well as Maharashtra (Jaiswal, 2017).

Geography of the area:

The district of Dhule, formerly known as West Khandesh lies in the Upper Tapi basin in the North West corner of Maharashtra is now split in two separate districts i.e. district Dhule and district Nandurbar from 1" July 1998. It lies between 20°28' and 20° 3 North latitude and 73° 47' and 75 l1 east longitudes.

It is bounded on the West by Gujarat state on the North by Madhya Pradesh, and on the east and south by Jalgaon and Nasik district respectively. The district is separated from Gujarat and Madhya Pradesh states by Satpudas and from the Deccan by the Satmala hill ranges and arm of the Sahyadri mountains stretching out in easterly directions.

One taluka from each district i.e. Sakri taluka from Dhule district and Navapur taluka from Nandurbar district are chosen for the systematic account of algae. Sakri shows 2998.9 km while Navapur 919.8 km.

Material and Methods:

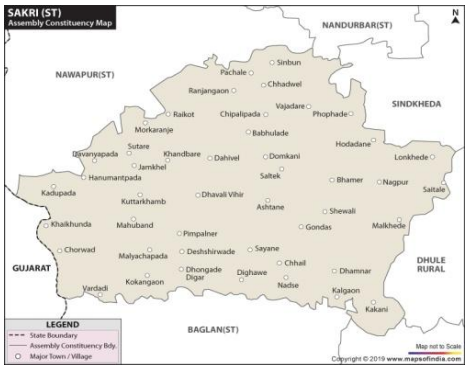
A total 320 collections were studied. The collections were made from December 2019 to January 2020 from 28 towns and village. The sampling sites were selected very carefully, so as to get maximum number of algal forms growing in the varied habitats. Another important aim of this method of selection is to correlates the species identification to the changes taking place in the habitats. Sampling was done from all possible place of collection like pools, puddles, tanks, ponds, lakes, talaws, dams, rain water, waste water passages (gutters), streamlets, rivers, moist soils, tree trunks i.e. bark, moist wall, from the body of submerged animals etc. Field note-book is maintained in which color of the algae, habit, habitat, P^H and temperature of water body were noted down on the spot. All collections were preserved in 4% commercial formalin. These preserved algae were later studied in detail. Camera Lucida of these algae was drawn. Distribution of taxa in India has been discussed.

Preparation of semi-permanent slides for algae:

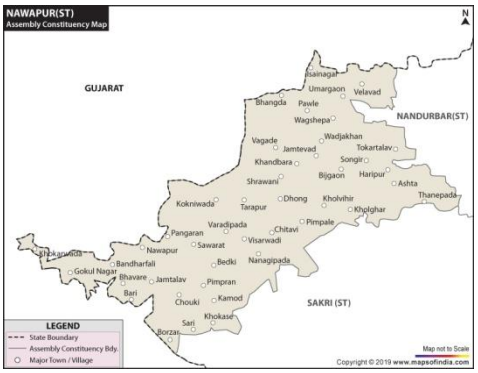
A drop of glycerin-formalin mountant (6 ml. Glycerin + 10 ml. of 40 % formaldehyde + 84 ml. of distilled water) was taken on slide, to which a drop of concentrated preserved sample was added and was covered by a cover slip of suitable size.

The specimens were identified with the help of available literature and standard research papers i.e. Distribution of taxa in India has been discussed. The quantitative availability of

species is also noted. For quantitative abundance abbreviations are used as VR- very rare, VVVR- very very very rare.



Map of Sakri talika



Map of Navapur taluka

Taxonomic Description:

***Sahaeropleineae* Fritsch.**

***Sphaeropleaceae* Kutzing.**

***Sphaeroplea* C. A. Agardh.**

***Sphaeroplea soleiroli* var. *crassisepta* (Rieth) Ramanathan** (Fig. 1)

Ramanathan K.R., 1964; P. 172, Pl. 45, F.S.T.

Filaments thinner than the species, 18-38 µ broad (above the cross wall) and 28 µ at the median region; cells length on an average 85-770 µ, cross wall often with irregular growth. Observed breadth of cell 18 µ, length 149 µ.

Habitat: - Floating in a pond. (RC)

Habit: Rangawali Dam; Date of collection: 23 October 2019

pH 8.0; Temperature: 27^0C ; CBN: - 288; VVVR;

Distribution: - Not recorded.

Ulvales

Ulvaceae

***Enteromorpha* Link.**

***Enteromorpha prolifera* (Fl. Den) Agardh** (Fig.2 a-b)

Collins, 1928; P. 122.

Printz, 1962; P. 90, Pl, 2, F 1-5

Biswas K., 1980; P. 73, Pl. 9, F. 89.

Filaments long, freely branched, branches narrower than the main filaments. 160 - 245 µ

in diam. cell polygonal, quadrangular, 10-25 u in diam., chloroplast parietal with a pyrenoid. Observed diam. of cells 15- 21 µ

Habitat: - Submerged in stagnant water. (VVR);

Habit: Bhamer; Date of collection: 3 February 2019

pH 8.0; Temperature: 10.2^{0}C; CBN: - 15; VVR

Distribution: - M.S. - Bombay: Borivali (Dixit, 1937) Kolhapur (Kamat, 1963, 1965) Alibag (Kamat, 1968).

Schizomeridaceae

***Schizomeris* Kutzing.**

***Schizomeris leibleinii* Kuetz** (Fig. 3 a-b)

Smith G.M., 1950, P. 192, F. 117.

Prasad B.N. and Srivastava P.N., 1963, Phycologia, 2, 148-156

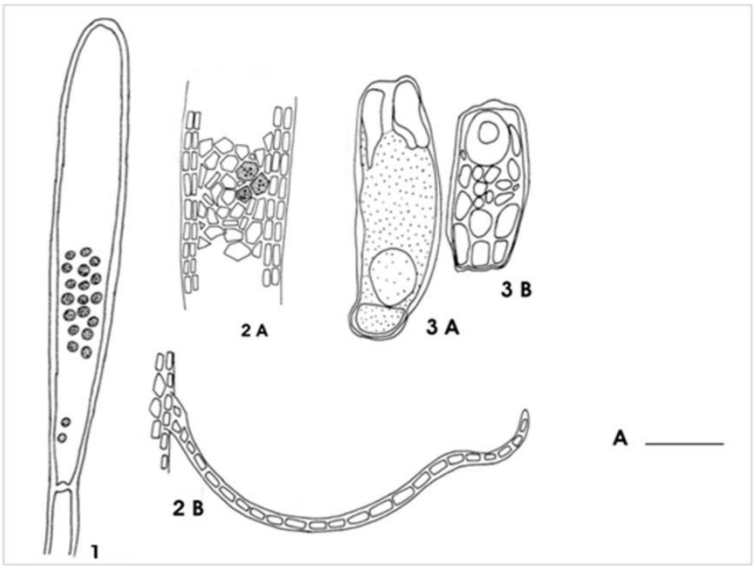
Plant body up to 88 µ long and 30 µ broad.

Habitat: - Free floating in well water. (VVR)

Habit: Navapur; Date of collection: 20 June 2019

pH 7.0; Temperature: 30.5^{0}C; CBN: - 130 A; VVR

Distribution: - M.S. - Nagpur (Kamat & Freitas, 1976); G.S. - Vallabh Vidyanagar (Shaikh & Vaidya, 1972); Bi- (Prasad & Srivastava, 1963).



Conclusion:

Three taxa belonging to three families collected from different places. Out of three taxa one is reported for the first time from India. During taxonomic evaluation of algae from this

region it is observed that all these three taxa are on the verge of extinct. A serious step should be taken for the conservation of algal wealth of this region. Otherwise in short period of time these species will be endemic for this region as well as Maharashtra (Jaiswal, 2017).

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ANIOXIDANTS: CLASSIFICATION, SOURCES AND SIGNIFICANCES: A REVIEW

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Oxygen is vital for several living organisms and it is also a source of endogenic oxidants. Oxidation is essential for healthy metabolic function. The oxidation process is responsible for purifying the blood. Adequate oxygen allows the body to recuperate during sleep, strengthening the system. Oxidation in living organisms produces energy to fuel biological process additionally to free radicals. Various reactive species which are either radicals or non-radicals capable of producing radical species are formed during normal metabolic processes (Anju *et al.*, 2016).

A radical is an atom or group of atoms that has an unpaired electron and is therefore unstable and highly reactive. An atom's chemical behavior is determined by the quantity of electrons in its outermost shell. When, however, the outermost shell isn't full, the atom is unstable. It will try to stabilize itself by either gaining or losing an electron to either fill or empty its outermost shell or it'll share its electrons by bonding with another atom that's also looking to complete its outer shell. Free radicals formed when one of these weak bonds between electrons is broken and an uneven number of electrons remain. The unpaired electron is chemically reactive which will steal an electron from a neighboring molecule to stabilize itself (Paradini, 1995).

Free radical reactions usually takes place as a chain reaction consisting of initiation, propagation and termination steps (Howard, 2012).

Initiation $RH \rightarrow R^{\cdot}$

Propagation $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$

$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$

Termination $ROO^{\cdot} + ROO^{\cdot} \rightarrow$ molecular product

Scheme: Steps of free radical reaction chain. RH- substrate molecule, → Single electron indicating the radical

Though the free radicals are fundamental to many biochemical processes and represent a of aerobic life and metabolism, they're unstable, violently reactive, potentially destructive and short lived. A radical is an unpaired electron in an outer orbit and harmful because it's in search for a pairing electron. The radical takes one electron from a stable molecule, successively the resulting chain reaction can injure tissues and impair their functions (Sivanandham, 2011) that is implicated in over many diseases like arthritis, cancer, ageing, cardiovascular and neurodegenerative diseases.

There are two important sources of reactive species generated within the biological system. One is that the interior factors normal cellular metabolism like mitochondrial electron transport, endoplasmic reticulum oxidation, enzymatic activity, prostaglandin synthesis and stimulated neutrophils. External factors include environmental sources such as radiations like X-rays and gamma rays besides, light or UV within the presence of oxygen an endogenous compound or a drug that acts as photo sensitizer, oxidant of engine exhaust, carbon tetrachloride, paracetamol, pesticides, transition metals, alcohols, pollutants, cigarette smoke etc. (Bandyopadhyay *et al.,* 1999; Tandon *et al.,* 2005).

In a normal healthy human body, the generation of pro-oxidants such as ROS and RNS are effectively kept in check by various levels of antioxidant defense. However, when the body gets exposed to adverse physico-chemical, environmental or pathological agents the delicately maintained balance is shifted in favor of pro-oxidants resulting in oxidative stresses. Though, organisms are able to synthesize proteins, small molecules and specific enzymes protecting the organism from harmful actions of the oxidants. Oxidative stress occurs in situations when natural antioxidant defense of the human body isn't sufficient to fight excessive generation of reactive species and cause oxidative damage. The damaged bio-molecules and cells can successively induce a spread of diseases (Halliwell, 2001).

Production of free radicals within the human body :Free radicals and other reactive oxygen species (ROS) are derived either from normal essential metabolic processes within the human body or from external sources like exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Enzymatic reactions, which function source of free radicals, include those involved within the respiratory chain, in phagocytosis. Free radicals can also be formed in non-enzymatic reactions of oxygen with organic compounds also as those initiated by ionizing reactions (Lobo *et al.,* 2010).

Table 1: Internal and External generated free radicals

Some internally generated sources of free radicals	Sources of free radicals from external environment
Xanthine oxidase	Cigarette smoke
Arachidonate pathways	Ozone
Peroxisomes	Radiation
Ischemia/reperfusion injury	Environmental pollutants
Inflammation	Certain drugs, pesticides
Exercise	Industrial solvents

Important pathological and Physiological roles of reactive oxygen species

The role of oxidative stress in the development of different types of diseases are presented below Fig. 1 and 2.

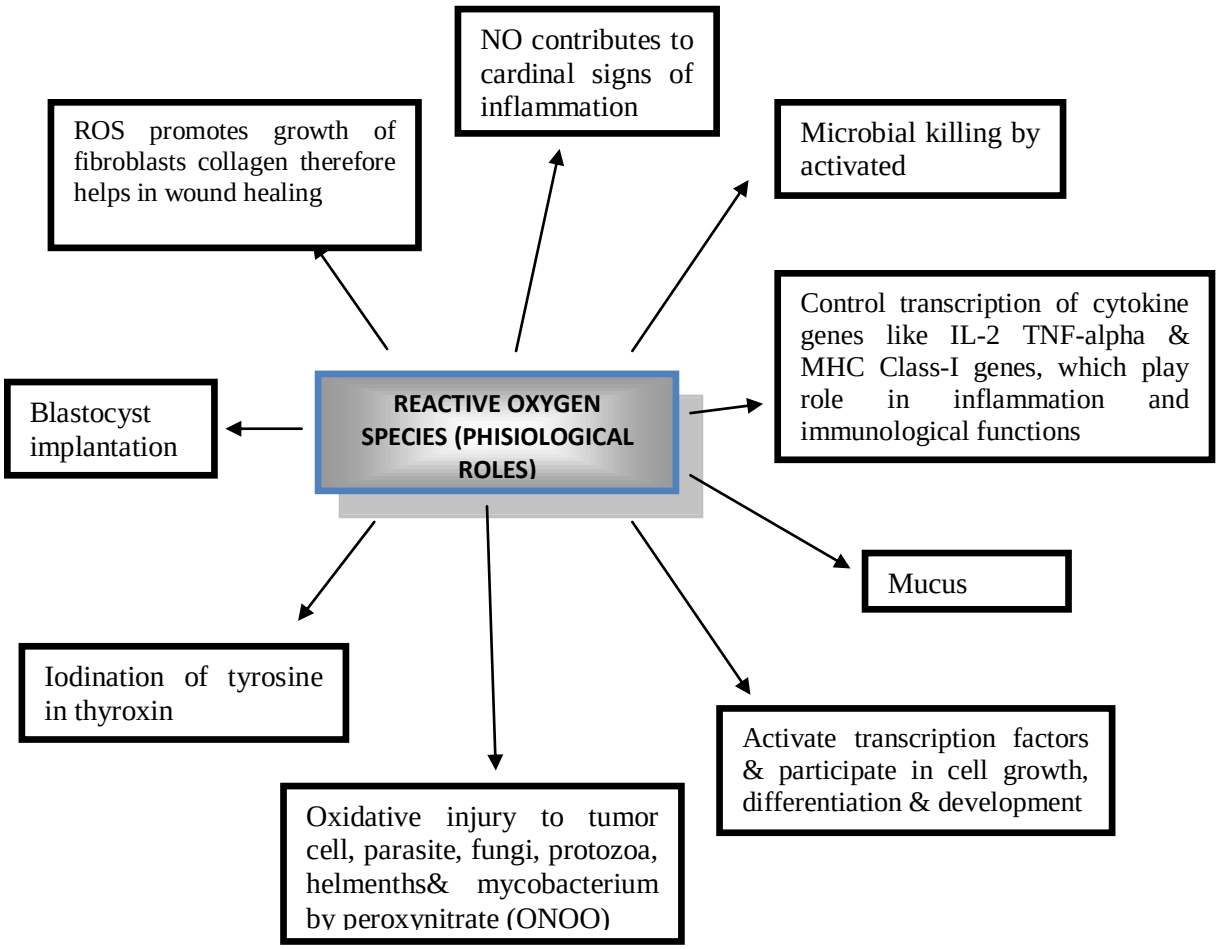

Figure 1: Pathological roles of free radicals (Tandon *et al.*, 2005)

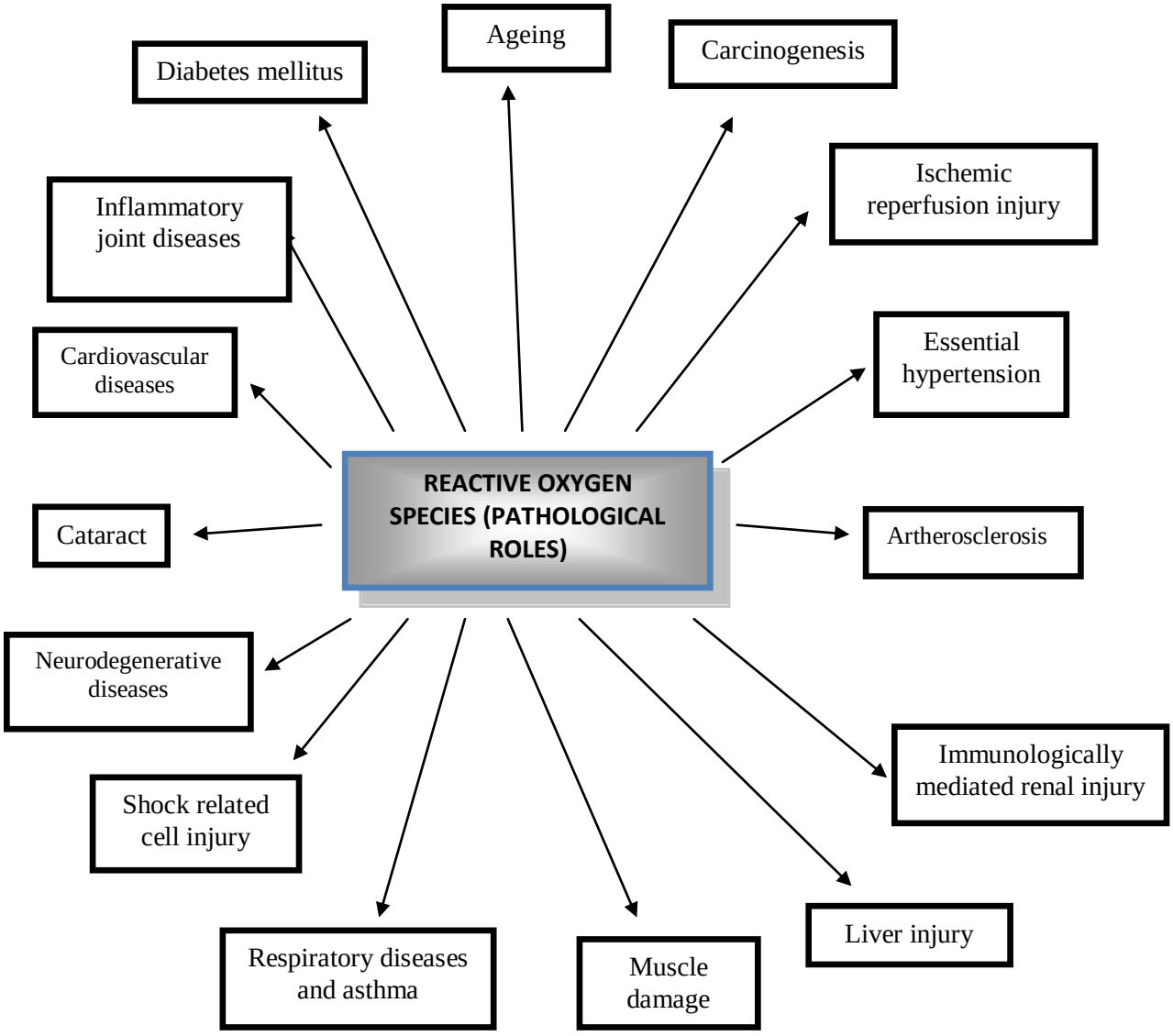

Figure 2: Physiological roles of free radicals (Tandon *et al.*, 2005)

Antioxidants:

An antioxidant could also be a molecule stable enough to donate an electron to a rampaging radical and neutralize it, thus reducing its capacity to cause damage. These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Anju *et al.*, 2016). Variety of such antioxidants, including glutathione, ubiquinol, and uric acid are produced during normal metabolism within the body. Other lighter antioxidants are found within the diet. Although there are several enzymes system within the body that scavenge free radicals, the principal micronutrient (vitamins) antioxidants are vitamin E, vitamin C (ascorbic acid) and β-carotene. The body cannot manufacture these micronutrients, hence they need to be supplied within the diet.

Antioxidants can decrease oxidative stress induced because of carcinogenesis by direct scavenging of ROS or by inhibiting cell proliferation. β-carotene could even be protective against cancer through its antioxidant function, because oxidative products can cause genetic damage. Thus, the photo protective properties of β-carotene may protect against ultraviolet induced carcinogenesis. β-carotene can also have anti-carcinogenic effect by altering the liver metabolism. Vitamin E, an important antioxidant, plays a task in immune competence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, the T-lymphocytes tumor necrosis factor production, inhibition of mutagen formation, repair of membranes in DNA and blocking micro cell line formation. (Mandal *et al.,* 2009).

Classification of Antioxidants:

Antioxidants are classified supported various criteria:

A. Classification supported their solubility

1. Soluble in lipids/fat (hydrophobic)
2. Soluble in water (hydrophilic)

Both of these are required by our body to guard the cells, since the interior of our cells and the fluids between them are composed of water, while the cell membranes themselves are mostly made of fat.

1. Lipid-soluble antioxidants are those which protect the cell membranes from lipid peroxidation. They're mostly located in cell membranes. Some examples of lipid-soluble antioxidants are vitamins A and E, carotenoids and lipoic acid.

2. Water-soluble antioxidants are found in aqueous fluids, like blood and thus the fluids within and around cells (cytosol or cytoplasmic matrix). Some examples of water-soluble antioxidants are vitamin C, polyphenols and glutathione.

B. Classification based on their nature:

1) Enzymatic antioxidants
2) Non-enzymatic antioxidants.

1) Enzymatic antioxidants benefits by breaking down and removing free radicals. They clean up hazardous oxidative products by converting them into peroxide, then into water. E.g.: SOD (SOD), Catalase (CAT), peroxidase (GSHpx) and glutathione reductase.

2) Non-enzymatic antioxidants

It is a category of the antioxidants which are not found within the body naturally but are required to be supplemented externally for the proper metabolism. Non-enzymatic antioxidants benefits

by interrupting radical chain reactions. Most antioxidants found in supplements and foods are non-enzymatic, which they supply support to enzymatic antioxidants (Kumar *et al.*, 2012).

E.g.: carotenoids, vitamin C, vitamin E, plant polyphenols and glutathione (GSH) minerals.

- ✓ Minerals: The body cells require them for the proper functioning of the enzymes. Their absences are known to affect the metabolism of many macromolecules.

Examples: selenium, copper, iron, zinc, manganese

- ✓ Vitamins: vitamins form the class of micro nutrients required for the proper functioning of the body's antioxidants enzymes system.

Examples: vitamin A, vitamin C, vitamin E and vitamin B.

- ✓ Carotenoid: They are fat soluble colored compounds found in fruits and vegetable

Examples: β-carotene, lycopene, lutein and zeaxanthin

- ✓ Polyphenols: Polyphenols is a class of the phytochemicals that possess marked antioxidant activities.

Examples: phenolic acids, flavonoids, gingerol, curcumin etc….

C. Classification supported their molecular size:

1) Small-molecular antioxidants work by mopping up or "scavenging" the reactive oxygen species and carrying them away by chemical neutralization.

E.g.: vitamins C and E, glutathione, lipoic acid, carotenoids

2) Large-protein antioxidants tend to work as enzymes and also as "sacrificial proteins," that absorb ROS and stop them from attacking essential proteins.

E.g.: albumin (Mercola, 2011).

D. Classification based on the source:

1. Endogenous antioxidants

   Bilirubin, Glutathione, NADPH, etc

2. Dietary antoxidants

   Vitamin C, Vitamin E, Flavonols, etc.

3. Metal binding proteins

   Albumin, Myoglobin, Transferrin, etc.

E.Classification based upon mechanism of action:

1. Catalytic systems to neutralize or divert ROS

   SOD, CAT, GPx

2. Binding of metal ions prevents production of ROS by Haber-Weiss reaction

   Ferritin, Caeruloplasmin, Catcchins

3. Self suicidal and chain breaking antioxidants scavenge, destroy ROS

Vitamin C, Vitamin E, Uric acid, etc.

4. Quenching ROS, chemical traps/ sinks to take in energy

Carotenoids, anthocyanidins

F. Classification based on their source and synthesis:

- Primary antioxidants
- Secondary antioxidants

Most antioxidants are present in plants and include vitamins A, C, E and carotenoids like beta-carotene, minerals, phenolic compounds and many other natural chemicals with antioxidant properties.Flavonoids are another powerful antioxidant and are contained in wine and tea.

Consuming a selection of natural foods like fruits, vegetables, cereals, legumes, nuts, seeds and whole grains - is that the simplest because they supply our body with antioxidants (Ndiamaka Okorie *et al.*, 2019)

Primary or natural antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following:

1. Antioxidants minerals - These are co-factor of antioxidant enzymes. Their absence will definitely affect metabolism of the various macromolecules like carbohydrates. Examples include selenium, copper, iron, zinc and manganese.

2. Antioxidants vitamins –It's needed for several body metabolic functions. They include vitamin C, vitamin E, vitamin B.

3. Phytochemicals - These are phenolic compounds that are secondary metabolites obtained from plants .These include flavonoids ,the phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark . Catechins are the foremost active antioxidants in green tea and sesamol. Carotenoids are fat soluble fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin A when the body lacks enough of the vitamin.

4. Low relative molecular mass compounds -These include lipid soluble antioxidants and water soluble antioxidants. Tocopherol, quinines, bilirubin and poly phenols come under lipid soluble antioxidants and vitamin C, acid and a couple of poly phenols come under water soluble antioxidants.

5. High relative molecular mass compounds -These includes proteins like albumin, transferin, ceruloplasmin. They restrict the assembly of metal catalysed free radicals.

Natural Antioxidant sources:

Beta-carotene: Orange foods like carrots, pumpkin, apricots, sweet potatoes and a couple of leafy greens.

Lutein - green, leafy vegetables like spinach.

Lycopene - tomatoes, watermelon, papaya.

Selenium - rice and wheat.

Vitamin A - carrots, sweet potato, milk, egg yolks.

Vitamin E -almonds, vegetable oils, mangoes, nuts, broccoli.

Vitamin C - available in many fruits and vegetables like parsley, broccoli, berries, oranges, cauliflower and kale. (Sardarodiyan *et al.*, 2016).

Secondary or synthetic antioxidants:

Among several samples of synthetic antioxidants permitted for food, there are 5 antioxidants that extends their use and spread around the world, namely Anisol Butyl Hydroxy(BHA), Butyl Hydroxy Toluene (BHT), Propyl gallate, tert-butyl Quinon (TBHQ) and tocopherols (Bunaciu *et al.*,2012).

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compound include:

a) Butylated hydroxyl anisole (BHA)

b) Butylatedhydroxyrotoluene (BHT)

c) Propyl gallate (PG) and metal chelating agent (EDTA)

d) Tertiary butyl hydroquinone (TBHQ)

e) Nordihydroguaretic acid (NDGA)

Benefits of Antioxidants:

Antioxidants are frequently discussed permanently health and preventing diseases. These powerful substances, which mostly come from the fresh fruits, and vegetables prohibit and in some cases even prevent the oxidation of other molecules within the body. The advantages of antioxidants are vital for good health, because if free radicals are not checked, they will cause a good array of sicknesses and chronic diseases.

There is a good sort of antioxidants found in nature, and since they're so varied, different antioxidants provide benefits to different parts of the body. For instance, beta-carotene (and other carotenoids) is extremely beneficial to eye health; lycopene is useful for helping maintain prostate health; flavonoids are especially beneficial for heart health; and pro-anthocyanins are beneficial for tract health (Sen *et al.*, 2010).

Antioxidants and skin health benefits:

When skin is exposed to high levels of ultraviolet, photo-oxidative damage is induced by the formation of various sorts of reactive species of oxygen, which damage cellular lipids, proteins, and DNA, and that they are considered to be the first contributors to erythema (sunburn), premature ageing of the skin, photodermatoses, and skin cancers.

Astaxanthin, beta-carotene combined with vitamin E has been shown to be one among the foremost powerful antioxidant combinations for shielding the skin

Antioxidants and system support:

Astaxanthin and Spirulina are shown to reinforce both the non-specific and specific system. Astaxanthin is that the single most powerful quencher of singlet oxygen, while Spirulina features a sort of antioxidants and other substances that are beneficial in boosting immunity.

Additional ways antioxidants help benefit one's health:

Increasing antioxidant intake is important for optimum health, especially in today's polluted world. Because the body just can't manage with the interior antioxidant production, an honest amount of those vitamins, minerals, phytochemicals, and enzymes must come from one's daily diet. Increased antioxidant intake can provide added protection for the body against:

- Heart problems
- Eye problems
- Memory problems
- Mood disorders
- Immune system problems (Hamid *et al.*, 2010)

Advantages and drawbacks of natural antioxidants and artificial antioxidants:

Synthetic antioxidants are effective, pure, comparatively cheap, easily obtainable, and harmless, if added at concentrations permitted by legislation. Their only disadvantage is that they're suspect of being chemicals. Therefore, most consumers prefer natural antioxidants as they believed to be free of chemicals. The advantage of the many natural antioxidants, which are components of human diet for several thousand years, is that men became adapted to them. Those, which are common components of food, aren't subject to any legislative restrictions. Another advantage is that there are many substances of antioxidant activity in human diet. Their choice is thus not restricted to a couple of compounds as within the case of synthetic antioxidants. Natural antioxidants are, however, complex mixtures of the many compounds of various activities, which can influence each other. Their composition differs consistent with the origin and year of crop. the simplest way for the appliance of natural antioxidants is their direct

addition as food ingredients with none fractionation, like vegetables, oilseed, herbs or spices, or only simply treated materials, like oilseed extracted meals or rosemary resins. Only such applications could compete with synthetic antioxidants. Other native antioxidants are proposed as food additives, which are components of herbs or drugs, but which aren't used as foods (Stoia and Oancea 2011; Bahare *et al.,* 2018).

Table 2: Advantages and disadvantages of natural antioxidants

Advantages	Disadvantages
Natural antioxidants are essential for maintain natural body health.	Natural antioxidants can add color and an after taste to food and can be less effective at slowing down the rate of rancidity than synthetic antioxidants.
Naturally occurring vitamins C, E and carotenoids reduce the risk of cancer and heart diseases by inhibiting the formulation of free radicals.	Natural antioxidants are more expensive and less effective than synthetic antioxidants and can also add unwanted color.
Beta-carotene can be used as an additive in margarine to provide it with a yellow color and act as a precursor for vitamin A synthesis	Policies regarding the safe use and labeling of food additives may be difficult to implement in developing countries and across International borders.

Table 3: Advantages and disadvantages of synthetic antioxidants

Advantages	Disadvantages
Synthetic antioxidants functions as food preservatives and prevent undesirable rotting and descent.	It cannot be recycled and reused by the organism, once they have donated their electron.
They were primarily added to edible fats and fat containing foods for their ability to prevent food from deteriorating.	After showing their antioxidant property, synthetic antioxidant tends to turn into harmful metabolic by-products.
BHA and BHT is a synthetic variant of vitamin E. It is incorporated into food to prevent free radical damage that can cause food to rot, elicit a change in taste and produce putrid foods.	These by-products, instead of decreasing oxidative stress on organism increase it.
Lower dose of synthetic antioxidant is needed in palm disease to achieve the minimum rancimat indication period in 6 hours.	There are no by-products in case of antioxidants.

(Augustyniak *et al.,* 2010; Pokerny *et al.,* 2011)

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COMPARATIVE STUDY ON DYEING OF COTTON AND SILK BY USING NATURAL DYE OBTAINED FROM THE FLOWERS OF *PYROSTEGIA VENUSTA* (KER GAWL.) MIERS

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Introduction:

Indian prehistoric times, use of natural dyes in coloring of food substrate, leather as well as natural protein fibers like wool, silk and cotton were known as major areas of application. However, growing concern due to environmental pollution caused by the synthetic dyes, a number of commercial dyers and small textile export houses have started looking at the possibilities of using natural dyes (Agarwal, 2009).

Natural dyes can be obtained from plant, minerals and animal sources Most of these sources produce very colorful effects that are so wonderful. Dye matter extracted from the different parts of the plants like roots, stems, leaves or berries and flowers lift up expectations for their use to develop various color shades (Vankar, 2007). The roots, nuts, berries, leaves, young shoots, flowers, outer, inner bark, heart wood and even seeds of plants can be the sources of natural dyes. Until the early 1900s, existence of over 1000 sources of plants based dyes have been discovered with some common sources (Korankye, 2010).

Pyrostegia venusta is scandent shrub with angled stem and opposite leaves. Flower of crimson-bright orange color born in corymbose cymes or racemes (Singh *et al.,* 2001). In present study, efficiency of dye extracted from flowers of *Pyrostegia venusta* was evaluated for development of variety of colors.

Materials and Methods:

The study is carried out in following steps:

a. Flowers of *Pyrostegia venusta* (Ker Gawl.) Miers. were collected from campus of the Government Vidarbha Institute of Science and Humanities, Amravati and identified by using

standard flora (Dhore, 2002). Fresh flower petals of *Pyrostegia venusta* (Orange color) were used for dye extraction and dyeing process (Figure 1).

Figure 1 : Plant Material

Pyrostegia venusta

b. 2% Ferrous ammonium sulphate (Mohr's salt) and 5% Copper sulphate solutions were used as a chemical mordants.

c. Extraction of dye was carried out using three solvents – water, ethanol and alcohol. About 10 gm of fresh flowers petals were crushed in mortar and pestle with 50 ml of solvent and the solution was filtered through muslin cloth to obtain dye.

d. The process of dyeing and mordanting done simultaneously. Dyeing was done by two methods: i.e. without mordanting and with mordanting. In first case Cotton and silk fabrics were directly dipped in a dye bath solution containing dye while in later case cotton and silk fabrics were first treated with either 2% Ferrous ammonium sulpahte or 5% Copper sulphate and then further dipped in a dye bath solution containing dye. After dyeing, the dyed material was dried at room temperature.

e. Dyed fabrics were washed with tap water and dried in air at room temperature.

f. Pantone shade color charts system was used for matching the natural colorants shade.

Results and Discussions:

Present study aimed to evaluate the efficiency of dye extracted from *Pyrostegia venusta* to generate variety of colors on cotton and silk fiber. Four colors were obtained in total by dyeing of cotton and silk without mordant (Table I). Ethanolic extract of *Pyrostegia venusta* found to exhibit darkest color on cotton (Colonial sage, Panton paint shade no. 615) while aqueous extract exhibit most lighter shade on silk (Ivory, Panton paint shade no.480) (Figure 2). Whereas alcoholic extract does not generate any color.

Table 1: Effect of aqueous and ethanolic extracts of *Pyrostegia venusta* on cotton and silk without mordant

Sr. No.	Materials	Extract	Color shade (Panton paints color chart)	Color
1	Cotton	Aqueous	468	Parchment
2	Silk	Aqueous	480	Ivory
3	Cotton	Ethanolic	615	Colonial sage
4	Silk	Ethanolic	453	Topaz bulsh

Different color shades obtained from aqueous, alcoholic and ethanolic extracts of *Pyrostegia venusta* with mordants ferrous ammonium sulphate (FAS) and Copper sulphate ($CuSO_4.5H_2O$) were tabulated in Table II (Figure 2).

Dark husk gold (Panton paint Shade No.5845) was the darkest shade obtained on cotton dyed with ethanol extract using mordant ferrous ammonium sulphate. Two most lighter shades obtained on silk dyed with ethanol extract and aqueous extract by using common mordant Copper sulphate were Light sand (Panton paint shade no.4545) and Coconut (Panton paint shade no. 481). Alcoholic extract does not yield any color shade on cotton or silk fiber with any mordant.

Table 2: Effects of aqueous and ethanolic extracts of *Pyrostegia venusta* on cotton and silk with mordant

Sr. No.	Material	Mordant	Extract	Color shade (Panton paints color chart)	Color
1	Cotton	FAS	Aqueous	454	Tan
2	Silk	FAS	Aqueous	482	Linen
3	Cotton	$CuSO_4.5H_2O$	Aqueous	207	Bone
4	Silk	$CuSO_4.5H_2O$	Aqueous	481	Coconut
5	Cotton	FAS	Ethanolic	5845	Dark husk gold
6	Silk	FAS	Ethanolic	5865	Bean sprout
7	Cotton	$CuSO_4.5H_2O$	Ethanolic	5855	Light husk gold
8	Silk	$CuSO_4.5H_2O$	Ethanolic	4545	Light sand

Indian subcontinent is rich in biodiversity and hence very little resources were evaluated for their possible dye yielding properties so far. Chavan and Ghosh (2015) evaluated dyeing capacity of floral parts of African marigold (*Tagetes erecta*) and found that color obtained on silk was excellent. Ghurde *et al.* (2013) obtained 10 wonderful shades when dye extracted from *Ixora coccinea* flowers was used as source of dye with Alum, Copper sulphate, ferrous sulphate and Stannous chloride as a mordant for cotton coloration. Tiwari and Bharat (2008) reviewed dye yielding potential of more than thirty plants Achanakmar-Amarkantak biosphere reserve.

Many workers found that dyes even possess antimicrobial properties. Das *et al.* (2011) reported antimicrobial activity of dyes obtained from *Acacia catechu*, *Pterocarpus marsupium*, *Toddalia asiatica* and *Ventilago denticulate* against four bacterial strains *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Vibrio cholerae*. Beiki *et al.*, (2017) successfully utilized main waste product obtained from walnut i.e. walnut husk as a source of dye which is capable of inhibiting growth of bacteria and fungus, proving its antimicrobial ability.

Many workers carry out successful dyeing practices by evaluating dye yielding potential of different plants like – *Hibiscus mutabilis* by Shanker and Vanker (2006), *Tecoma stans* by Chandra *et al.* (2012), *Holarrhena antidysentrica* by Deshmukh (2012), *Woodfordia fruticose* by Grover and Patni (2011), Red calico (Alternanthera bittzickiana) by Khan *et al.* (2014), *Butea monosperma* by Mall *et al.* (2017), mangrove bark (*Rhizophora apiculata*) by Punrattanasin *et al.* (2013) etc.

Figure 2 : Notable color variation obtained by using dye extracted from *Pyrostegia venusta*

P. S. No. – Panton Paint Shade Number

As compared to aqueous extract, ethanolic extract exhibit darker color shade. Alcoholic extract fails to generate any color shade in both dyeing procedures i.e. with and without mordant. Darker coloration obtained on cotton fiber as compare to silk in both cases. Four colors obtained when cotton and silk fabrics were directly immersed in dye solution out of which Colonial sage was the darkest one while Ivory was the most lighter shade obtained. About eight different color shades were obtained in dyeing with mordant treatment. It was found that Ferrrous ammonium sulphate exhibit darker coloration on cotton than the Copper sulphate. Dark husk gold was the darkest shade obtained by dyeing with mordant Ferrous ammonium sulphate. Silk exhibit two lighter coloration i.e. Light sand and Coconut by dyeing with mordant Copper sulphate.

Conclusion:

Plants has become more reliable sources for extraction of dye due to increased attention gain by polluting effects and health-hazards of synthetic dyes. Natural dyes are non-toxic and low pollution resources with minimum side effects which made them usable even in day to day food products.

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STUDY OF MYCOFLORA OF SUNFLOWER SEEDS (*HELIANTHUS ANNUS*)

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Abstract:

Sunflower seeds are used in present investigation of fungal association. The study aimed to isolate and identify seed Borne fungi associated with store cultivars of sunflower.seed samples were brought from oil Seed Research Station. The seed samples were and store in cloth bag. The standard blotter paper and Agar plate methods were used to identify the fungal diversity of seed Borne fungi. The fungal genera such as Aspergillus, Curvularia, Fusarium, Alternaria was found to be prominently associated with seeds. The mean percent incidence of infection was more on Agar plate method. The intensity of fungi where found to be increased during the storage period. The presence of mycoflora deteriorates the seed quality such as seed viability, seed germination, and growth of the seedlings, which leads to apparent losses in production and productivity. These also create the infection causes health hazards therefore consumption avoided.

Keywords: *Helianthus annuus*, Aspergillus, Fusarium, Alternaria

Introduction:

Seed health plays anvital role in successful cultivation and yield exploration of a crop.Fungi are the main component of microflora associated with seeds and are the main cause ofdeterioration and loss observed during storage (Tanaka *et al.*, 2001) Seed health plays an important role in successful cultivation and yield exploration of a crop. Sunflower (*Helianthus annuus* L.) is one of the most popular oilseed crops grown in India.

Sunflower seeds contain 20% of protein and35-40- oil,itconstitute rich source ofunsaturated fats, It also comprises fiber as an important nutrients, selenium, copper, zinc, vitamin E and B complex as (Afzal *et al.*, 2010).

Materials and Methods:

Collection of seed samples Sunflower seeds were collected from farmers and local markets of Aurangabad and the mycoflora associated with seeds were detected by following Agar plate (Musket, 1948; Agarwal, 1976; ISTA, 1966) method. Approximately 1 kg of Seeds were collected from each, brought to the laboratory and dried in paper bags. Pods were then hand shelled and divided into sub samples. After these samples were used for further study for analyses of seed mycoflora.

Detection of seed mycoflora:

Standard Blotter Paper Method:

In this method Sterilized blotting paper discs of 90mm diameter were placed in sterile Petri plates and moistened with sterile distilled water. The excess water was drained off from the plates. Seeds were transferred to the plates containing moist blotting paper discs. Ten seeds per plate were placed at equidistance and the plates were kept for incubator at 24 ± 2° C for seven days for incubation

Agar plate method (ISTA, 1966):

In this method AgarAgar medium was prepared and sterilized in an autoclave. After that about 20 ml of the medium was distributed to each of the sterile Petri plate under aseptic conditions. 10-12 Sunflower seeds were transferred to the plates containing PDA medium. Ten seeds per plate were placed at equidistance in a circular fashion aseptically. One hundred seeds from each sample were placed in the plates in four replications. The Petri plates were incubated at 25 ± 2°C in the incubator for seven days and observed every day for the growth of fungi. Per cent infection was assessed as suggested by Jha (1995) and the per cent incidence of each species was calculated as follows.

Isolation and identification of fungal species:

Fungi identification was carried out based on macro morphological characteristics like surface coloration of colonies and colony texture and micro morphological characteristics like conidial head, conidia shape and shape of vesicle. Isolates were observed and identified based on morphological feature by using illustrated kingdomoffungi .Fungal colonies that grew rapidly and produced Aspergillus species. Many dense mycelial/hyphal growth are categorized as Fusarium species. There are Microconidia also observed and after sclerotial formation the entire colony turn into black color were classified as Macrophominaspecies..colonies of Penicillium. Black colored mass of Alternaria species.

Results and Discussion:

Table 1: Percent Incidence of Mycoflora on Sunflower (*Hellianthus annus*)

Sr.No.	Fungi Isolated	Standard Blotter Method	Agar Plate Method
1.	*Aspergillus flavus*	12.10	13.0
2.	*Aspergillus niger*	12.65	15.5
3.	*Alternaria alternata*	13.10	14.3
4.	*Curvularia lunata*	12.76	13.0
5	*Fusarium moniliforme*	7.45	8.2
6.	*Fusarium oxysporum*	6.90	7.2
7.	*Fusarium semitectum*	7.0	7.2
8.	*Penicillumdigitatum*	5.75	6.3
9.	*Trichoderma spp.*	4.56	5.0
10.	*Alternaria,alternata*	5.0	5.3
11.	*Macrophomina,*	4.0	4.8
12.	*Stemphylium sp.*	2.0	2.1

The incidence of seed borne fungi associated with Sunflower seeds.Thefungal genera such as Aspergillus, Curvularia, Fusarium, Alternaria was found to be prominently associated with seeds. The mean percent incidence of infection was more on Agar plate method as compared to Standard Blotter paper method. The intensity number of fungi was found to be increased during the storage period.The seeds plays vital role in cropproduction .Generally Seed mycoflora greatly affects the seed germination for healthy crop. In present study, the results shows that a total of six fungal species viz., *A. flavus, A. niger, Fusarium, Alternaria, Macrophomina, Penicillium* were found to be associated with the seeds of sunflower seeds. Out of these fungal species *A. niger*was predominant while, the least was *Penicillium*

Conclusion:

Sunflower (Helianthus annuus L.), considered a commercial oil crop all over the world, the crop is widely cultivated in Egypt and in many countries all over the world. Sunflower seeds used for production of edible oils as well as for seed consumption. If the crop is infected by seed mycoflora and these pathogens may affect the crop resulting in a deterioting the seed quantity as well as quality. There is direct impact of storage fungi on the economical part which is need of

hour. The different effects of storage fungi on sunflower oil in order to increase oil yield and crop quality, makes fit for human consumption.

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A REVIEW ON BIOACTIVE NITROGENOUS COMPOUNDS

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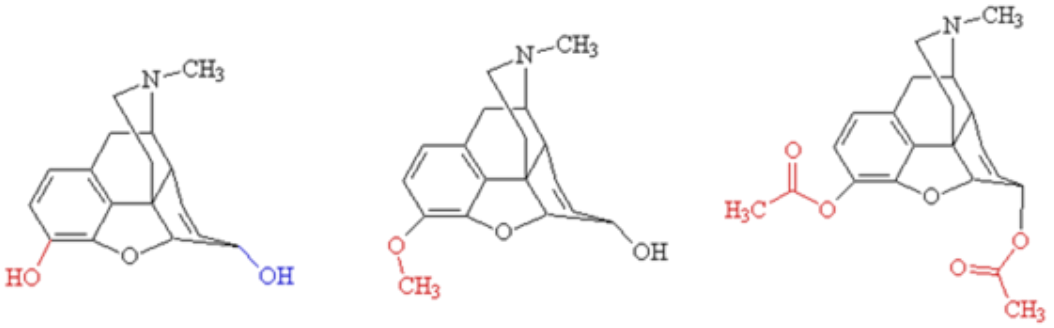
Abstract:

The article put the views about the biologically active N-heterocyclic compounds. The emphasis have been given on occurrence, synthesis, application, pharmacological properties of nitrogen containing heterocycles which leads to generate the library of the nitrogenous compounds

Introduction:

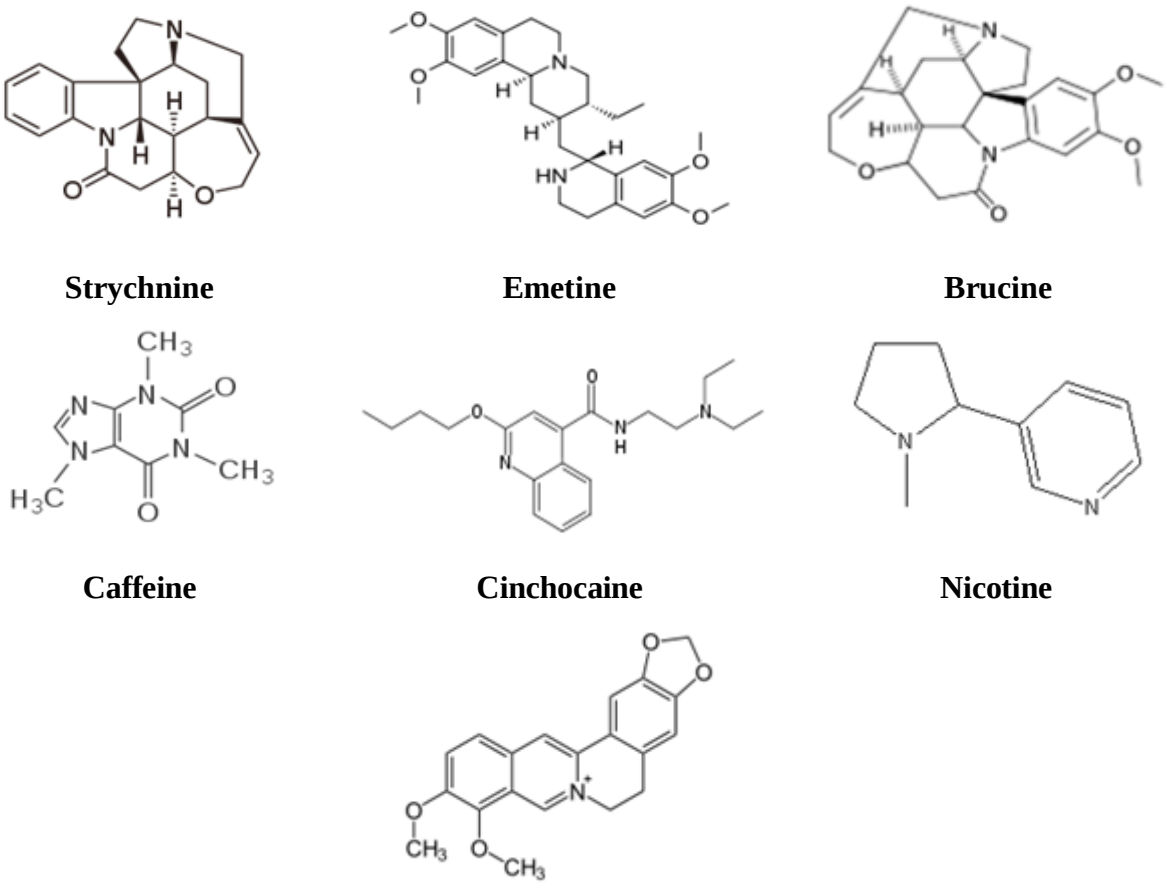
Synthetic organic chemistry deals with the creation of carbon-containing molecules, hydrocarbon and their derivatives. These compounds may contain any number of other elements, including hydrogen, nitrogen, oxygen, the halogens as well as phosphorus, silicon and sulfer (Roberts and Caserio, 1964; Morrison et al., 1992) which may find important constituents of manyproducts thatuse in diverse areas such as medicine, food, agriculture, dyes, paints, polymer and petroleumindustry etc. They form the basis of almost all earthly life processes.

An enormous development in the field of organic synthesis has occurred since the early 19th century when the first synthetic organic molecule, urea, was reported (Morrison et al., 1992) in 1828 by Fredrich Wohlar. The reaction is known as Wohlarsynthesis (Wohler, 1828). Presently, immense numbers of reactions which can be used for the synthesis of compounds with different levels of structural complexity are reported. However, in order to meet the demands for cost-efficient and environmentally benign processes for the preparation of effective products, further improvements of existing methods as well as development of new synthetic methodology are still needed. The ideal synthetic pathway includes atom-efficient, high yielding, simple, nontoxic, and inexpensive reaction steps.



Morphine **Codeine** **Heroin**

Figure 1.1: Structures of Morphine, Codeine and Heroin



Strychnine **Emetine** **Brucine**

Caffeine **Cinchocaine** **Nicotine**

Berberine

Figure 1.2: Some of the Active Alkaloides

In the field of organic chemistry, Nitrogenous compounds play a major role. Nitrogeneous compounds make up the framework of many natural products especially alkaloids. Alkaloids are nitrogen containing organic substances of natural origin. Humans have been using alkaloids as drugs, medicines, teas and poisons for 4000 years. The plants which contained arrow

poisons were used in hunting or in dealing with enemies. Such poisons are still in use in Africa and South America. Alkaloids were isolated from arrow poison have been used in the treatment of glaucoma and myasthenia gravis, as a muscle relaxant, in anesthesia and as an antihypertensive (Roberts and Wink, 1998). The first crude drug (Opium – also called as afim) was obtained from the opium poppy, which had been used for its analgesic and narcotic properties for around 6000 years ago. Raw opium contains some 20 alkaloid substances, one of which ismorphine, in a typical yield of 10%. In 1805, Friedrich Serturner isolated morphine from opium.

Between the years 1817 and 1820 many biologically active compounds were isolated from plants, including styrchnine, emetine, brucine, caffeine, quinine, cinchocine and colchicine. This pioneering work formed the cornerstone of all that has occurred in alkaloid chemistry to the present (Kutchan, 1996). The number of alkaloids that had been isolated and identified was 200 in 1939 and by 1989, 10,000 alkaloids wereknown. Currently, there are over 27000 known alkaloids (Wink, 1998; Asano, 2008). Since this type of compounds often shows interesting biological activities and also possess fascinating structures (Fig. 1.2), they have for a long time attracted attention as synthetic targets from organic chemists.

Heterocycles:

Heterocyclic compounds could be any cyclic organic compound with at least one atom being an element other than carbon. This is a simple explanation because some heterocyclic compounds have properties similar to other aliphatic compounds and can therefore not be classified as heterocycles (Schmid, 1996; Usifoh, 2010). The importance of heterocycle is apparent in the wealth and variety of such compounds that occur naturally or are prepared on commercial scale by drugs and dyes companies. Many heterocycles found to have important physiological functions in plant and animals. Heterocycles may contain one or more heteroatoms in a ring. The heterocyclic rings constitute systems of central importance in theoretical, synthetic organic, bioorganic, and medicinal chemistry. Among the entire heterocycles, Nitrogen containing heterocycles plays important in number of natural and biologically active substances and acts versatile synthetic intermediates. Therefore, the development of efficient methods for the synthesis and elaboration of nitrogen containing heterocycles are inviting ongoing challenges.

Most of the N-heterocyclic compounds occur naturally and they functions as essential importance to living systems as it involves in numerous biochemical reactions and physiological

processes, such as provision of energy, transmission of nerve impulses, sight, metabolism and transfer of hereditary information etc. The reason that nature utilizes so many nitrogenous heterocyclic compounds lies in their remarkable properties. Nitrogen-containing heterocycles can behave like acids (the -NH group of pyrroles), bases (the *N*-group of pyridine), or be amphoteric (imidazoles) (Zaman *et al.*, 2005).

Therefore, the study of *N*-heterocyclic chemistry focuses especially on unsaturated derivatives and the prevalence of work and applications involves unstrained 5- and 6-membered rings. Another large class of heterocycles are fused to benzene rings, which for pyridine, pyrrole, imidazoleare quinoline, benzothiophene, indole, benzimidazole respectively.

- **Classification:**

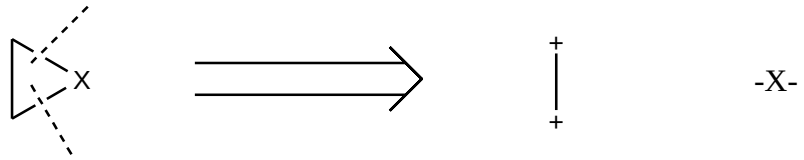
On the basis ring size, N-heterocycles can be classified in following group:

3-Membered rings:

Common 3-membered heterocycles with *one* heteroatom are:

Heteroatom	Saturated	Unsaturated
With *one* heteroatom	Aziridine	Azirine
With *two* heteroatoms	___	Diazirine

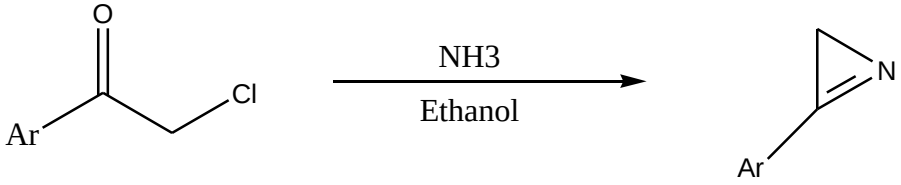
Heterocycles with three atoms in the ring are more reactive because of ring strain. Those containing one heteroatomare, in general, stable. Those with two heteroatoms are more likely to occur as reactive intermediates.



Synthesis of Azirine:

e.g:

Azirines are three membered heterocyclic unsaturated (i.e. they contain a double bond) compounds containing a nitrogen atom and related to the saturated analogue aziridine. They are highly reactive yet are found in a number of natural products such as the antibiotic azirinomycin.

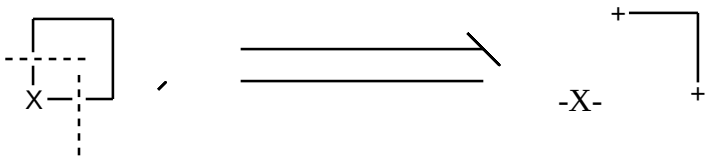


4-Membered ring:

Compounds with one heteroatom:

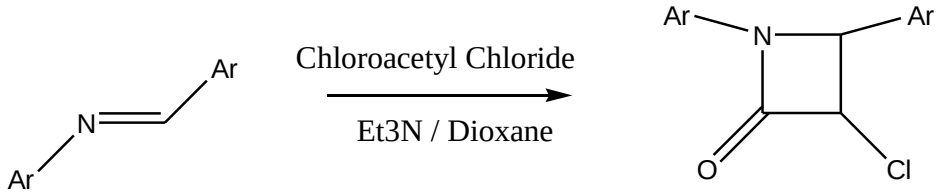
Heteroatom	Saturated	Unsaturated
With *one* heteroatom	Azetidine	Azete
With *two* heteroatoms	Diazetidine	___

Synthesis of four membered heterocycles:



Synthesis of Azetidinone:

Azetidinones which are part of antibiotics structure are known to exhibit interesting biological activities. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activities.



5-Membered rings:

With heterocycles containing five atoms, the unsaturated compounds are frequently more stable because ofaromaticity.

Five-membered rings with *one* heteroatom:

Heteroatom	Saturated	Unsaturated
With *one* heteroatom :		
Nitrogen	Pyrrolidine	Pyrrole

The 5-membered ring compounds containing *two* heteroatoms, at least one of which is nitrogen, are collectively called the azoles. Thiazoles and isothiazoles contain a sulfur and a nitrogen atom in the ring. Dithiolanes have two sulfur atoms.

Heteroatom	Saturated	Unsaturated
Nitrogen/ Nitrogen	1) Imidazolidine 2) Pyrazolidine	1) Imidazole 2) Pyrazole
Nitrogen/Oxygen	1) Oxazolidine 2) Isoxazolidine	1) Oxazole 2) Isoxazole
Nitrogen/Sulfur	1) Thiazolidine 2) Isothiazolidine	1) Thiazole 2) Isothiazole

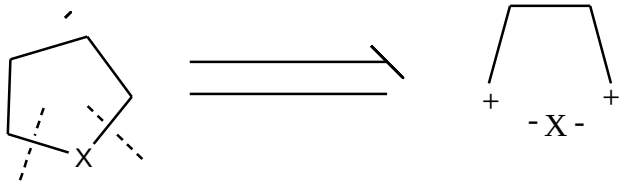
A large group of 5-membered ring compounds with threeheteroatomsalso exists. One example is dithiazoles thatcontain two sulfur and a nitrogen atom.

Heteroatom	Saturated	Unsaturated
3 ×Nitrogen	—	Triazole
2 ×Nitrogen/ 1 ×Oxygen	—	Oxadiazole
2 ×Nitrogen/ 1 × Sulfur	—	Thiadiazole
1 ×Nitrogen/ 2 × Sulfur	—	Dithiazole

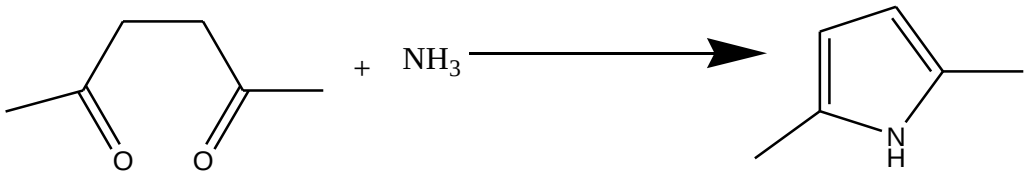
Five-member ring compounds with four heteroatoms:

Heteroatom	Saturated	Unsaturated
4 ×Nitrogen	—	Tetrazole

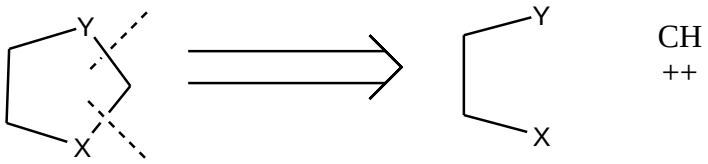
Synthesis of five membered heterocycles:

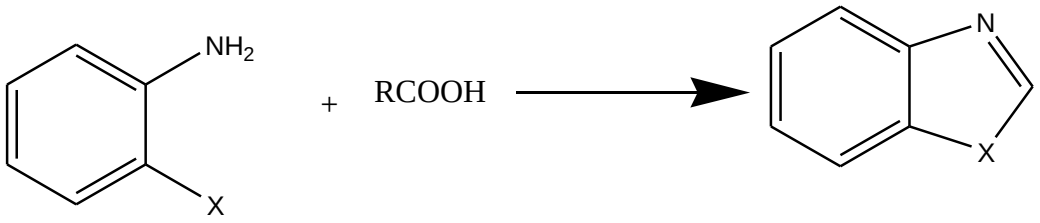


Synthesis of Pyrrol:



Synthesis of five membered heterocles containing two heteroatoms:





Benzimidazole is a bicyclic compound which consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound in nature is-N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B_{12} (Barker *et al.*, 1960).

With 5-heteroatoms, the compound may be considered inorganic rather than heterocyclic. Pentazole is the allnitrogen heteroatom unsaturated compound.

6-Membered ring:

Six-membered rings with a *single* heteroatom:

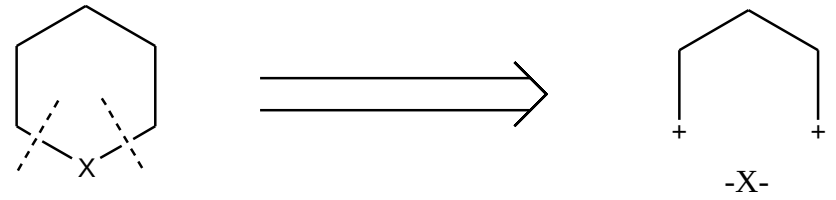
Heteroatom	**Saturated**	**Unsaturated**
With one heteroatom :		
Nitrogen	Piperidine	Pyridine
With two heteroatom (at least one is nitrogen) :		
Nitrogen/ Nitrogen	Piperazine	Diazine
Nitrogen/Oxygen	Morpholine	Oxazine
Nitrogen/Sulfur	Thiomorpholine	Thiazine

The 6-membered ring compounds containingthree and four Nitrogenheteroatoms

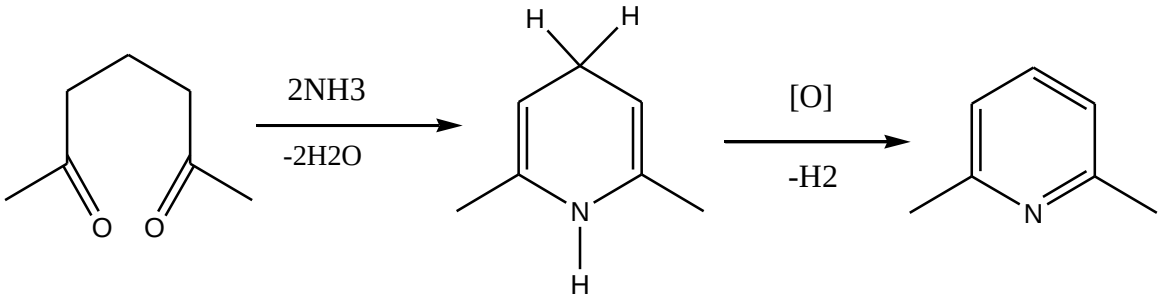
Heteroatom	**Saturated**	**Unsaturated**
With three heteroatom :		
Nitrogen	—	Traizine
With Four heteroatom :		
Nitrogen	—	Tetrazine

The hypothetical compound with six nitrogen heteroatoms would be hexazine.

General Synthesis of Six membered ring:



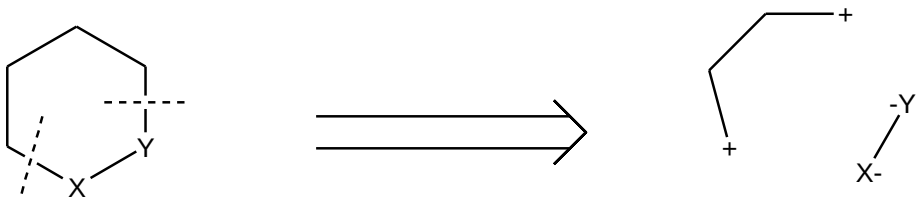
Synthesis of pyridine by 1,5-Dicarbonyl compounds can be prepared via Michael addition of enones with ammonia would make dihydropyridine, followed by separate oxidation.



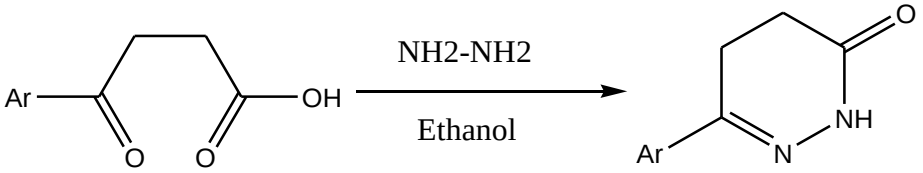
Pyridine is a basic heterocyclic organic compound. Pyridine was discovered in 1849 by the Scottish chemist Thomas Anderson as one of the constituents of bone oil. It is a colorless, highly flammable, weakly alkaline, water soluble liquid with a distinctive, unpleasant fish-like odor (Encyclopedia Britannica).

Synthesis of Six membered ring containing two heteroatom:

Synthesis of 1,2disubstituted six membered heterocycles



e.g.- Synthesis of pyridazine



Pyridazine is a heteroaromatic organic compound. The pyridazine structure is found within a number of herbicides such as Credazine, Pyridafol and Pyridate. It is also found within

the structure of several pharmaceutical drugs such as cefozopran, cadralazine, minaprine, pipofezine, hydralazine and cilazapril.

7-Membered rings:

With 7-membered rings, the heteroatom must be able to provide an empty pi orbital (e.g., boron) for "normal"aromatic stabilization to be available; otherwise, homoaromaticity may be possible.

Heterocyclic rings systems that are formally derived by fusion with other rings, either carbocyclic or heterocyclic, have a variety of common and systematic names. For example, with the benzo-fused unsaturated nitrogen heterocycles, pyrrole provides indole or isoindole depending on the orientation. The pyridine analog is quinoline or isoquinoline. For azepine, benzazepine is the preferred name. Likewise, the compounds with two benzene rings fused to the central heterocycle are carbazole, acridine, and dibenzoazepine.

Heteroatom	**Saturated**	**Unsaturated**
With Oneheteroatom :		
Nitrogen	Azepane	Azepine
With Twoheteroatom :		
2 ×Nitrogen	Homopiperazine	Diazepine
1 ×Nitrogen/ 1 × Sulfur	—	Thiazepine

History of Heterocyclic Chemistry:

The history of heterocyclic chemistry began in the 1800s, in step with the development of organic chemistry. Somenoteworthy developments (Campaigne, 1986):

- 1818: Brugnatelli isolates alloxan from uric acid (Lenzen, 2008)**.**
- 1834: Runge obtains pyrrole ("fiery oil") by dry distillation of bones
- 1906: Friedlander synthesizes indigo dye, allowing synthetic chemistry to displace a large agricultural industry (Steingruber, 2004).
- 1936: Treibs isolates chlorophyl derivatives from crude oil, explaining the biological origin of petroleum (Kvenvolden, 2006).
- 1951: Chargaff's rules are described, highlighting the role of heterocyclic compounds (purines and pyrimidines) in the genetic code (Szybalski *et al.,* 1996).

Importance of Heterocycles:

Heterocycles are an important and a large proportion of natural products contain them many pharmaceuticals and agrochemicals contain at least one heterocyclic unit (Moody, 1998). Heterocyclic systems are important building-blocks for new materials possessing interesting electronic, mechanical or biological properties (Eguchi, 2006).

Heterocycles display intrinsic reactivity which enables rich, versatile and productive transformations. Taking into account their ubiquitous presence of heterocycles in natural products and drugs, the development of new, fast and efficient preparative protocols for heterocycles remain an urgent task in medicinal chemistry and are actually life-saving agents. The progress made in synthesis in the last twenty years can be considered phenomenal. Many non-natural products have been imagined and prepared in the laboratory. The most important representation of each class of heterocycles have been synthesized, some of them several times using different reactions or strategies. These compounds are extensively distributed in nature and are essential to life since they play a vital role in the metabolism of all living cells.

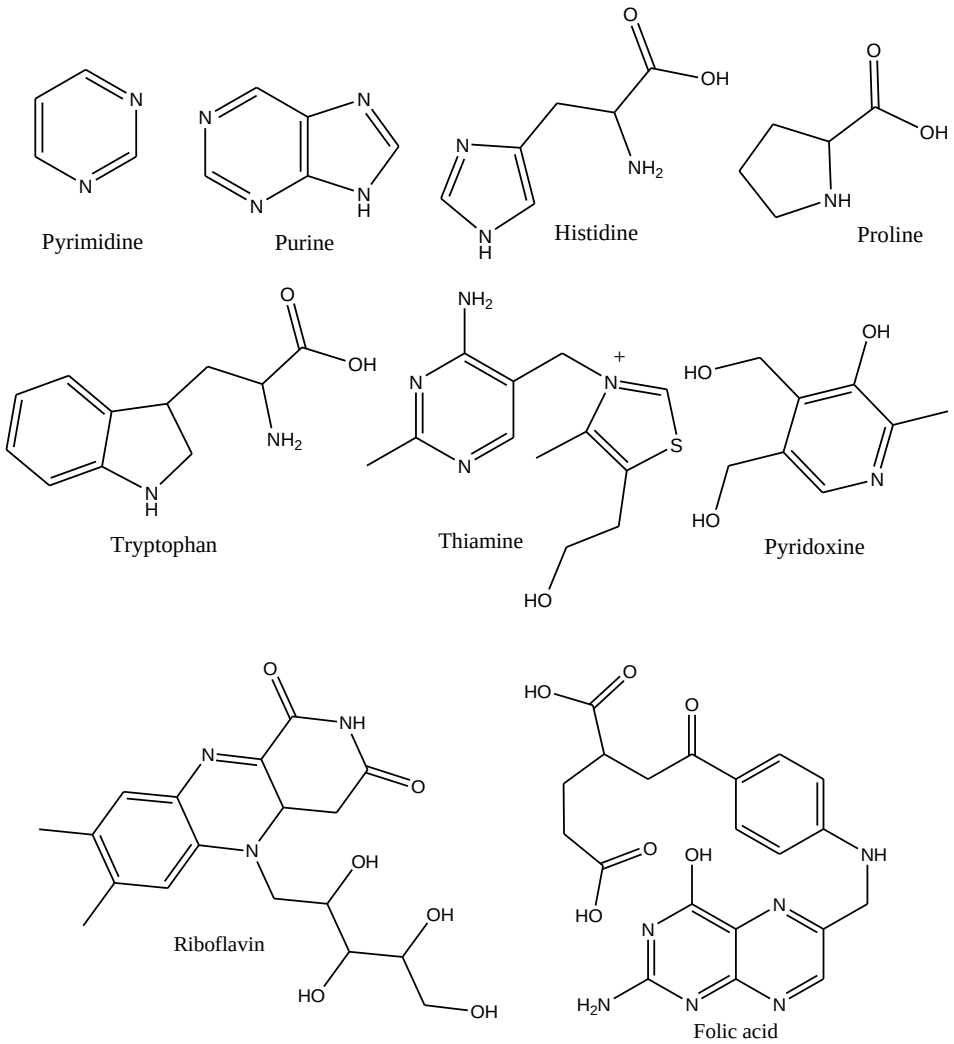


Figure 1.3: Some of the Essential N-heterocycles in Human System

For eg- the following heterocyclic compounds, - the pyrimidine and purine -bases of the genetic material DNA, the essential amino acidsproline, histidine and tryptophan, also the vitamins and coenzymes precursors thiamine, riboflavin, pyridoxine, folic acid and biotin; the B12 and E. families of vitamins; the photosynthesizing pigment chlorophyll; the oxygen transporting pigment hemoglobin and its breakdown products, the bile pigments; the hormones kinetin, heteriauxin, serotonin, histamine andmethoxatin etc.

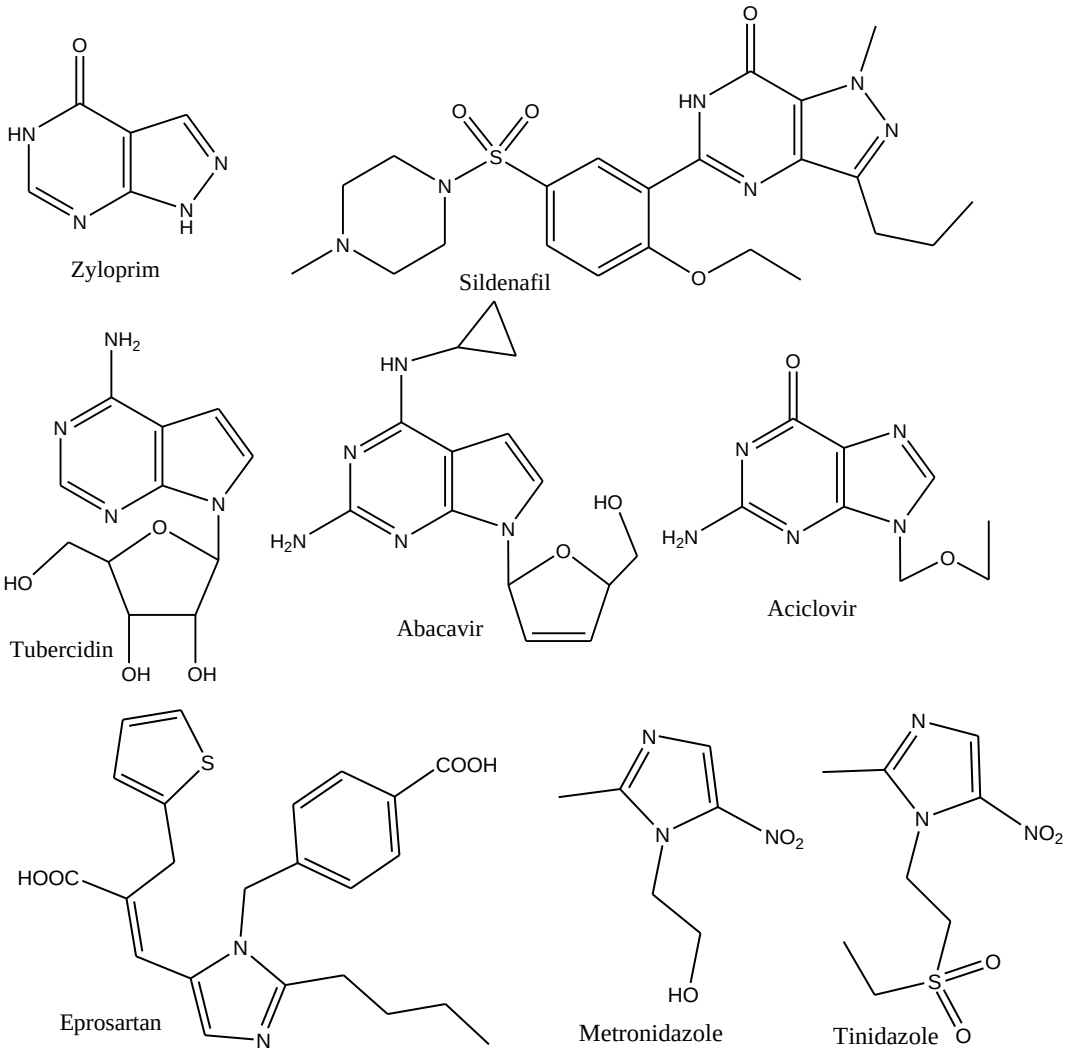


Figure 1.4: N-heterocycle-based Pharmaceuticals

There is large number of synthetic heterocyclic compounds with other important practical applications as, dyestuffs, copolymers, solvents, photographic sensitizer and developers, antioxidant and vulcanization accelerators in the rubber industry and many are valuable intermediates in the synthesis.

Besides their abundance in nature, N-heterocyclic compounds are of important units in pharmaceuticals many of which are in regular clinical use. Viz. Zyloprim used primarily to treathyperuricemia (excess of uric acid in blood plasma) and useful in chronic gout to prevent future attacks known as gout therapeutic21, Sildenafil (inhibitor of type VcGMP phosphodiesterase, Viagra), Tubercidin (anticancer agent), Abacavir (anti HIV agent), Aciclovir (antiviral agent for the treatment of Herpes) and Eprosartan (antihypertensive agent) (**Fig.1.4**). Heterocyclic nitroimidazole pharmaceuticals, such as metroindazole and tinidazole, work as magic bullets for the treatment of trichomonas infections (**Fig. 1.4**).

In addition, N-heterocyclic compounds like -lactams make up the core structure of antibiotics, such as penicillin G and Cephalosporin C. δ -Lactams [22,23]have found applications as HIV protease inhibitors (**Fig. 1.5**).

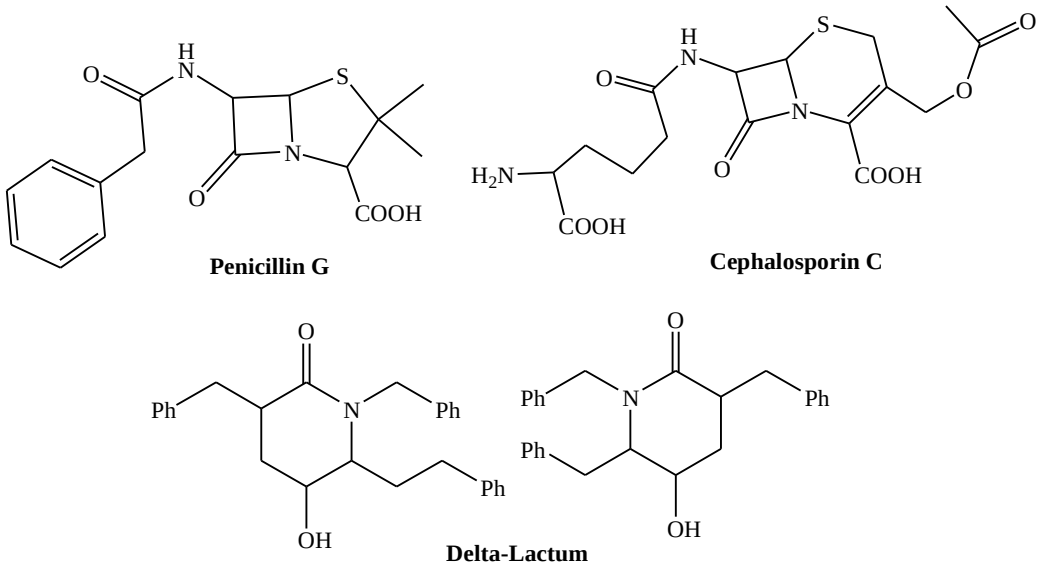
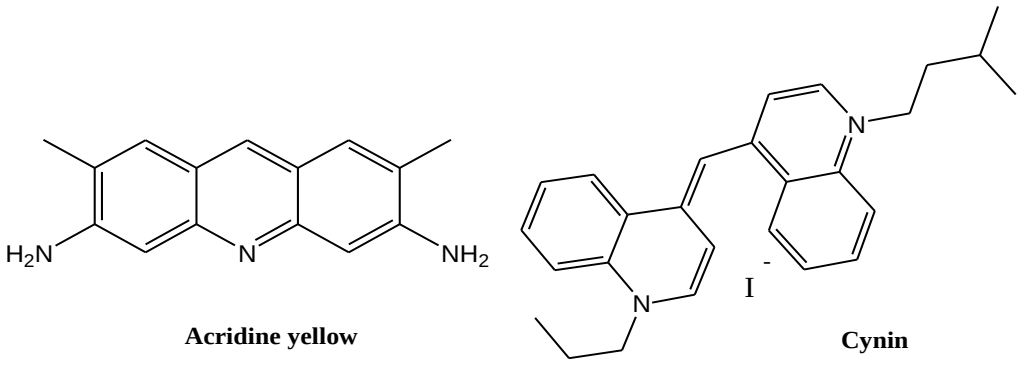


Figure 1.5: Some β and δ - Lactams containing biologically important compounds

N -heterocycles also make the key cores of herbicides, fungicides, dyes (e.g. acridine yellow G & purple coloured cyanine dye) and pigments (e.g. haemin, obtained from hemoglobin) (**Fig. 1.6**).



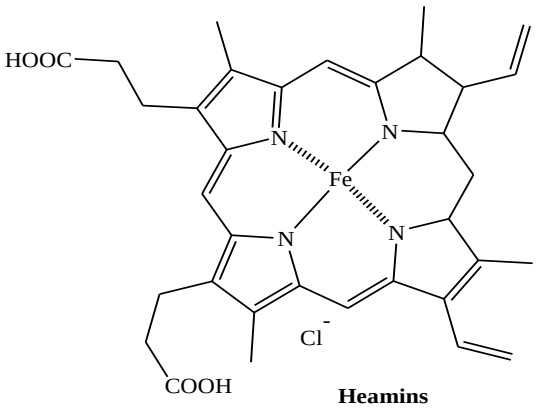


Figure 1.6: N-Heterocyclebased dyes and pigment

Aim of the studies:

Therefore, the main scope of this study is to develop synthetic methodologies, which generates bioactive nitrogen-containing heterocycles.

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ALLELOPATHIC EFFECT OF *CHROMOLAENA ODORATA* ON ANTIOXIDATIVE POTENTIAL OF SOME COASTAL PLANTS

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Abstract:

The present study intended to investigate the effect of aqueous extract from *Chromoleana odorata* L. on the non enzymatic antioxidant mechanism of some mangrove and mangrove associate plants. The activities of antioxidant such as DPPH Radical Scavenging potential, Ferric Ion Reducing Antioxidant Power (FRAP) and Reducing power were markedly affected due to leaf extract treatments of *C. odorata.* Ranmodi (*Chromolaena odorata*) has allelopathic potential that alters on antioxidative potential of other plants and helps to *Chromolaena odorata* to struggle with others.leaf leachate and leaf extract both treatments of Siam weed were found responsible to change significantly enhance antioxidant levels/ potential.

Keywords: *Chromoleana odorata*, DPPH activity, antioxidant potential, FRAP and Reducing power.

Introduction:

Chromolaena odorata (Linn) R.M. King and H. Robinson from family Asteraceae recognized as Siam weed is a fast-growing perennial, diffuse and jumbling shrub resident to Central and Southern America then brought into the tropical locales of Asian, African and the Pacific where it is an intrusive weed. This plant in Sawantwadi taluka, District Sindhudurg, Maharashtra, is commonly known as Ranmodi. This obtrusive weed is acquainted with numerous spots, either deliberately as a fancy plant or coincidentally, it is currently viewed as a noticeable amid the most perilous weeds introduce on earth because of its exceedingly intrusive and allelopathic nature (Vaisakh and Pandey, 2012; Otarigho and Morenikeji, 2013). This weed

suppresses crops and other plants in its surroundings by competing for nutrients and water, over-shading and allelopathy (Wilson *et al.*, 2014).

The literature on the infuence of *C. odorata* or its extracts on the growth of other coastal plants is quite scanty. Keeping in mind the above background, the current investigation was carried out to evaluate the allelopathic effects of aqueous leaf extract and leaf litter leachate of Siam weed on antioxidative parameters of some mangroves and associated plants.

Antioxidative assay helps to count antioxidant capacity of particular organism specifically plants which presently have huge demand in world of pharmaceutical biology and medicine, therefore is investigated further largely in bioscience (Cao and Prior, 1998 and Pellegrini *et al.*, 2003). As per described by Halliwell and Gutteridge (1999) and Hou *et al.* (2003) an antioxidant are that material which transparently delays or thwarts oxidation of that compounds by binding the free radicals.

Material and Methods:

Senescent leaves of *Chromolaena odorata* L. were collected from the coastal area of Sawantwadi taluka, Maharashtra. The senecent leaf litter was weighted, washed altogether with tap water and blotted to drythen 200g leaf material was soaked in 1 litre of distilled water for 24 hours at room temperature. Following 24 hours leachate was separated through Whatman No.1 filter paper. The leaf leachate (filtrate) was put away in icebox until utilized for additionally examines.

The antioxidant activities of plant extracts and the standard were evaluated based on the free radical searching impact of the steady 1, 1-diphenyl-2-picrylhydrazyl (DPPH) - free radical movement by changed strategy (Braca et al., 2002), FRAP antioxidant activityachieved according to the approach portrayed by Benzie and Strain (1996) and Electron donating capacity of an antioxidant is measured by using reducing power assay (Yen and Chen, 1995).

Result and Discussion:

Synthetic antioxidant has toxic influence on human as bioaccumulation in tissue leads to liver damage and carcinogenesis. Therefore there was need to find alternative sources of it hence herbal and natural sources of it are ultimately plants which are under research for it (Velioglu *et al.*, 1998; Pinelo *et al.*, 2004 and Rubilar *et al.*, 2006). Such natural antioxidants are used in food demands as inhibitors of lipid peroxidation (Scherer and Godoy, 2009).

In the present investigation influence of allelochemicals of *Chromolaena odorata* an antioxidative property of some coastal plant species has been examined by different methods.

The purpose of this study was also to evaluate impact of allelochemicals of *Chromolaena odorata* on different antioxidant capacity measurements in coastal plant species by radical scavenging ability, FRAP ability and Reducing Power assay.

DPPH radical scavenging activity:

1,1-Diphenyl-2-picrylhydrazyl, is a class of unwavering organic radical. The aptitude of biological reagents to chase DPPH radicals, can be expressed as its enormity of antioxidation ability. The antioxidant activities in natural products from plant and microbial sources and the standard were assessed on the basis of scavenging effect of the stable DPPH free radical activity by adapted technique (Peng *et al.*, 2000; Braca *et al.*, 2002; Shyur *et al.*, 2005). Different workers investigated radical scavenging activity from different plants as Mohammed *et al.*, (2010) and Bhawya and Anilkumar (2010) as 35 41% and 50% in *Anastaticum hierochantica* and *Hypaene thebaica* and *Tinospora cordifolia* respectively, Aparadh *et al.* (2012) noticed 35%, 37%, 47%, 57% variation in DPPH scavenging activities in different *Cleome* species DPPH activity, Ravindran *et al.* (2012) investigated 50% in *Excoecaria agallocha* and 83% in *Kandelia candel* mangrove species for radical scavenging activity. According to these workers DPPH activity was in the range 20 to 67 %.

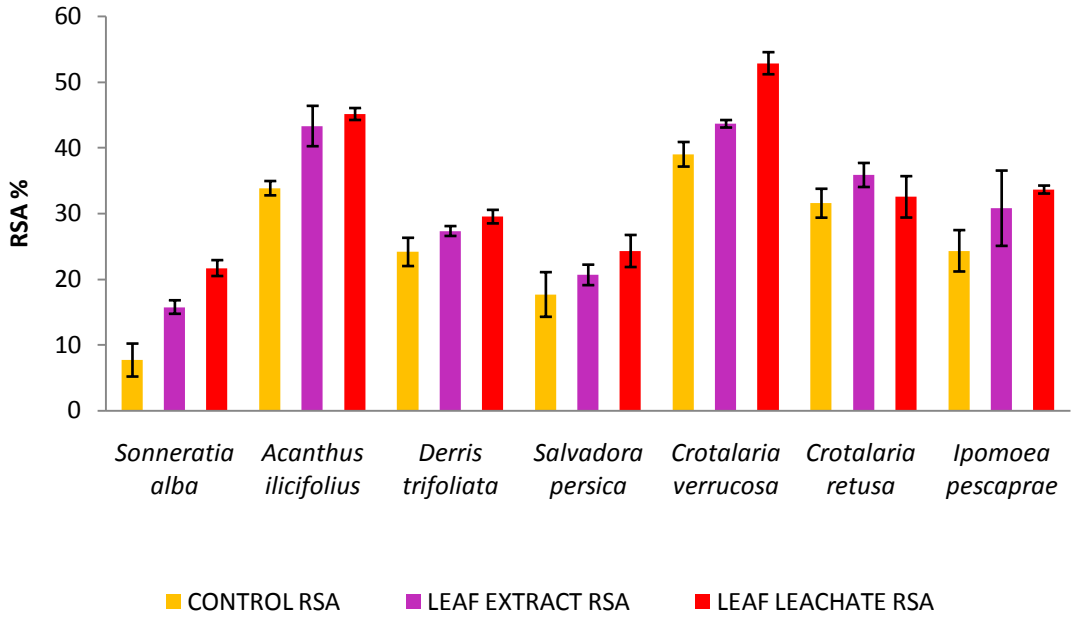
In the present investigation DPPH radical scavenging action of different coastal Mangroves and their associates have been studied and allelochemical influence of *Chromolaena odorata* through leaf extract and leaf leachate treatment on this property are depicted in Fig. 1. It is evident from result that under controlled condition DPPH scavenging activity found highest in *Crotalaria verrucosa* (38.98 ± 1.874%) that lowest in *Sonneratia alba* (7.66 ±2.508%).It is clear from result that leaf leachate treatment is responsible for enhancing RSA among all studied species. As same that of control under leaf extract and leaf leachate treatment of *Chromolaena odorata* ,both on *Crotalaria verrucosa* shows highest RSA (i.e.43.62 ± 0.573 % and 52.84 ±1.684 % respectively) and that lowest observed in *Sonneratia alba* (i.e. 15.73 ± 1.026 % 21.66 ±1.214 % respectively).it is observed in present study thatallelopathic effect of *Chromolaena odorata* on RSA of *Sonneratia alba* indicates there is maximum enhance under leaf leachate treatment (up to 282.81%) as compare with control one. i.e. 182.81 % enhance in activity as compare with control one. Under leaf extract treatment of same 205.43% enhance in RSA i.e. 105.43% enhance as compare to control one. Under same treatments RSA activity in *Acanthus ilicifolius* there is higher elevation observed under leaf leachate treatment i.e. 33.40 % than leaf extract treatment i.e.27.97 % as compare to control one. In case of *Derris trifoliata* for same treatment same kind of results observed i.e highest enhance under leaf leachate treatment of

Chromolaena odorata (22.27%) than leaf extract treatment of same (13.22%) as compare to control condition. In *Salvadora persica* also found same response as that of *Sonneratia alba* and *Derris trifoliata*. There is higher enhancement in RSA found under leaf leachate than that of leaf extract treatment (37.52 % and 16.90 % respectively) as compare to control of same. As contrast to leaf extract treatment there is 17.65 % elevation observed in RSA of *Salvadora persica*. In case of *Crotalaria verrucosa* also found higher elevation in RSA activity under leaf leachate treatment of *Chromolaena odorata* (i.e. 35.56 %) than leaf extract treatment of same (i.e. 11.91 %) as compare to control one. As comparing these both treatments there is 21.13 % enhancement in activity of RSA in leaf leachate treatment of *Chromolaena odorata* on *Crotalaria verrucosa* as compare with leaf extract treatment of same.

Allellopahic effect of *Chromolaena odorata* on *Crotalaria retusa* are depicted in Fig.1. It is evident from result that in contrast with other studied species here exact reverse pattern observed. i.e. Elevation in radical scavenging activity is observed higher in leaf extract treatment than that of leaf leachate treatment and control one. Under leaf leachate treatment there is enhancement in RSA activity of *Crotalaria retusa* observed as compare to control one i.e. 3.08 % while as compare to leaf extract treatment there is 9.28 % decline in RSA activity. Under leaf extract treatment of *Chromolaena odorata* on *Crotalaria retusa* there is elevation of RSA activity observed from 31.53 ± 2.20 % to 35.83 ± 2.20 %.

In case of *Ipomoea pes-caprae*, leaf leachate treatment of *Chromolaena odorata* causes higher elevation or enhancement in radical scavenging activity (24.29 to 33.61 ± 0.61 %) than that of leaf extract treatment of same (24.29 to 30.77 %) in contrast with control one. It indicates that there is 38.36 % enhance at leaf leachate treatment and 26.68 % elevation under leaf extract treatment in antioxidant potential of *Ipomoea pes-caprae*. By comparing these values it is clear that antioxidant potential in *Ipomoea* species get upto 9.23 % enhanced under leaf leachate treatment as compare to leaf extract treatment. It can be concluded that allelochemicals released by *Chromolaena odorata* improve greatly the bleaching action of free radicals in studied plants, which ultimately cause higher development in the Antioxidative capacity of these plants. This experiment also suggest that allelochemicals released by *Chromolaena odorata* have exhibited potent antioxidant improving effect in influenced plants by inhibiting free radicals which can supply as a intoxicating resource in chemo protective therapy.

Fig. 1. Allelopathic effect of *Chromolaena odorata* on radical scavenging activity of some mangrove and their associates



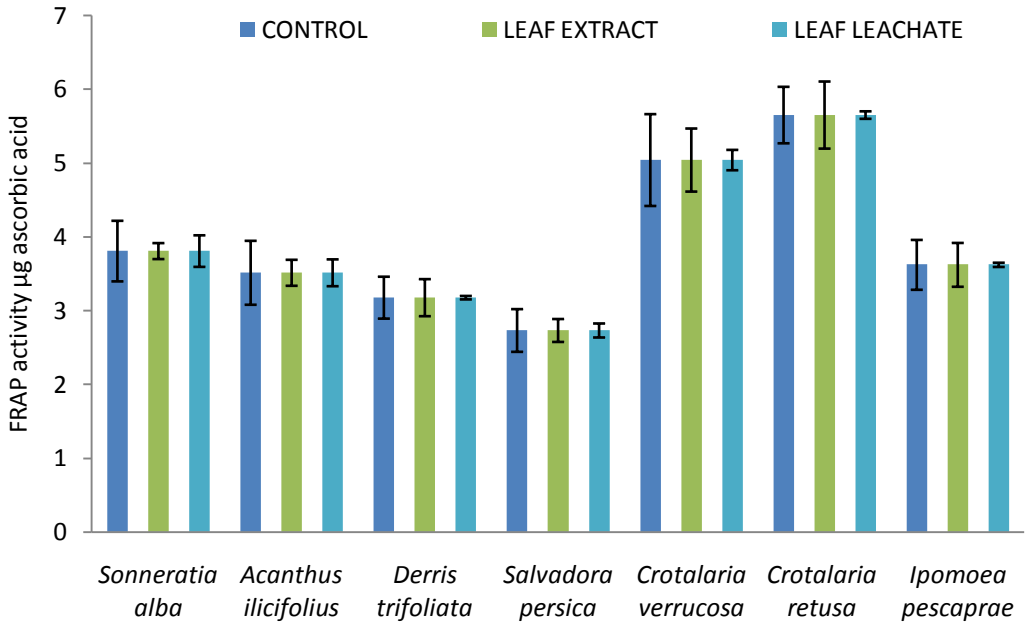
Ferric-reducing antioxidant power (FRAP):

The effect of allelopathic treatment (leaf extract and leaf leachate) of *Chromolaena odorata* on ferric reducing antioxidant plasma activity of some coastal plants is presented in Fig 2.Tsai *et al.* (2002) reported FRAP has been utilized to know antioxidant status of plant extracts. The higher the FRAP value the greater is the antioxidant activity. Thiapong *et al.* (2006), detected that FRAP tests was pessimistically allied with B-carotene content.

It is evident from results (Fig.2), under controlled condition FRAP activity found highest 5.651±0.382 µg ascorbic acid found in *Crotalaria retusa* and that lowest (2.732±0.285 µg ascorbic acid) activity found in *Salvadora persica* among all studied plants. The FRAP activity in other studied plants is found as 3.808 ±0.410 µg ascorbic acid in *Sonneratia alba*, 3.514±0.433 µg ascorbic acid in *Acanthus ilicifolius*, 3.177±0.284 µg ascorbic acid in *Derris trifoliate*, 2.732±0.285 µg ascorbic acid in *Salvadora persica* and that in *Ipomoea pes-capra*eas 3.622±0.337 µg ascorbic acid. Similar range of FRAP reported by Chan *et al.* (2007) as 54.5 ± 2.8 mg GAE/g FRAP at lower altitude and 50.4 ± 12.9 8 mg GAE/g FRAP at higher altitude from young tea leaves and by Dordevic *et al.* (2010) in *Fagopyrum esculentum*, 49.43 ± 0.49 nmol Fe2+/mg; *Hordeum vulgare*, 15.56 ± 0.67 nmol Fe2+/mg; *Triticum durum*, 12.15 ± 0.60 nmol Fe2+/mg and *Secale cereale* 8.94 ± 0.86 nmol Fe2+/mg. Surveswaran *et al.* (2007)

evaluated FRAP activity as in *Aloe littoralis* Baker 8.68µmol/g DW, *Murraya exotica* L. 1.80 µmol/g DW, *Vitex negundo* 1.44 µmol/g DW and *Viola serpens* Wall. Ex Ging. 0.91 µmol/g DW. Deighton *et al.* (2000) noted 191±33mM TEAC FRAP activity in *Rubus coreanus*, 20711±432mM TEAC in *R. hunanensis*, and 51289±635mM TEAC FRAP in *R. lambertianus.*

Fig. 2. Allelopathic effect of *Chromolaena odorata* on FRAP activity of some coastal plants



From the results of current investigation it is clear that both allelopathic treatments of *Chromolaena odorata* are responsible to alter the FRAP activity of all studied plants. As compare to controlled condition in leaf extract treatment of *Chromolaena odorata* FRAP activity found decreased in all studied coastal plants except *Crotalaria* species. Highest decrease by 14.90% found in *Acanthus ilicifolius* (i.e. 2.991±0.176 FRAP activity) and lowest decline 5.51% found in *Salvadora persica* (i.e. 2.582±0.155 FRAP activity) as compare to control of same. In other studied plants viz. *Sonneratia alba, Derris trifoliate* and *Ipomoea pes-caprae*same kind of decline in FRAP activity observed by 11.49%, 9.48% and 8.91% respectively. While in *Crotalaria* species elevation in FRAP activity found in same leaf extract treatment as 3.13% increase FRAP activity in *Crotalaria verrucosa* (i.e. 5.199±0.427 FRAP activity) and 5.71% increase FRAP activity in *Crotalaria retusa* (i.e. 5.974±0.455FRAP activity) as compare to control one.

In case of leaf leachate treatment also FRAP activity found decreased in most of studied plants except *Salvadora persica* and *Crotalaria retusa.* In these two plants FRAP activity found elevated by 3.94% in *Salvadora persica* and *by 7.23%* in *Crotalaria retusa.* While all other studied plants shows decline in FRAP activity as 7.53% loss in *Sonneratia alba,* 15.10% loss in *Acanthus ilicifolius,* 16.36% fall down in *Crotalaria verrucosa.* The highest 18.06% reduction in FRAP activity observed in *Derris trifoliate* (3.177±0.284 to 2.603±0.381 µg ascorbic acid) and that lowest 5.74% loss of FRAP activity found in *Ipomoea pes-caprae* (3.622±0.337 to 3.414±0.118 µg ascorbic acid) as compare to control of same. Dudonne *et al.,* (2009) reported that there is always strong correlation between TPC and FRAP and recommended that phenolic derivatives present in the plants are the major contributors of antioxidant potential. It is clear from results that among the all studied plant species appreciable enhancement in FRAP activity in the leaf tissue is noticed in case of *Crotalaria verrucosa* and *Crotalaria retusa* under leaf extract treatment and that in *Salvadora persica* and *Crotalaria retusa* under leaf leachate treatment of same plant *Chromolaena odorata*.

Reducing power:

In present study allelopathic potential of *Chromoleana odorata*have been studied on reducing power assay of few coastal plants. Effect of leaf extract and leaf leachate treatments of *Chromolaena odorata* on few coastal plant species have been recorded in Fig. 3. It is evident from results that among all studied plant reducing power potential found elevated under both of these treatments of *Chromolaena odorata*.

In reducing power assay, the yellow color of reaction solution transforms to various tints of green and blue, counting on the reducing power of every compound which is due to the conversion of the ferricyanide complex to the ferrous form during the exploit of breaking the free radical chain by contributing H^+ atom. Absorbance of Fe2+ can be measured at 700 nm which helpful to count antioxidative potential / reducing power of sample solution (Yildirim *et al.*, 2000; Zou *et al.*, 2004; Singh and Rajini, 2004). Higher the absorbance, greater will be intensity and greater will be antioxidant capacity (Zou *et al.*, 2004).

In the current investigation among studied plant, highest reducing power potential found in *Crotalaria rutusa* and lowest in *Sonneratia alba* at control condition. Abs at 700 nm directly proportional to reducing power potential of plants i.e. increase in absorbance at thin wavelength indicates increase in reducing power of plant. Highest abs 1.221 of *Crotalaria verrucosa* indicates highest reducing power of this plant among all studied plant and that lowest 0.466 OD

in *Sonneratia alba*. The reducing power potential pattern observed in studied plants is as *Crotalaria verrucosa>Acanthus ilicifolius>Ipomoea pes-caprae>Derris trifoliate >Crotalaria retusa>Salvadora persica>Sonneratia alba* at controlled condition.

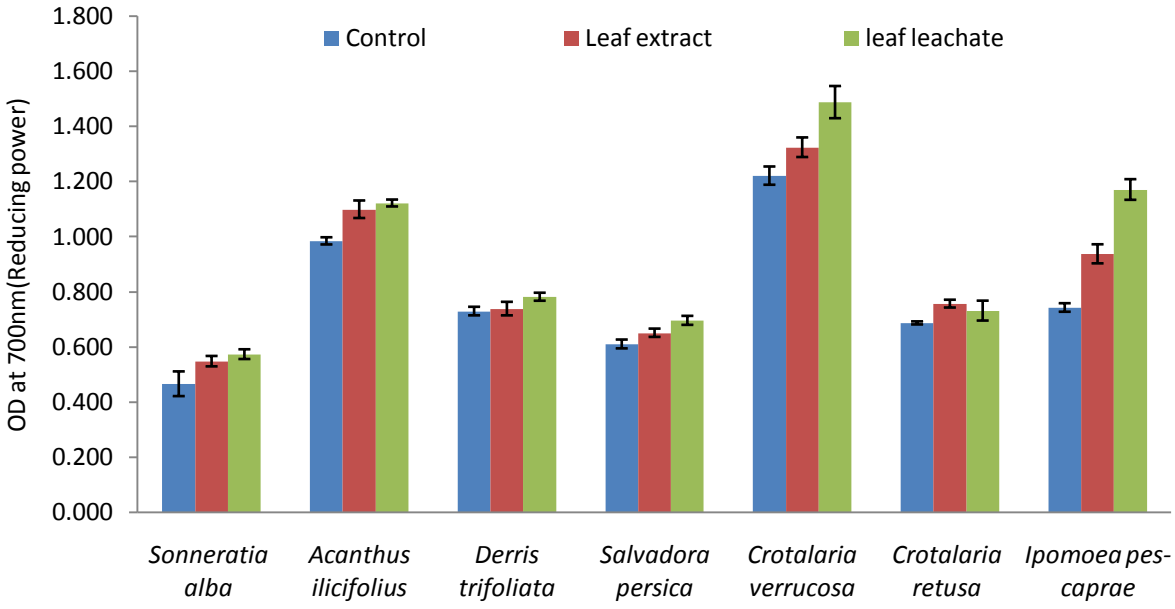
In leaf extract treatment of *Chromolaena odorata* elevation in absorption found among all studied plants indicate leaf extract treatment responsible to increase reducing power potential of all these plants. Under this treatment highest elevation 26.20% found in reducing power of *Ipomoea pes-caprae* and that lowest increase is observed in *Derris trifoliata* (1.233%) as compare to control one and other studied plants. Under these treatments other plants also showed increase in reducing power viz. 17.58% elevation in *Sonneratia alba*. 11.64% elevation in *Acanthus ilicifolius*, 6.66% enhancement in *Salvadorapersica*, 8.43% elevation in *Crotalaria verrucosa* and that 10.19% elavation in reducing power of *Crotalriaretusa* as compare to control one of same.

In leaf leachate treatment this potential found highly or maximum increase than leaf extract and control treatment in all studied plants. At leaf leachate treatment highest increase 57.60% observed in *Ipomoeapes-caprae*, while lowest elevation 6.50% observed in *Crotalaria retusa*. For *Crotalaria retusa* leaf leachate treatment found less effective than leaf extract treatment to increase reducing power potential of this plant. Under leaf leachate treatment of *Chromolaenaodorata* other studied plants found showing maximum elevation in their reducing power potential viz.23.02% in *Sonneratia alba*, that 13.95% increase in *Acanthus ilicifolius*, 7.12% improvement in *Derristri foliata*, 14.03% increase in *Salvadora persica* and 21.83% elevation in reducing power potential observed in *Crotalaria verrucosa*.

Various workers studied reducing power in various plants as Bursal and Koksal (2011) from *Rhuscoriaria* L., Ebrahimzadeh and Bahramian (2009) from *Crataeguspentaegyna* subsp. *Elburensis*, Deore*et al*. (2009) in *Lagenaria siceraria*, Hajimahmoodi *et al.* (2008) in 6 Iranian olive cultivars, Lu *et al.* (2010) from green tea, Aparadh (2011) in different *Cleome* species.

From overall outcome it can be reasoned that leaf leachate treatment of *Chromolaena odorata* highly alters antioxidative potential of other plants than leaf extract treatment of same. But both treatments are responsible to change significantly in antioxidative potential of other plants i.e. reducing power potential. It can be concluded that both studied treatments responsible to enhance in hydrogen donating capability as described by Shimada *et al*. (1992).

Fig. 3. Allelopathic effect of *Chromolaena odorata* reducing power potential of some coastal plants



Conclusion:

FRAP activity, reducing power potential and DPPH radical scavenging action of diverse coastal plants and their associates influences by allelochemicals of *Chromolaena odorata* through leaf extort and leaf leachate dealing. The allelochemicals secreted by *Chromolaena odorata* improve greatly the bleaching action of charged particles in studied plants, which ultimately cause higher development in the RSA capability of studied plants.Due to both treatments all studied plants had displayed a mild enhancement in chelating activity. From the results (Fig. 1, 2 and 3), it can be confirmed that leaf leachate conduct of *C. odorata* highly alters antioxidative potential of other plants than leaf extract treatment. But both treatments were found responsible to change significantly reducing power potential. Hence it can be said that allelochemicals of *C. odorata* may helpful to enhance remedial prospective of influenced plant aligned with assorted syndromes.This experiment also suggest that allelochemicals released by *Chromolaena odorata* have exhibited potent antioxidant improving effect in influenced plants by inhibiting free radicals which can supply as a intoxicating resource in chemo protective therapy.

While FRAP activity found decreased in most of studied plants under both treatment except *Salvadorapersica*and*Crotolaria retusa.* The Siam weed has allelopathic potential that by all ways affect on antioxidative potential of other plants and helps to *Chromolaena odorata* to

struggle with others.From general outcome it can be reasoned that *Chromolaena odorata* has allelopathic potential that directly or indirectly affect on antioxidative potential of other plants and helps to *Chromolaena odorata* to compete with others.

Acknowledgement:

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RESEARCH INTERVENTIONS AND ADVANCEMENTS IN PLANT SCIENCES

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